Key References

The complete reference list is available online at Expert Consult.

2. Azad AF. Epidemiology of murine typhus. *Annu Rev Entomol*. 1990;35:553–569.
8. Dumler JS, Taylor JP, Walker DH. Clinical and laboratory features of murine typhus in south Texas, 1980 through 1987. *JAMA*. 1991;266:1365–1370.
10. Pieracci EG, Evert N, Drexler NA, et al. Fatal flea-borne typhus in Texas: a retrospective case series, 1985-2015. *Am J Trop Med Hyg*. 2017;96:1088–1093.
15. McLeod MP, Qin X, Karpathy SE, et al. Complete genome sequence of *Rickettsia typhi* and comparison with sequences of other rickettsiae. *J Bacteriol*. 2004;186:5824–5855.
22. Rahman MS, Gillespie JJ, Kaur SJ, et al. *Rickettsia typhi* possesses phospholipase A2 enzymes that are involved in infection of host cells. *PLoS Pathog*. 2013;9:e1003399.
26. Traub R, Wisseman CL. The ecology of murine typhus—a critical review. *Trop Dis Bull*. 1978;75:237–317.
28. Irons JV, Bohls SW, Thurman DCJ, et al. Probable role of the cat flea, *Ctenocephalides felis,* in transmission of murine typhus. *Am J Trop Med Hyg*. 1944;s1-24:359–362.
32. Blanton LS, Vohra RF, Bouyer DH, et al. Reemergence of murine typhus in Galveston, Texas, USA, 2013. *Emerg Infect Dis*. 2015;21:484–486.
39. Murray KO, Evert N, Mayes B, et al. Typhus group rickettsiosis, Texas, USA, 2003-2013. *Emerg Infect Dis*. 2017;23:645–648.
58. Whiteford SF, Taylor JP, Dumler JS. Clinical, laboratory, and epidemiologic features of murine typhus in 97 Texas children. *Arch Pediatr Adolesc Med*. 2001;155:396–400.
60. Jensenius M, Han PV, Schlagenhauf P, et al. Acute and potentially life-threatening tropical diseases in western travelers—a GeoSentinel multicenter study, 1996-2011. *Am J Trop Med Hyg*. 2013;88:397–404.
64. Farhang-Azad A, Traub R, Baqar S. Transovarial transmission of murine typhus rickettsiae in *Xenopsylla cheopis* fleas. *Science*. 1985;227:543–545.
65. Walker DH, Parks FM, Betz TG, et al. Histopathology and immunohistologic demonstration of the distribution of *Rickettsia typhi* in fatal murine typhus. *Am J Clin Pathol*. 1989;91:720–724.
66. Binford CH, Ecker HD. Endemic (murine) typhus: report of autopsy findings in three cases. *Am J Clin Pathol*. 1947;17:797–806.
67. Paris DH, Chansamouth V, Nawtaisong P, et al. Coagulation and inflammation in scrub typhus and murine typhus—a prospective comparative study from Laos. *Clin Microbiol Infect*. 2012;18:1221–1228.
70. Walker DH, Popov VL, Feng HM. Establishment of a novel endothelial target mouse model of a typhus group rickettsiosis: evidence for critical roles for gamma interferon and CD8 T lymphocytes. *Lab Invest*. 2000;80:1361–1372.
71. Rollwagen FM, Dasch GA, Jerrells TR. Mechanisms of immunity to rickettsial infection: characterization of a cytotoxic effector cell. *J Immunol*. 1986;136:1418–1421.
74. Walker DH, Ismail N. Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events. *Nat Rev Microbiol*. 2008;6:375–386.
85. Tsioutis C, Zafeiri M, Avramopoulos A, et al. Clinical and laboratory characteristics, epidemiology, and outcomes of murine typhus: a systematic review. *Acta Trop*. 2017;166:16–24.
86. Stuart BM, Pullen RL. Endemic (murine) typhus fever: clinical observations of 180 cases. *Ann Intern Med*. 1945;23:520–536.
90. Silpapojakul K, Ukkachoke C, Krisanapan S, et al. Rickettsial meningitis and encephalitis. *Arch Intern Med*. 1991;151:1753–1757.
100. Hechemy KE, Stevens RW, Sasowski S, et al. Discrepancies in Weil-Felix and microimmunofluorescence test results for Rocky Mountain spotted fever. *J Clin Microbiol*. 1979;9:292–293.
106. Walker DH, Feng HM, Ladner S, et al. Immunohistochemical diagnosis of typhus rickettsiosis using an anti-lipopolysaccharide monoclonal antibody. *Mod Pathol*. 1997;10:1038–1042.
108. Paris DH, Dumler JS. State of the art of diagnosis of rickettsial diseases: the use of blood specimens for diagnosis of scrub typhus, spotted fever group rickettsiosis, and murine typhus. *Curr Opin Infect Dis*. 2016;29:433–439.
114. Sorvillo FJ, Gondo B, Emmons R, et al. A suburban focus of endemic typhus in Los Angeles County: association with seropositive domestic cats and opossums. *Am J Trop Med Hyg*. 1993;48:269–273.
118. Rolain JM, Maurin M, Vestris G, et al. In vitro susceptibilities of 27 rickettsiae to 13 antimicrobials. *Antimicrob Agents Chemother*. 1998;42:1537–1541.
119. Dumler JS. Clincial disease: current treatment and new challenges. In: Palmer GH, Azad AF, eds. *Intracellular Pathogens II: Rickettsiales*. Washington, DC: ASM Press; 2012:1–39.
120. Gikas A, Doukakis S, Pediaditis J, et al. Comparison of the effectiveness of five different antibiotic regimens on infection with *Rickettsia typhi:* therapeutic data from 87 cases. *Am J Trop Med Hyg*. 2004;70:576–579.
125. Raoult D, Drancourt M. Antimicrobial therapy of rickettsial diseases. *Antimicrob Agents Chemother*. 1991;35:2457–2462.
127. Cross R, Ling C, Day NP, et al. Revisiting doxycycline in pregnancy and early childhood—time to rebuild its reputation? *Expert Opin Drug Saf*. 2016;15:367–382.

References

1. Hone FS. A series of cases closely resembling typhus fever. *Med J Aust*. 1922;I:1–13.
2. Azad AF. Epidemiology of murine typhus. *Annu Rev Entomol*. 1990;35:553–569.
3. Marshall GS. *Rickettsia typhi* seroprevalence among children in the southeast United States: tick-borne infections in children study (ticks) group. *Pediatr Infect Dis J*. 2000;19:1103–1104.
4. Tay ST, Ho TM, Rohani MY, et al. Antibodies to *Orientia tsutsugamushi, Rickettsia typhi,* and spotted fever group rickettsiae among febrile patients in rural areas of Malaysia. *Trans R Soc Trop Med Hyg*. 2000;94:280–284.
5. Lledo L, Gegundez MI, Saz JV, et al. Prevalence of antibodies to *Rickettsia typhi* in an area of the center of Spain. *Eur J Epidemiol*. 2001;17:927–928.
6. Daniel SA, Manika K, Arvanmdou M, et al. Prevalence of *Rickettsia conorii* and *Rickettsia typhi* infections in the population of northern Greece. *Am J Trop Med Hyg*. 2002;66:76–79.
7. Boomstrom A, Beier MS, Macaluso JA, et al. Geographic association of *Rickettsia felis*-infected opossums with human murine typhus, Texas. *Emerg Infect Dis*. 2002;8:549–554.
8. Dumler JS, Taylor JP, Walker DH. Clinical and laboratory features of murine typhus in south Texas, 1980 through 1987. *JAMA*. 1991;266:1365–1370.
9. Duffy PE, Le Guillouzic H, Gass RF, et al. Murine typhus identified as a major cause of febrile illness in a camp for displaced Khmers in Thailand. *Am J Trop Med Hyg*. 1990;43:520–526.
10. Pieracci EG, Evert N, Drexler NA, et al. Fatal flea-borne typhus in Texas: a retrospective case series, 1985-2015. *Am J Trop Med Hyg*. 2017;96:1088–1093.
11. Tsioutis C, Chaliotis G, Kokkini S, et al. Murine typhus in elderly patients: a prospective study of 49 patients. *Scand J Infect Dis*. 2014;46:779–782.
12. Azad AF, Radulovic S, Higgins JA, et al. Flea-borne rickettsioses: ecologic considerations. *Emerg Infect Dis*. 1997;3:319–327.
13. Comer JA, Paddock CD, Childs JE. Urban zoonoses caused by *Bartonella, Coxiella, Ehrlichia,* and *Rickettsia* species. *Vector Borne Zoonotic Dis*. 2001;1:91–118.
14. Bouyer DH, Stenos J, Crocquet-Valdes P, et al. *Rickettsia felis:* molecular characterization of a new member of the spotted fever group. *Int J Syst Evol Microbiol*. 2001;51:339–347.
15. McLeod MP, Qin X, Karpathy SE, et al. Complete genome sequence of *Rickettsia typhi* and comparison with sequences of other rickettsiae. *J Bacteriol*. 2004;186:5824–5855.
16. Walker DH, Yu XJ. Progress in rickettsial genome analysis from pioneering of *Rickettsia prowazekii* to the recent *Rickettsia typhi*. *Ann N Y Acad Sci*. 2005;1063:13–25.
17. Sears KT, Ceraul SM, Gillespie JJ, et al. Surface proteome analysis and characterization of surface cell antigen (Sca) or autotransporter family of *Rickettsia typhi*. *PLoS Pathog*. 2012;8:e1002856.
18. Uchiyama T, Kawano H, Kusuhara Y. The major outer membrane protein rOmpB of spotted fever group Rickettsiae functions in the rickettsial adherence to and invasion of Vero cells. *Microbes Infect*. 2006;8:801–809.
19. Radulovic S, Troyer JM, Beier MS, et al. Identification and molecular analysis of the gene encoding *Rickettsia typhi* hemolysin. *Infect Immun*. 1999;67:6104–6108.
20. Rahman MS, Ammerman NC, Sears KT, et al. Functional characterization of a phospholipase A2 homolog from *Rickettsia typhi*. *J Bacteriol*. 2010;192:3294–3303.
21. Rennoll-Bankert KE, Rahman MS, Gillespie JJ, et al. Which way in? The RalF Arf-GEF orchestrates *Rickettsia* host cell invasion. *PLoS Pathog*. 2015;11:e1005115.
22. Rahman MS, Gillespie JJ, Kaur SJ, et al. *Rickettsia typhi* possesses phospholipase A2 enzymes that are involved in infection of host cells. *PLoS Pathog*. 2013;9:e1003399.
23. Rahman MS, Simser JA, Macaluso KR, et al. Functional analysis of *secA* homologues from Rickettsiae. *Microbiology*. 2005;151:589–596.
24. Ammerman NC, Rahman MS, Azad AF. Characterization of sec-translocon-dependent extracytoplasmic proteins of *Rickettsia typhi*. *J Bacteriol*. 2008;190:6234–6242.
25. Kaur SJ, Rahman S, Ammerman NC, et al. TolC-dependent secretion of an ankyrin repeat-containing protein of *Rickettsia typhi*. *J Bacteriol*. 2012;194:4920–4932.
26. Traub R, Wisseman CL. The ecology of murine typhus—a critical review. *Trop Dis Bull*. 1978;75:237–317.
27. Kuo CC, Wardrop N, Chang CT, et al. Significance of major international seaports in the distribution of murine typhus in Taiwan. *PLoS Negl Trop Dis*. 2017;11:e0005430.
28. Irons JV, Bohls SW, Thurman DCJ, et al. Probable role of the cat flea, *Ctenocephalides felis,* in transmission of murine typhus. *Am J Trop Med Hyg*. 1944;s1-24:359–362.
29. Civen R, Ngo V. Murine typhus: an unrecognized suburban vectorborne disease. *Clin Infect Dis*. 2008;46:913–918.
30. Blanton LS, Idowu BM, Tatsch TN, et al. Opossums and cat fleas: new insights in the ecology of murine typhus in Galveston, Texas. *Am J Trop Med Hyg*. 2016;95:457–461.
31. Acuna-Soto R, Calderon-Romero L, Romero-Lopez D, et al. Murine typhus in Mexico City. *Trans R Soc Trop Med Hyg*. 2000;94:45.
32. Blanton LS, Vohra RF, Bouyer DH, et al. Reemergence of murine typhus in Galveston, Texas, USA, 2013. *Emerg Infect Dis*. 2015;21:484–486.
33. Hidalgo M, Montoya V, Martinez A, et al. Flea-borne rickettsioses in the north of Caldas province, Colombia. *Vector Borne Zoonotic Dis*. 2013;13:289–294.
34. Teoh YT, Hii SF, Stevenson MA, et al. Serological evidence of exposure to *Rickettsia felis* and *Rickettsia typhi* in Australian veterinarians. *Parasit Vectors*. 2017;10:129.
35. Pratt HD. The changing picture of murine typhus in the United States. *Ann N Y Acad Sci*. 1958;70:516–527.
36. Hill EL, Morlan HB. Evaluation of county-wide DDT dusting operations in murine typhus control. *Public Health Rep*. 1948;63:1635–1653.
37. Wiley JS. Recent developments in murine typhus fever control. *Am J Public Health Nations Health*. 1946;36:974–983.
38. Erickson T, da Silva J, Nolan MS, et al. Newly recognized pediatric cases of typhus group rickettsiosis, Houston, Texas, USA. *Emerg Infect Dis*. 2017;23:2068–2071.
39. Murray KO, Evert N, Mayes B, et al. Typhus group rickettsiosis, Texas, USA, 2003-2013. *Emerg Infect Dis*. 2017;23:645–648.
40. Adjemian J, Parks S, McElroy K, et al. Murine typhus in Austin, Texas, USA, 2008. *Emerg Infect Dis*. 2010;16:412–417.
41. Maina AN, Fogarty C, Krueger L, et al. Rickettsial infections among *Ctenocephalides felis* and host animals during a flea-borne rickettsioses outbreak in Orange County, California. *PLoS ONE*. 2016;11:e0160604.
42. Older JJ. The epidemiology of murine typhus in Texas, 1969. *JAMA*. 1970;214:2011–2017.
43. Dzul-Rosado K, Lugo-Caballero C, Tello-Martin R, et al. Direct evidence of *Rickettsia typhi* infection in *Rhipicephalus sanguineus* ticks and their canine hosts. *Open Vet J*. 2017;7:165–169.
44. Phongmany S, Rolain JM, Phetsouvanh R, et al. Rickettsial infections and fever, Vientiane, Laos. *Emerg Infect Dis*. 2006;12:256–262.
45. Dupont HT, Brouqui P, Faugere B, et al. Prevalence of antibodies to *Coxiella burnetti, Rickettsia conorii,* and *Rickettsia typhi* in seven African countries. *Clin Infect Dis*. 1995;21:1126–1133.
46. Bernabeu-Wittel M, Pachón J, Alarcón A, et al. Murine typhus as a common cause of fever of intermediate duration: a 17-year study in the south of Spain. *Arch Intern Med*. 1999;159:872–876.
47. Hidalgo M, Salguero E, de la Ossa A, et al. Murine typhus in Caldas, Colombia. *Am J Trop Med Hyg*. 2008;78:321–322.
48. Yang WH, Dong T, Zhang HL, et al. Murine typhus in drug detoxification facility, Yunnan Province, China, 2010. *Emerg Infect Dis*. 2012;18:1388–1390.
49. Murdoch DR, Woods CW, Zimmerman MD, et al. The etiology of febrile illness in adults presenting to Patan Hospital Kathmandu, Nepal. *Am J Trop Med Hyg*. 2004;70:670–675.
50. Blacksell SD, Sharma NP, Phumratanaprapin W, et al. Serological and blood culture investigations of Nepalese fever patients. *Trans R Soc Trop Med Hyg*. 2007;101:686–690.
51. Zimmerman MD, Murdoch DR, Rozmajzi PJ, et al. Murine typhus and febrile illness, Nepal. *Emerg Infect Dis*. 2008;14:1656–1659.
52. Pradhan R, Shrestha U, Gautam SC, et al. Bloodstream infection among children presenting to a general hospital outpatient clinic in urban Nepal. *PLoS ONE*. 2012;7:e47531.
53. Gasem MH, Wagenaar JF, Goris MG, et al. Murine typhus and leptospirosis as causes of acute undifferentiated fever, Indonesia. *Emerg Infect Dis*. 2009;15:976–977.
54. Bhengsri S, Baggett HC, Edouard S, et al. Sennetsu neorickettsiosis, spotted fever group, and typhus group rickettsioses in three provinces in Thailand. *Am J Trop Med Hyg*. 2016;95:43–49.
55. Mayxay M, Sengvilaipaseuth O, Chanthongthip A, et al. Causes of fever in rural southern Laos. *Am J Trop Med Hyg*. 2015;93:517–520.
56. Prabhu M, Nicholson WL, Roche AJ, et al. Q fever, spotted fever group, and typhus group rickettsioses among hospitalized febrile patients in northern Tanzania. *Clin Infect Dis*. 2011;53:e8–e15.
57. Hamaguchi S, Cuong NC, Tra DT, et al. Clinical and epidemiological characteristics of scrub typhus and murine typhus among hospitalized patients with acute undifferentiated fever in northern Vietnam. *Am J Trop Med Hyg*. 2015;92:972–978.
58. Whiteford SF, Taylor JP, Dumler JS. Clinical, laboratory, and epidemiologic features of murine typhus in 97 Texas children. *Arch Pediatr Adolesc Med*. 2001;155:396–400.
59. Gikas A, Doukakis S, Pediaditis J, et al. Murine typhus in Greece: epidemiological, clinical, and therapeutic data from 83 cases. *Trans R Soc Trop Med Hyg*. 2002;96:250–253.
60. Jensenius M, Han PV, Schlagenhauf P, et al. Acute and potentially life-threatening tropical diseases in western travelers—a GeoSentinel multicenter study, 1996-2011. *Am J Trop Med Hyg*. 2013;88:397–404.
61. Walter G, Botelho-Nevers E, Socolovschi C, et al. Murine typhus in returned travelers: a report of thirty-two cases. *Am J Trop Med Hyg*. 2012;86:1049–1053.
62. Raby E, Dyer JR. Endemic (murine) typhus in returned travelers from Asia, a case series: clues to early diagnosis and comparison with dengue. *Am J Trop Med Hyg*. 2013;88:701–703.
63. Azad AF, Traub R. Experimental transmission of murine typhus by *Xenopsylla cheopis* flea bites. *Med Vet Entomol*. 1989;3:429–433.
64. Farhang-Azad A, Traub R, Baqar S. Transovarial transmission of murine typhus rickettsiae in *Xenopsylla cheopis* fleas. *Science*. 1985;227:543–545.
65. Walker DH, Parks FM, Betz TG, et al. Histopathology and immunohistologic demonstration of the distribution of *Rickettsia typhi* in fatal murine typhus. *Am J Clin Pathol*. 1989;91:720–724.
66. Binford CH, Ecker HD. Endemic (murine) typhus: report of autopsy findings in three cases. *Am J Clin Pathol*. 1947;17:797–806.
67. Paris DH, Chansamouth V, Nawtaisong P, et al. Coagulation and inflammation in scrub typhus and murine typhus—a prospective comparative study from Laos. *Clin Microbiol Infect*. 2012;18:1221–1228.
68. Silpapojakul K, Mitarnun W, Ovartlarnporn B, et al. Liver involvement in murine typhus. *QJM*. 1996;89:623–629.
69. Khairallah M, Yahia SB, Toumi A, et al. Ocular manifestations associated with murine typhus. *Br J Ophthalmol*. 2009;93:938–942.
70. Walker DH, Popov VL, Feng HM. Establishment of a novel endothelial target mouse model of a typhus group rickettsiosis: evidence for critical roles for gamma interferon and CD8 T lymphocytes. *Lab Invest*. 2000;80:1361–1372.
71. Rollwagen FM, Dasch GA, Jerrells TR. Mechanisms of immunity to rickettsial infection: characterization of a cytotoxic effector cell. *J Immunol*. 1986;136:1418–1421.
72. Crist AE Jr, Wisseman CL Jr, Murphy JR. Characteristics of lymphoid cells that adoptively transfer immunity to *Rickettsia mooseri* infection in mice. *Infect Immun*. 1984;44:55–60.
73. Murphy JR, Wiseman CL Jr, Fiset P. Mechanisms of immunity in typhus infection: analysis of immunity to *Rickettsia mooseri* infection of guinea pigs. *Infect Immun*. 1980;27:730–738.
74. Walker DH, Ismail N. Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events. *Nat Rev Microbiol*. 2008;6:375–386.
75. Feng HM, Whitworth T, Popov V, et al. Effect of antibody on the rickettsia-host cell interaction. *Infect Immun*. 2004;72:3524–3530.
76. Older JJ. The epidemiology of murine typhus in Texas, 1969. *JAMA*. 1970;214:2011–2017.
77. Silpapojakul K, Chupuppakarn S, Yuthasompob S, et al. Scrub and murine typhus in children with obscure fever in the tropics. *Pediatr Infect Dis J*. 1991;10:200–203.
78. Hernandez Cabrera M, Angel-Moreno A, Santana E, et al. Murine typhus with renal involvement in Canary Islands, Spain. *Emerg Infect Dis*. 2004;10:740–743.
79. Koliou M, Psaroulaki A, Georgiou C, et al. Murine typhus in Cyprus: 21 paediatric cases. *Eur J Clin Microbiol Infect Dis*. 2007;26:491–493.
80. Wilde H, Pornsilapatip J, Sokly T, et al. Murine and scrub typhus at Thai-Kampuchean border displaced persons' camps. *Trop Geogr Med*. 1991;43:363–369.
81. Fergie JE, Purcell K, Wanat D. Murine typhus in South Texas children. *Pediatr Infect Dis J*. 2000;19:535–538.
82. Silpapojakul K, Chayakul P, Krisanapan S, et al. Murine typhus in Thailand: clinical features, diagnosis, and treatment. *Q J Med*. 1993;86:43–47.
83. Tselentis Y, Babalis TL, Chrysanthis D, et al. Clinicoepidemiological study of murine typhus on the Greek island of Evia. *Eur J Epidemiol*. 1992;8:268–272.
84. Anyfantakis D, Doukakis S, Papadakis M, et al. Liver function test abnormalities in murine typhus in Greece: a retrospective study of 165 cases. *Infez Med*. 2013;21:207–210.

85. Tsioutis C, Zafeiri M, Avramopoulos A, et al. Clinical and laboratory characteristics, epidemiology, and outcomes of murine typhus: a systematic review. *Acta Trop*. 2017;166:16–24.
86. Stuart BM, Pullen RL. Endemic (murine) typhus fever: clinical observations of 180 cases. *Ann Intern Med*. 1945;23:520–536.
87. Samara Y, Shaked Y, Maier MK. Delayed neurologic display in murine typhus: report of two cases. *Arch Intern Med*. 1989;149:949–951.
88. Gikas A, Kokkini S, Tsioutis C, et al. Murine typhus in children: clinical and laboratory features from 41 cases in Crete, Greece. *Clin Microbiol Infect*. 2009;15:211–212.
89. Vander T, Medvedovsky M, Valdman S, et al. Facial paralysis and meningitis caused by *Rickettsia typhi* infection. *Scand J Infect Dis*. 2003;35:886–887.
90. Silpapojakul K, Ukkachoke C, Krisanapan S, et al. Rickettsial meningitis and encephalitis. *Arch Intern Med*. 1991;151:1753–1757.
91. Tsiachris D, Deutsch M, Vassilopoulos D, et al. Sensorineural hearing loss complicating severe rickettsial diseases: report of two cases. *J Infect Dis*. 2008;56:74–76.
92. Vallejo-Maroto I, Garcia-Morillo S, Wittel MB, et al. Aseptic meningitis as a delayed neurologic complication of murine typhus. *Clin Microbiol Infect*. 2002;8:826–827.
93. Hudson HL, Thach AB, Lopez PF. Retinal manifestations of acute murine typhus. *Int Ophthalmol*. 1997;21:121–126.
94. Moy WL, Ooi ST. Abducens nerve palsy and meningitis by *Rickettsia typhi*. *Am J Trop Med Hyg*. 2015;92:620–624.
95. van der Vaart TW, van Thiel PP, Juffermans NP, et al. Severe murine typhus with pulmonary system involvement. *Emerg Infect Dis*. 2014;20:1375–1377.
96. Dittrich S, Rattanavong S, Lee SJ, et al. Orientia, rickettsia, and leptospira pathogens as causes of CNS infections in Laos: a prospective study. *Lancet Glob Health*. 2015;3:e104–e112.
97. Howard A, Fergie J. Murine typhus in South Texas children: an 18-year review. *Pediatr Infect Dis J*. 2018.
98. Segura F, Pons I, Pla J, et al. Shell-vial culture and real-time PCR applied to *Rickettsia typhi* and *Rickettsia felis* detection. *World J Microbiol Biotechnol*. 2015;31:1747–1754.
99. Zavala-Castro JE, Dzul-Rosado KR, Peniche-Lara G, et al. Isolation of *Rickettsia typhi* from human, Mexico. *Emerg Infect Dis*. 2014;20:1411–1412.
100. Hechemy KE, Stevens RW, Sasowski S, et al. Discrepancies in Weil-Felix and microimmunofluorescence test results for Rocky Mountain spotted fever. *J Clin Microbiol*. 1979;9:292–293.
101. Afzal Z, Kallumadanda S, Wang F, et al. Acute febrile illness and complications due to murine typhus, Texas, USA. *Emerg Infect Dis*. 2017;23:1268–1273.
102. Aouam A, Toumi A, Ben Brahim H, et al. Epidemiological, clinical and laboratory features of murine typhus in central Tunisia. *Med Mal Infect*. 2015;45:124–127.
103. Phetsouvanh R, Thojaikong T, Phoumin P, et al. Inter- and intra-operator variability in the reading of indirect immunofluorescence assays for the serological diagnosis of scrub typhus and murine typhus. *Am J Trop Med Hyg*. 2013;88:932–936.
104. Phetsouvanh R, Blacksell SD, Jenjaroen K, et al. Comparison of indirect immunofluorescence assays for diagnosis of scrub typhus and murine typhus using venous blood and finger prick filter paper blood spots. *Am J Trop Med Hyg*. 2009;80:837–840.
105. La Scola B, Rydkina L, Ndihokubwayo JB, et al. Serological differentiation of murine typhus and epidemic typhus using cross-absorption and Western blotting. *Clin Diagn Lab Immunol*. 2000;7:612–616.
106. Walker DH, Feng HM, Ladner S, et al. Immunohistochemical diagnosis of typhus rickettsiosis using an anti-lipopolysaccharide monoclonal antibody. *Mod Pathol*. 1997;10:1038–1042.
107. Blanton LS, Lea AS, Kelly BC, et al. An unusual cutaneous manifestation in a patient with murine typhus. *Am J Trop Med Hyg*. 2015;93:1164–1167.
108. Paris DH, Dumler JS. State of the art of diagnosis of rickettsial diseases: the use of blood specimens for diagnosis of scrub typhus, spotted fever group rickettsiosis, and murine typhus. *Curr Opin Infect Dis*. 2016;29:433–439.
109. Chao CC, Belinskaya T, Zhang Z, et al. Development of recombinase polymerase amplification assays for detection of *Orientia tsutsugamushi* or *Rickettsia typhi*. *PLoS Negl Trop Dis*. 2015;9:e0003884.
110. Dittrich S, Castonguay-Vanier J, Moore CE, et al. Loop-mediated isothermal amplification for *Rickettsia typhi* (the causal agent of murine typhus): problems with diagnosis at the limit of detection. *J Clin Microbiol*. 2014;52:832–838.
111. Miller ES, Beeson PB. Murine typhus fever. *Medicine (Baltimore)*. 1946;25:1–15.
112. Green JS, Singh J, Cheung M, et al. A cluster of pediatric endemic typhus cases in Orange County, California. *Pediatr Infect Dis J*. 2011;30:163–165.
113. Keysary A, Strenger C. Use of enzyme-linked immunosorbent assay techniques with cross-reacting human sera in diagnosis of murine typhus and spotted fever. *J Clin Microbiol*. 1997;35:1034–1035.
114. Sorvillo FJ, Gondo B, Emmons R, et al. A suburban focus of endemic typhus in Los Angeles County: association with seropositive domestic cats and opossums. *Am J Trop Med Hyg*. 1993;48:269–273.
115. Vallee J, Thaojaikong T, Moore CE, et al. Contrasting spatial distribution and risk factors for past infection with scrub typhus and murine typhus in Vientiane City, Lao PDR. *PLoS Negl Trop Dis*. 2010;4:e909.
116. Karen G, Itzhaki A, Oron C, et al. Evaluation of the anti-rickettsial activity of fluoroquinolones. *Drugs*. 1995;49:208–210.
117. Keysary A, Itzhaki A, Rubinstein E, et al. The in-vitro anti-rickettsial activity of macrolides. *J Antimicrob Chemother*. 1996;38:727–731.
118. Rolain JM, Maurin M, Vestris G, et al. In vitro susceptibilities of 27 rickettsiae to 13 antimicrobials. *Antimicrob Agents Chemother*. 1998;42:1537–1541.
119. Dumler JS. Clincial disease: current treatment and new challenges. In: Palmer GH, Azad AF, eds. *Intracellular Pathogens II: Rickettsiales*. Washington, DC: ASM Press; 2012:1–39.
120. Gikas A, Doukakis S, Pediaditis J, et al. Comparison of the effectiveness of five different antibiotic regimens on infection with *Rickettsia typhi:* therapeutic data from 87 cases. *Am J Trop Med Hyg*. 2004;70:576–579.
121. Gomez J, Molina Boix M, Banos V, et al. Murine typhus. Efficacy of treatment with ciprofloxacin. *Enferm Infecc Microbiol Clin*. 1992;10:377, [in Spanish].
122. Eaton M, Cohen MT, Shlim DR, et al. Ciprofloxacin treatment of typhus. *JAMA*. 1989;262:772–773.
123. Strand O, Stromberg A. Ciprofloxacin treatment of murine typhus. *Scand J Infect Dis*. 1990;22:503–504.
124. Van Der Kleij FG, Gansevoort RT, Kreeftenberg HG, et al. Imported rickettsioses: think of murine typhus. *J Intern Med*. 1998;243:177–179.
125. Raoult D, Drancourt M. Antimicrobial therapy of rickettsial diseases. *Antimicrob Agents Chemother*. 1991;35:2457–2462.
126. Laferl H, Fournier PE, Seiberl G, et al. Murine typhus poorly responsive to ciprofloxacin: a case report. *J Travel Med*. 2002;9:103–104.
127. Cross R, Ling C, Day NP, et al. Revisiting doxycycline in pregnancy and early childhood—time to rebuild its reputation? *Expert Opin Drug Saf*. 2016;15:367–382.
128. McGready R, Prakash JA, Benjamin SJ, et al. Pregnancy outcome in relation to treatment of murine typhus and scrub typhus infection: a fever cohort and a case series analysis. *PLoS Negl Trop Dis*. 2014;8:e3327.
129. Jolley JA, Pelayo R, Hatfield TJ, et al. Murine typhus in a pregnant woman. *Obstet Gynecol*. 2010;116:541–543.

191 *Orientia tsutsugamushi* (Scrub Typhus)

Daniel H. Paris

SHORT VIEW SUMMARY

Definition
- Scrub typhus is a serious rickettsial illness characterized by an eschar at the mite bite site, possible skin rash, and an initial flulike febrile illness with complications such as respiratory and renal failure, meningoencephalitis, and severe multiorgan failure.

Epidemiology
- *Orientia* spp. are predominantly spread across the Asia-Pacific region, but emerging evidence suggests a worldwide distribution in tropical and subtropical zones. Scrub typhus is the leading cause of treatable undifferentiated febrile illness in many parts of Southeast Asia.

Microbiology
- Scrub typhus is caused by *Orientia* spp.—most commonly *Orientia tsutsugamushi*—an obligate intracellular pathogen that is transmitted to humans by trombiculid mites.

Diagnosis
- Historically the gold standard diagnosis of scrub typhus was based on serology, but the added value of polymerase chain reaction (PCR) in the early bacterial dissemination phase of disease has led to a combination of "PCR plus serology" for most rickettsial diseases.

Therapy
- Scrub typhus is easily treated with appropriate antibiotics (doxycycline, azithromycin, and chloramphenicol), and upon clinical suspicion early consideration of empirical treatment is justified.

Prevention
- The many heterogeneous strains of *O. tsutsugamushi* and the lack of long-term protective natural immunity pose difficulties in vaccine development. Currently, no licensed vaccines or vector-control efforts are available for scrub typhus.

HISTORY

The earliest clinical reports of possible scrub typhus date back to the Chinese manual *Zhouhofang* in 313 BC. In 1810 the association of a febrile illness with mite transmission was made in the Niigata Prefecture in Japan, which led to the first clinical definition of tsutsugamushi fever. Historically, the occurrence of scrub typhus was associated with its dominant presence in Asia, in an area defined as the "Tsutsugamushi Triangle," but emerging reports of *Orientia* spp. and clinical cases reported from regions in Africa, Europe, and South America suggest a wider global distribution in tropical to subtropical regions (Fig. 191.1). The recent description of confirmed human cases in Chile has led to a paradigm shift in the epidemiology of scrub typhus.[1] Today, scrub typhus is probably the world's most relevant rickettsial disease based on its disease burden, and although the understanding of its global distribution is expanding, the available literature remains limited.[2,3]

DEFINITION

Scrub typhus is a vector-borne infectious disease caused by the obligate intracellular bacterium *Orientia tsutsugamushi*. The initial clinical presentation includes nonspecific "flulike" symptoms such as fever, fatigue, frontal headaches, myalgia, cough, restlessness, and insomnia. In many cases an inoculation lesion may appear, termed eschar or "tache noire" (Fig. 191.2A–B). However, in areas where scrub typhus is common and the population is regularly exposed, the presence of an eschar may occur less frequently; this is likely due to preexisting cellular immunity against *Orientia* spp.[4,5] Similarly, a diffuse, macular or maculopapular skin rash can develop within 3 to 8 days after the onset of fever. Confusion and gastrointestinal symptoms are common, and complications include respiratory and renal failure, meningitis or meningoencephalitis, and, very rarely, disseminated intravascular coagulation. Severe scrub typhus manifested with severe multiorgan failure in up to a third of hospitalized patients in India, with an average 24% mortality rate.[2,6] In Lao patients with central nervous system (CNS)–related complications, a mortality rate of 14% was observed,[2,7] and scrub typhus during pregnancy was associated with poor maternal and fetal outcomes (Laos and Thai-Myanmar border), with approximately a third of cases resulting in either abortion or stillbirth.[2,8,9] However, to what extent such outcomes depend on the strain of *Orientia,* immune competence, and genetics of the patient remains to be defined.

In most endemic areas of scrub typhus, the following differential diagnosis for "typhus-like illnesses" should be considered: malaria, murine typhus, dengue, leptospirosis, Q fever, typhoid, melioidosis, and chikungunya fever; malaria and meningitis should always be ruled out first.

ECOLOGY AND EPIDEMIOLOGY

Scrub typhus is a zoonotic disease, and *Orientia* spp. are transmitted to humans by trombiculid mites (phylum Arthropoda, family Trombiculidae, genus *Leptotrombidium*) via the bite of the larval stage, termed *chiggers* (see Fig. 191.2C–D). Mites represent both vectors and major reservoirs of *Orientia* spp. in nature; bacteria can be passed on transstadially through all stages in the life cycle and maintained transovarially over multiple generations. The life cycle of trombiculid mites takes place in the soil, ranging from mountainous heights (i.e., Himalayas), semiarid regions, jungle, and shrubby fringes to river banks and beaches at sea level. Humans play no role in the *Orientia* mite life cycle and are dead-end hosts; this is relevant because any human-based preventive interventions such as treatment or vaccines will not affect disease incidence rates. Scrub typhus can exhibit a pronounced seasonality, with high transmission peaks before and after the rainy season in regions of Southeast Asia, with a regular year-round transmission common in tropical and subtropical regions.[7]

Scrub typhus is a truly neglected tropical disease. Prospective causes-of-fever studies have shown it to be a leading cause of treatable nonmalarial febrile illness in many regions of Asia, affecting mainly rural populations but occurring increasingly also in urban settings, especially in fast-growing metropolitan areas in China and Korea.[2,7,10,11] Given the median seroprevalence of 22%, approximately every fifth person carries antibodies against *Orientia* spp. in Asia. All five countries with a passive national surveillance system in place have reported an apparent rise in

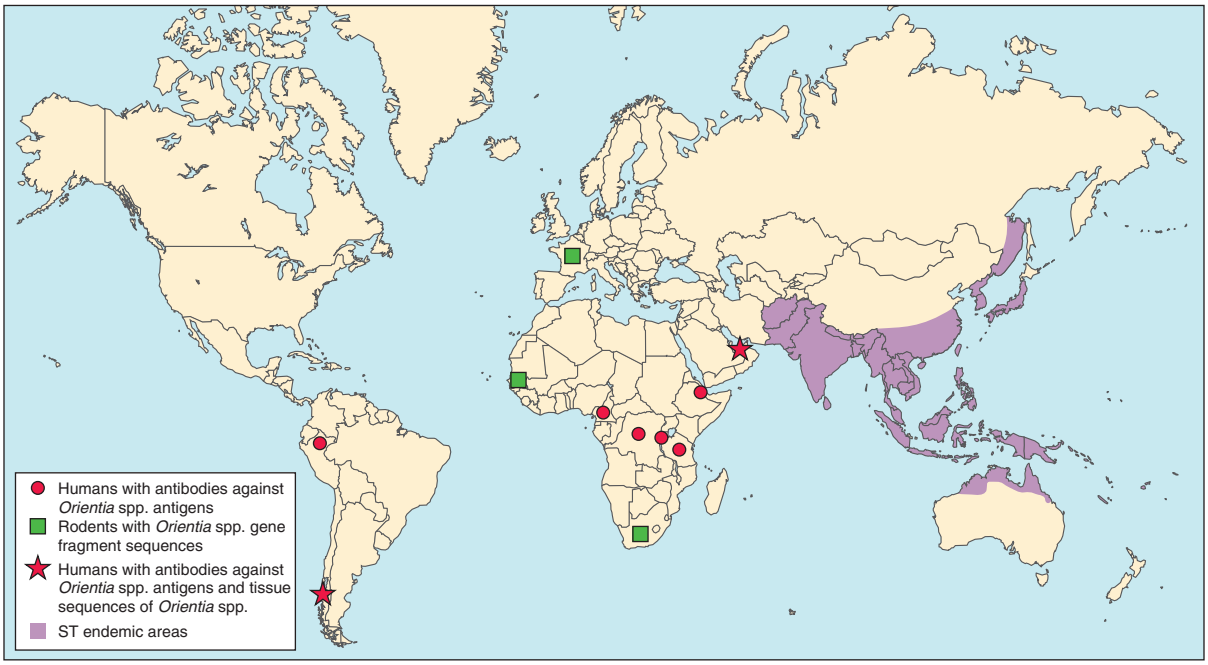

FIG. 191.1 World distribution of scrub typhus cases and *Orientia* spp. Originally scrub typhus was believed to be restricted to the "Tsutsugamushi Triangle," with most cases occurring in the Asia-Pacific region *(shaded in purple).* However, serologic evidence from Kenya, Congo, and Cameroon, recent reports of *Orientia* spp. in febrile patients from the Arabian Peninsula and Chile (polymerase chain reaction [PCR], culture and sequencing), and reports in rodents from southern France, South Africa, and Senegal (PCR) are suggestive of a more worldwide distribution along the tropical/subtropical belt. *(Modified from Jiang J, Richards AL. Scrub typhus: no longer restricted to the tsutsugamushi triangle.* Trop Med Infect Dis. *2018;3:E11.)*

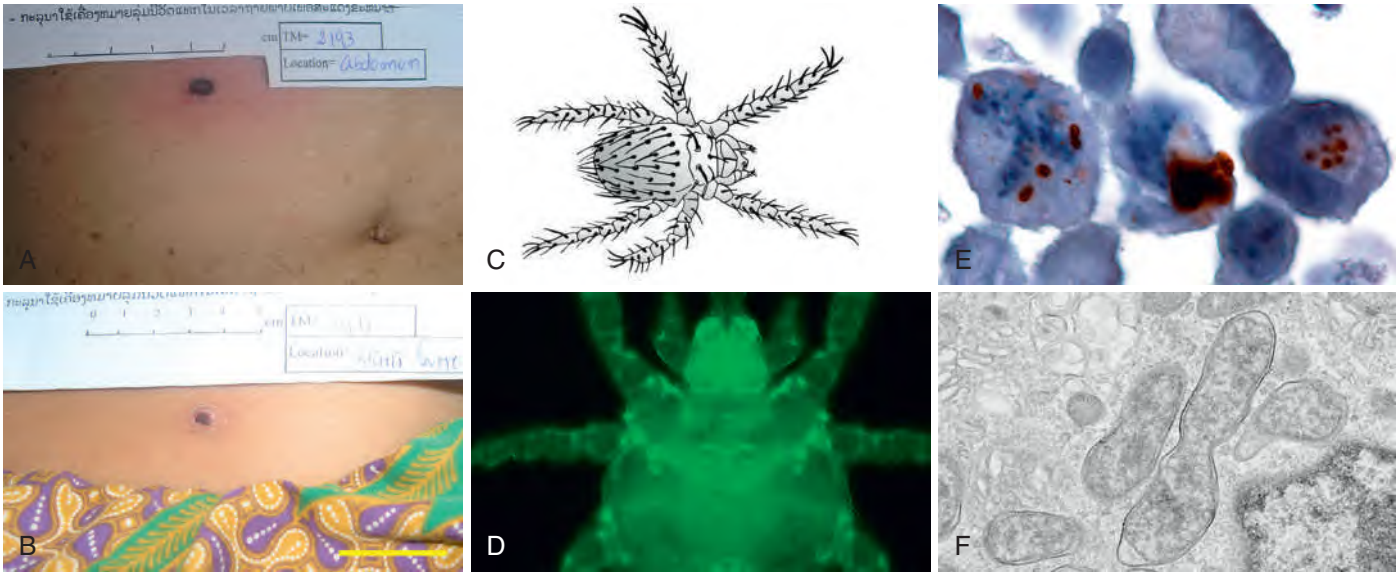

FIG. 191.2 Images of scrub typhus eschars, trombiculid mites, and *Orientia tsutsugamushi* bacteria. (A, B) Scrub typhus eschars typically occur along areas of restrictive clothing, (e.g., belts, collars, sarongs, or elastic bands of bras) or in axillae, in groin areas, or under the breasts. Detection requires careful examination and often special counseling by physicians owing to the cultural sensitivities of certain body areas involved. Chiggers—the larval stages of trombiculid mites—transmit *Orientia tsutsugamushi* to humans via their saliva during feeding (C). These mites are very small (larvae, approximately 0.2–0.3 mm; adults, approximately 2–3 mm) and have autofluorescent properties under ultraviolet light (D). (E) *O. tsutsugamushi* are obligate intracellular bacteria requiring in vitro cell culture; here the pathogens colored *brown* multiply in the perinuclear region of COS-1 cells. The bacterial double-membrane has a thicker outer leaflet in *Orientia* spp. than the inner one, as seen in (F), where *O. tsutsugamushi* is undergoing mitosis in L929 cells. *(Images A and B were generously provided by Dr. Rattanaphone Phetsouvane from Laos, and image D by Dr. Rawadee Kumlert from Thailand.)*

minimum disease incidence of scrub typhus over the past 8 to 10 years, with the highest being in China.[2] The mortality of untreated cases varies widely around a median of 6% to 8%, while current reports place mortality for treated scrub typhus at 1.4%.[2,10] However, much higher overall mortality is associated with complications such as CNS involvement (14% mortality)[12] and multiorgan dysfunction (24%)[6] in adults, while the high number of pregnancy miscarriage rates associate with poor maternal and neonatal outcomes.[8]

MICROBIOLOGY

Orientia spp. are obligate intracellular gram-negative coccobacillary bacteria that acquire their nutrition (adenosine triphosphate [ATP] and glycogen) primarily from the cytoplasm and thus locate to the microtubule organization center (MTOC) of the host cell. The *Orientia* bacterial membrane differs from that of its closest relatives, the rickettsia, by its outer membrane, which is considerably thicker than its inner one (see Fig. 191.2E–F). The typical constituents of gram-negative

cell walls—peptidoglycan and lipopolysaccharide—are not evident in *Orientia*,[13] and a major outer surface protein, the 56-kDa type-specific antigen (TSA), which contains four hypervariable regions, is mainly responsible for the organism's antigenic heterogeneity.[14]

To date two human-pathogenic *Orientia* spp. are known: the widely recognized and dominant *O. tsutsugamushi* and the recently described *Orientia chuto,* originating from the United Arabic Emirates.[15] In the past, serology-based strain characterization revealed the dramatic antigenic diversity of *O. tsutsugamushi.*[14,16] Genotyping efforts based on the immunodominant TSA 56-kDa gene demonstrate even greater diversity, but the combined data suggest that the clinically most relevant and common strains found in scrub typhus endemic areas are Karp, Gilliam, Kato, and TA716.[14] These data and new approaches involving whole-genome sequencing or antigenic cartography will provide insight into relevant antigens and cross-reactivity of antibodies, to inform diagnostic and vaccine development efforts in the future.[17]

The size of the *O. tsutsugamushi* genome is approximately 2 Mb, and among all sequenced bacterial genomes it contains the highest number of repetitive elements; an impressive 47% of the genome consists of repeats derived from integrative, conjugative, and transposable elements, generated by extensive gene degradation and amplification.[18–21] The underlying reasons for the pronounced proliferation of mobile elements and the selective pressures driving the process remain to be investigated. It is hoped that population genetics of genome sequences derived from mites, rodents, and humans will shed light on this intriguing conundrum.[19,20]

DIAGNOSIS

Historically, the gold standard diagnosis of scrub typhus was based on serology, but the added value of DNA-based pathogen detection in the early bacterial dissemination phase of disease has resulted in a combination of "polymerase chain reaction (PCR) plus serology" for most rickettsial diseases. The notoriously unreliable discontinued Weil-Felix OX-K agglutination test—discovered in 1916 and based on the cross-reactivity of anti-*Orientia* antibodies to *Proteus mirabilis,* specifically the OX-K (Kingsbury) strain—was replaced by the indirect immunofluorescence assay (IFA) in the 1960s.[22–24] The IFA was improved with new diagnostic positivity criteria defined in the 1970 and 1980s, and represented the gold standard for many years.[25,26] Only recently has the apparent lack of consensus for standardization of antigens and diagnostic positivity criteria led to the requirement of a dynamic titer rise (paired fourfold rising immunoglobulin M [IgM] or IgG titers) as a positivity criterion.[27] The development of enzyme-linked immunosorbent assays (ELISAs) using either culture-derived *O. tsutsugamushi* antigens or recombinant proteins to detect *Orientia*-specific antibodies has contributed to improved diagnostic accuracy and enabled higher throughput, over the often cumbersome IFAs and indirect immunoperoxidase test.[28,29] Point-of-care rapid diagnostic tests (RDTs) targeting anti-*Orientia* IgM and IgG are under validation, and it is hoped that future development will include antigen capture–based RDTs to enable early diagnosis at the patient bedside. Of note, the preexisting IgM and IgG in human populations of disease-endemic regions may interfere with the serological diagnosis during the acute phase. The presence of background immunity due to repeated exposure can lead to false positivity, especially in the absence of a convalescent serum sample, which would enable detection of a dynamic rise in titer (greater or equal to a fourfold rise).

The onset of the use of PCR has triggered a paradigm shift in rickettsial diagnostics; the long-term reliance on IFAs, which notably led to underdiagnosis and inappropriate therapies, is being replaced by the more objective ELISAs coupled with PCR assays to expand the diagnostic detection window toward early stages of infection. With their high diagnostic accuracies, PCR assays now represent a central pillar in scrub typhus diagnosis. Common target genes are the 56-kDa, 47-kDa, 16S rRNA, and *groEL* genes. This composite diagnostic approach has led to improved use of adequate antirickettsial therapy and reduction of morbidity and mortality, while providing more robust evidence in sample characterization for diagnostic and vaccine development.

The time from onset of symptoms to health care presentation is documented as "days of fever prior to admission"; this is of high value, because this number informs on the state of disease progression at admission and can guide the optimal use of diagnostics. Usually the bacteremia window can last up to 10 days from fever onset, and the antibody response in immune-naïve individuals is not generated until after approximately 7 to 10 days of fever.[30,31] Ideally, both a PCR assay and a serology test (i.e., RDT, ELISA) should be performed for optimal diagnosis (Fig. 191.3).

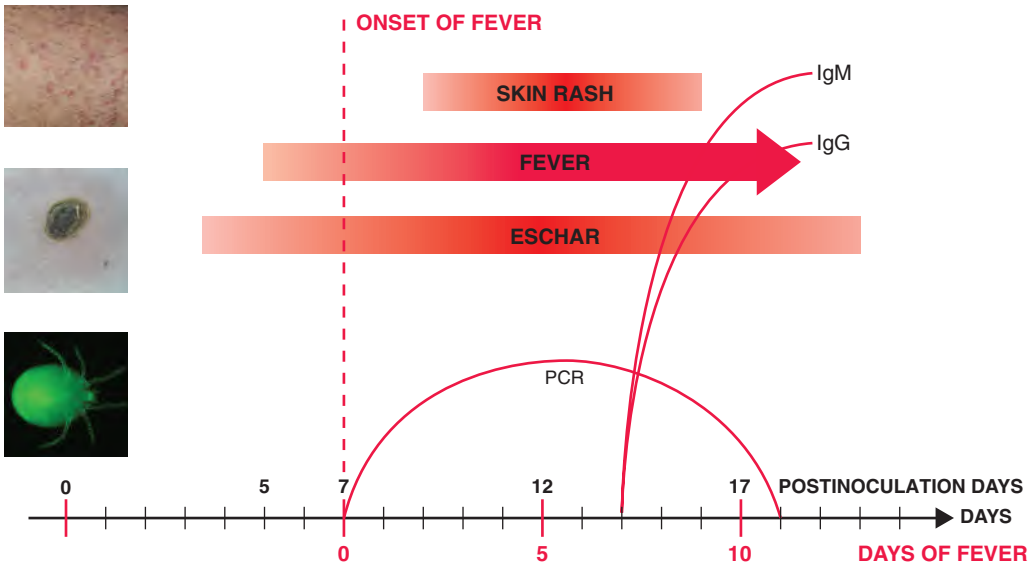

FIG. 191.3 Disease dynamics of scrub typhus clinical features, bacteremia, and anti–*Orientia tsutsugamushi* antibodies. Scrub typhus time course dynamics in relation to post–mite inoculation days *(black numbers)* and post–fever onset days *(red numbers)* are illustrated along the time axis (days). The day of the mite bite is shown as day 0 *(black).* The mite is shown in the lime green figure in the *bottom inset* as photographed under ultraviolet light. A skin rash can appear after 2 to 3 days of fever but is not always present *(upper inset).* After the mite bite, an eschar *(middle inset)* starts to form 2 to 3 days before fever onset and remains until approximately 14 days later. The bacteremia phase persists for about 10 days after onset of fever; during this time, polymerase chain reaction (PCR) assay and culture success is likely. Although anti-*Orientia* antibodies can appear as early as after 3 to 5 days of fever—and IgM can rise earlier, but often together with IgG in endemic areas—serology is likely to be the better modality to diagnose scrub typhus after approximately 7 days of fever. Patients with a history of 5 to 7 days of fever can be PCR, rapid diagnostic test, and serology positive. Because these numbers vary according to the inoculation dose (higher inoculation dose = faster dynamics), it is wise to always perform both PCR assay and serology for diagnosis of scrub typhus. *IgG,* Immunoglobulin G; *IgM,* immunoglobulin M.

If available, eschar swab or crust samples are excellent noninvasive diagnostic specimens; their high bacterial loads and separation from the circulation support early accurate molecular diagnosis until late in the disease course, even after initiation of treatment.[32] Culture of *Orientia* spp. from blood is difficult and can take several weeks owing to the fastidious nature of the bacteria, necessitating cell culture and Biosafety Level 3 facilities.

IMMUNE RESPONSE

Scrub typhus is a systemic vasculopathy with prominent infiltrates of lymphocytes, monocytes, and macrophages leading to prominent perivascular cuff formation (Fig. 191.4). *Orientia*-infected target cells include the endothelium and antigen-presenting cells, of which a subpopulation of infected monocytes has the propensity to enter the circulation and disseminate the pathogens in a "Trojan horse" phenomenon.[33] This bacteremia phase coincides with the onset of fever and lasts for approximately 10 days (see Fig. 191.3). Unfortunately, the natural immune response to scrub typhus is short-lived and is poorly cross-protective among different strains; the acquired immune protection can wane within months, resulting in symptomatic illness on reexposure to heterologous (different) strains, but could last for over a year for a homologous (same strain) reexposure.[34] The natural immune response against *Orientia* is a combination of synergistic humoral and cell-mediated immune responses; whereas the humoral component is characterized by a predominantly homologous protection with poor cross-protection to other strains, the cell-mediated immune response supports predominantly homologous protection, with initial short-lived heterotypic protection, that wanes over time. Investigations of the balance between these two arms in the context of bacterial dissemination dynamics will be crucial for the future success of vaccine design.

With appropriate treatment, patients with scrub typhus usually clear their fever within 48 hours; however, in northern Thailand and southern India, delayed fever clearance times and high case-fatality rates of 12% to 13% have been described.[35,36] Currently, ongoing investigations regarding the possible association of poor clinical responses with potential antibiotic resistance of *Orientia* spp. in northern Thailand and southern India are underway.

THERAPY

Scrub typhus is easily treated with appropriate antibiotics; these are doxycycline (preferred), azithromycin, chloramphenicol, or a combination of two. Early empirical treatment is justified upon clinical suspicion, and a response with reduction of fever within 48 hours of administration of doxycycline is often observed in scrub typhus.

The standard regimen is doxycycline 100 mg given orally twice daily for 7 days. Tetracycline is not commonly used, but 500 mg q6h for 7 days is an alternative regimen. Azithromycin has comparable efficacy to doxycycline and is usually administered as a 3-day regimen with a loading dose of 1000 mg or 500 mg on the first day, followed by 500 or 250 mg daily for another 2 days, respectively. Azithromycin remains the preferred drug during pregnancy or for children, owing to the fear of tetracycline side effects. Chloramphenicol is an excellent drug and a good alternative to doxycycline; it is administered as 500 mg q6h in adults or 50 to 75 mg/kg/day in children for 7 days. Unfortunately, the hematologic side effects (1:21,600) and the very rare occurrence of gray baby syndrome in premature infants (circulatory collapse) has led to significant reduction in its use.

Drug combinations of doxycycline plus either azithromycin or chloramphenicol are beneficial in cases with a delayed treatment response; reduction of fever clearance time also reduces adverse outcomes, especially in pregnancy, and no negative interactions have been reported to date. Decisions regarding combinations including cotreatment with rifampicin should consider the potential reduction of drug levels, and adequate dosage adjustments should be considered.

When clinical suspicion of typhoid or typhus arises, either a high-dose regimen of azithromycin (typhoid dosage) or chloramphenicol can be considered, because this would cover both infections. For uncomplicated scrub typhus, shorter regimens have been evaluated; 3 days of doxycycline performed equally as well as 7-day regimens, with comparable fever clearance times and no documented treatment failures.[37] Azithromycin is an effective alternative for potentially doxycycline-resistant scrub typhus with prolonged fever clearance times, as reported from northern Thailand.[38]

PREVENTION

Scrub typhus is among the top 10 causes of acute and potentially life-threatening diseases among ill returning western travelers from the tropics.[39] With improving awareness of medical staff and the availability of better diagnostics, increasing numbers of returning travelers with scrub typhus are being reported, including severe and fatal cases.[40,41]

Most reported cases are acquired after travel to Southeast Asia, as the disease-transmitting trombiculid chigger mites are most prevalent in scrub vegetation and tall grass of rural areas where tourists, local residents, and military personnel are exposed. Prevention is limited to the rigorous use of insect repellents, such as *N,N*-diethyl-meta-toluamide (DEET), which provides effective but short-lasting protection against ticks and mites during travel in rural areas of endemic countries. No licensed vaccines are available.

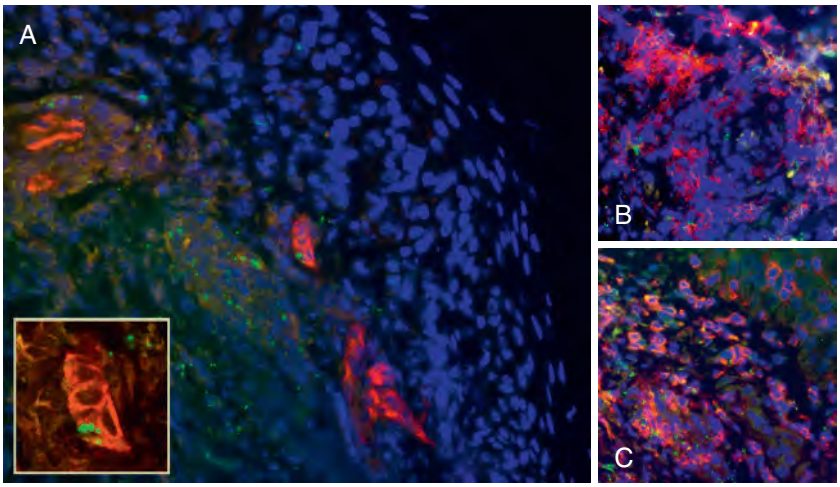

FIG. 191.4 *Orientia tsutsugamushi (green)* in skin and perivascular infiltrates. *O. tsutsugamushi (green)* in perivascular infiltrates at the superficial dermal-epidermal junction of the skin. The red-stained cells are endothelial cells of the vasculature; the inset represents a laser scanning micrograph (LSM) localizing the bacteria to the vasculature (A, with *inset*). (B and C) Two cross-sectional images of the typical cuff-forming infiltrates around smaller blood vessels consisting of large numbers of red-stained mononuclear cells (CD14-positive monocytes/macrophages); B and T cells (CD3 positivity) are shown. (TM2193, magnification ×400, LSM inset ×1000. Double-immunolabeling: CD31 in red [A], CD14 in red [B] and CD3 in red [C]; *O. tsutsugamushi* in green; DAPI nuclear counterstain in blue.)

Key References

The complete reference list is available online at Expert Consult.

1. Weitzel T, Dittrich S, Lopez J, et al. Endemic scrub typhus in South America. *N Engl J Med*. 2016;375:954–961.
2. Bonell A, Lubell Y, Newton PN, et al. Estimating the burden of scrub typhus: a systematic review. *PLoS Negl Trop Dis*. 2017;11:e0005838.
3. Paris DH, Shelite TR, Day NP, et al. Unresolved problems related to scrub typhus: a seriously neglected life-threatening disease. *Am J Trop Med Hyg*. 2013;89:301–307.
4. Paris DH, Chattopadhyay S, Jiang J, et al. A nonhuman primate scrub typhus model: protective immune responses induced by pKarp47 DNA vaccination in cynomolgus macaques. *J Immunol*. 2015;194:1702–1716.
7. Mayxay M, Castonguay-Vanier J, Chansamouth V, et al. Causes of non-malarial fever in Laos: a prospective study. *Lancet Glob Health*. 2013;1:e46–e54.
8. McGready R, Prakash JA, Benjamin SJ, et al. Pregnancy outcome in relation to treatment of murine typhus and scrub typhus infection: a fever cohort and a case series analysis. *PLoS Negl Trop Dis*. 2014;8:e3327.
10. Taylor AJ, Paris DH, Newton PN. A systematic review of mortality from untreated scrub typhus (*Orientia tsutsugamushi*). *PLoS Negl Trop Dis*. 2015;9:e0003971.
12. Dittrich S, Rattanavong S, Lee SJ, et al. Orientia, rickettsia, and leptospira pathogens as causes of CNS infections in Laos: a prospective study. *Lancet Glob Health*. 2015;3:e104–e112.
14. Kelly DJ, Fuerst PA, Ching W-M, et al. Scrub typhus: the geographic distribution of phenotypic and genotypic variants of *Orientia tsutsugamushi*. *Clin Infect Dis*. 2009;48(suppl 3):S203–S230.
16. Oaks EV, Rice RM, Kelly DJ, et al. Antigenic and genetic relatedness of eight *Rickettsia tsutsugamushi* antigens. *Infect Immun*. 1989;57:3116–3122.
18. Cho NH, Kim HR, Lee JH, et al. The *Orientia tsutsugamushi* genome reveals massive proliferation of conjugative type IV secretion system and host-cell interaction genes. *Proc Natl Acad Sci USA*. 2007;104:7981–7986.
24. Fletcher W. Typhus-like fevers of unknown aetiology, with special reference to the Malay states. *Proc R Soc Med*. 1930;23:1021–1030.
27. Blacksell SD, Bryant NJ, Paris DH, et al. Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: a lack of consensus leads to a lot of confusion. *Clin Infect Dis*. 2007;44:391–401.
31. Paris DH, Blacksell SD, Nawtaisong P, et al. Diagnostic accuracy of a loop-mediated isothermal PCR assay for detection of *Orientia tsutsugamushi* during acute Scrub Typhus infection. *PLoS Negl Trop Dis*. 2011;5:e1307.
32. Paris DH, Dumler JS. State of the art of diagnosis of rickettsial diseases: the use of blood specimens for diagnosis of scrub typhus, spotted fever group rickettsiosis, and murine typhus. *Curr Opin Infect Dis*. 2016;29:433–439.
34. Valbuena G, Walker DH. Approaches to vaccines against Orientia tsutsugamushi. *Front Cell Infect Microbiol*. 2012;2:170.
37. Panpanich R, Garner P. Antibiotics for treating scrub typhus. *Cochrane Database Syst Rev*. 2002;(3):CD002150.
41. Jensenius M, Fournier PE, Raoult D. Rickettsioses and the international traveler. *Clin Infect Dis*. 2004;39:1493–1499.

References

1. Weitzel T, Dittrich S, Lopez J, et al. Endemic scrub typhus in South America. *N Engl J Med*. 2016;375:954–961.
2. Bonell A, Lubell Y, Newton PN, et al. Estimating the burden of scrub typhus: a systematic review. *PLoS Negl Trop Dis*. 2017;11:e0005838.
3. Paris DH, Shelite TR, Day NP, et al. Unresolved problems related to scrub typhus: a seriously neglected life-threatening disease. *Am J Trop Med Hyg*. 2013;89:301–307.
4. Paris DH, Chattopadhyay S, Jiang J, et al. A nonhuman primate scrub typhus model: protective immune responses induced by pKarp47 DNA vaccination in cynomolgus macaques. *J Immunol*. 2015;194:1702–1716.
5. Paris DH, Richards AL, Day NPJ. *Orientia spp. Molecular Medical Microbiology*. 2nd ed. Elsevier Ltd; 2014. ISBN: 978-0-12-677530-3.
6. Peter JV, Sudarsan TI, Prakash JA, et al. Severe scrub typhus infection: clinical features, diagnostic challenges and management. *World J Crit Care Med*. 2015;4:244–250.
7. Mayxay M, Castonguay-Vanier J, Chansamouth V, et al. Causes of non-malarial fever in Laos: a prospective study. *Lancet Glob Health*. 2013;1:e46–e54.
8. McGready R, Prakash JA, Benjamin SJ, et al. Pregnancy outcome in relation to treatment of murine typhus and scrub typhus infection: a fever cohort and a case series analysis. *PLoS Negl Trop Dis*. 2014;8:e3327.
9. Chansamouth V, Thammasack S, Phetsouvanh R, et al. The aetiologies and impact of fever in pregnant inpatients in Vientiane, Laos. *PLoS Negl Trop Dis*. 2016;10:e0004577.
10. Taylor AJ, Paris DH, Newton PN. A systematic review of mortality from untreated scrub typhus (*Orientia tsutsugamushi*). *PLoS Negl Trop Dis*. 2015;9:e0003971.
11. McGready R, Ashley EA, Wuthiekanun V, et al. Arthropod borne disease: the leading cause of fever in pregnancy on the Thai-Burmese border. *PLoS Negl Trop Dis*. 2010;4:e888.
12. Dittrich S, Rattanavong S, Lee SJ, et al. Orientia, rickettsia, and leptospira pathogens as causes of CNS infections in Laos: a prospective study. *Lancet Glob Health*. 2015;3:e104–e112.
13. Amano K, Tamura A, Ohashi N, et al. Deficiency of peptidoglycan and lipopolysaccharide components in *Rickettsia tsutsugamushi*. *Infect Immun*. 1987;55:2290–2292.
14. Kelly DJ, Fuerst PA, Ching W-M, et al. Scrub typhus: the geographic distribution of phenotypic and genotypic variants of *Orientia tsutsugamushi*. *Clin Infect Dis*. 2009;48(suppl 3):S203–S230.
15. Izzard L, Fuller A, Blacksell SD, et al. Isolation of a novel *Orientia* species (*O. chuto* sp. nov.) from a patient infected in Dubai. *J Clin Microbiol*. 2010;48:4404–4409.
16. Oaks EV, Rice RM, Kelly DJ, et al. Antigenic and genetic relatedness of eight *Rickettsia tsutsugamushi* antigens. *Infect Immun*. 1989;57:3116–3122.
17. James SL, Blacksell SD, Nawtaisong P, et al. Antigenic relationships among human pathogenic *Orientia tsutsugamushi* isolates from Thailand. *PLoS Negl Trop Dis*. 2016;10:e0004723.
18. Cho NH, Kim HR, Lee JH, et al. The *Orientia tsutsugamushi* genome reveals massive proliferation of conjugative type IV secretion system and host-cell interaction genes. *Proc Natl Acad Sci USA*. 2007;104:7981–7986.
19. Darby AC, Cho NH, Fuxelius HH, et al. Intracellular pathogens go extreme: genome evolution in the Rickettsiales. *Trends Genet*. 2007;23:511–520.
20. Nakayama K, Kurokawa K, Fukuhara M, et al. Genome comparison and phylogenetic analysis of *Orientia tsutsugamushi* strains. *DNA Res*. 2010;17:281–291.
21. Nakayama K, Yamashita A, Kurokawa K, et al. The Whole-genome sequencing of the obligate intracellular bacterium Orientia tsutsugamushi revealed massive gene amplification during reductive genome evolution. *DNA Res*. 2008;15:185–199.
22. Bozeman FM, Elisberg BL. Serological diagnosis of scrub typhus by indirect immunofluorescence. *Proc Soc Exp Biol Med*. 1963;112:568–573.
23. Weil E, Felix A. Zur serologischen diagnose des fleckfiebers. *Wien Klin Wochenschr*. 1916;29:3–36.
24. Fletcher W. Typhus-like fevers of unknown aetiology, with special reference to the Malay states. *Proc R Soc Med*. 1930;23:1021–1030.
25. Brown GW, Shirai A, Rogers C, et al. Diagnostic criteria for scrub typhus: probability values for immunofluorescent antibody and Proteus OXK agglutinin titers. *Am J Trop Med Hyg*. 1983;32:1101–1107.
26. Robinson DM, Brown G, Gan E, et al. Adaptation of a microimmunofluorescence test to the study of human *Rickettsia tsutsugamushi* antibody. *Am J Trop Med Hyg*. 1976;25:900–905.
27. Blacksell SD, Bryant NJ, Paris DH, et al. Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: a lack of consensus leads to a lot of confusion. *Clin Infect Dis*. 2007;44:391–401.
28. Blacksell SD, Lim C, Tanganuchitcharnchai A, et al. Optimal cutoff and accuracy of an IgM enzyme-linked immunosorbent assay for diagnosis of acute scrub typhus in Northern Thailand: an alternative reference method to the IgM immunofluorescence assay. *J Clin Microbiol*. 2016;54:1472–1478.
29. Blacksell SD, Tanganuchitcharnchai A, Nawtaisong P, et al. Diagnostic accuracy of the InBios scrub typhus detect enzyme-linked immunoassay for the detection of IgM antibodies in Northern Thailand. *Clin Vaccine Immunol*. 2015;23:148–154.
30. Blacksell SD, Paris DH, Chierakul W, et al. Prospective evaluation of commercial antibody-based rapid tests in combination with a loop-mediated isothermal amplification PCR assay for detection of *Orientia tsutsugamushi* during the acute phase of scrub typhus infection. *Clin Vaccine Immunol*. 2012;19:391–395.
31. Paris DH, Blacksell SD, Nawtaisong P, et al. Diagnostic accuracy of a loop-mediated isothermal PCR assay for detection of *Orientia tsutsugamushi* during acute Scrub Typhus infection. *PLoS Negl Trop Dis*. 2011;5:e1307.
32. Paris DH, Dumler JS. State of the art of diagnosis of rickettsial diseases: the use of blood specimens for diagnosis of scrub typhus, spotted fever group rickettsiosis, and murine typhus. *Curr Opin Infect Dis*. 2016;29:433–439.
33. Paris DH, Phetsouvanh R, Tanganuchitcharnchai A, et al. *Orientia tsutsugamushi* in human scrub typhus eschars shows tropism for dendritic cells and monocytes rather than endothelium. *PLoS Negl Trop Dis*. 2012;6:e1466.
34. Valbuena G, Walker DH. Approaches to vaccines against Orientia tsutsugamushi. *Front Cell Infect Microbiol*. 2012;2:170.
35. Mathai E, Rolain JM, Verghese GM, et al. Outbreak of scrub typhus in southern India during the cooler months. *Ann N Y Acad Sci*. 2003;990:359–364.
36. Watt G, Chouriyagune C, Ruangweerayud R, et al. Scrub typhus infections poorly responsive to antibiotics in Northern Thailand. *Lancet*. 1996;348:86–89.
37. Panpanich R, Garner P. Antibiotics for treating scrub typhus. *Cochrane Database Syst Rev*. 2002;(3):CD002150.
38. Watt G, Kantipong P, Jongsakul K, et al. Azithromycin activities against *Orientia tsutsugamushi* strains isolated in cases of scrub typhus in Northern Thailand. *Antimicrob Agents Chemother*. 1999;43:2817–2818.
39. Jensenius M, Han PV, Schlagenhauf P, et al. Acute and potentially life-threatening tropical diseases in western travelers–a GeoSentinel multicenter study, 1996-2011. *Am J Trop Med Hyg*. 2013;88:397–404.
40. Nachega JB, Bottieau E, Zech F, et al. Travel-acquired scrub typhus: emphasis on the differential diagnosis, treatment, and prevention strategies. *J Travel Med*. 2007;14:352–355.
41. Jensenius M, Fournier PE, Raoult D. Rickettsioses and the international traveler. *Clin Infect Dis*. 2004;39:1493–1499.

192 *Ehrlichia chaffeensis* (Human Monocytotropic Ehrlichiosis), *Anaplasma phagocytophilum* (Human Granulocytotropic Anaplasmosis), and Other Anaplasmataceae

J. Stephen Dumler and David H. Walker

SHORT VIEW SUMMARY

Definition
- Ehrlichiosis and anaplasmosis are infections caused by intracellular bacteria in the Anaplasmataceae family.

Microbiology
- Organisms of the family Anaplasmataceae of the order Rickettsiales are obligately intracellular gram-negative bacteria.
- All members of the Anaplasmataceae survive within vacuoles of host cells generally derived from the bone marrow, but also occasionally endothelial cells.
- *Ehrlichia chaffeensis, Ehrlichia canis, Ehrlichia muris,* and *Neorickettsia helminthoeca* infect mostly monocytes and macrophages in blood and tissues of mammalian hosts.
- *Anaplasma phagocytophilum* and *Ehrlichia ewingii* infect mostly neutrophils and other granulocytes in the blood of mammalian hosts.
- The mammalian target cell of *Candidatus* Neoehrlichia mikurensis is not known.

Epidemiology
- Human monocytotropic ehrlichiosis (HME) and human *E. ewingii* infection are distributed predominantly in south-central and eastern North America, where *Amblyomma americanum* ticks and white-tailed deer *(Odocoileus virginianus),* respectively, serve as vectors and reservoirs.
- Human granulocytic anaplasmosis (HGA) is distributed worldwide, especially in northern latitudes of North America, Europe, and Asia, where *Ixodes persulcatus* clade ticks or *Haemaphysalis longicornis* ticks (China) and multiple small mammals serve as vectors and reservoirs.
- Human *E. muris* subsp. *eauclairensis* infection is distributed in the upper Midwest of the United States (primarily Wisconsin and Minnesota), where *Ixodes scapularis* ticks and white-footed mice and other small mammals serve as vectors and reservoirs.
- Human *Anaplasma capra* infections are documented only in the northeast parts of China, and transmission is likely by the bites of *I. persulcatus* ticks.
- Human *Candidatus* N. mikurensis infection occurs in Europe and northern China, where *I. persulcatus* clade ticks and small mammals serve as vectors and reservoirs. The presentation of disease may differ (thrombotic and embolic events) in patients with B-cell malignancies and autoimmune disease, or as subclinical to moderately severe febrile disease in immunocompetent patients and subjects.
- Sennetsu neorickettsiosis occurs in eastern and southeastern Asia, where digenean trematodes serve as vectors and aquatic animals, mostly fish, serve as reservoirs through which infection is acquired by means of oral transmission.

Clinical Manifestations
- Frequent early manifestations include fever, headache, myalgia, nausea, and vomiting.
- Rash is infrequent or rare.
- The clinical course is usually uncomplicated, but severe complications can include a septic shock–like syndrome, respiratory distress, meningoencephalitis, renal failure, and death, particularly with HME.
- Leukopenia, thrombocytopenia, and elevations in serum hepatic aminotransferase and C-reactive protein levels are frequent findings.

Diagnosis
- Early diagnosis of HME, HGA, and other human infections caused by bacteria in the Anaplasmataceae family is based on clinical suspicion and epidemiologic clues.
- Rapid laboratory confirmation can sometimes be achieved through blood smear examination for morulae (inclusions) in circulating leukocytes or detection of bacterial DNA in blood by means of polymerase chain reaction assay.
- Seroconversion or fourfold increase in antibody titer from the acute to the convalescent phase confirms the diagnosis retrospectively when the specific tests are available.
- Early therapy improves outcomes and prevents severe complications or sequelae.

Therapy
- Doxycycline, 100 mg twice daily for 5 to 10 days until fever is resolved, is the treatment of choice.
- Chloramphenicol should not be used.
- Rifampin has been successfully used in children.

Prevention
- Prevention is directed toward avoidance of tick exposures and bites and early removal of attached ticks. Neorickettsiosis could be prevented by avoidance of uncooked or fermented fish food products in regions where the disease occurs.

Until 1987, infections by members of the family Anaplasmataceae, including the genera *Ehrlichia, Anaplasma,* and *Neorickettsia,* were known mainly as veterinary diseases (Table 192.1). Canine ehrlichiosis was first described in 1935 by Donatien and Lestoquard in Algeria. This disease is produced by *Ehrlichia canis,* which is transmitted to dogs by *Rhipicephalus sanguineus* ticks. The disease is characterized by fever associated with the presence of clusters of small Giemsa-stained organisms in circulating monocytes. *Ehrlichia* spp. generally have a tick vector and tropism for macrophages, granulocytes, or sometimes endothelial cells, where they grow within cytoplasmic membrane–bound vacuoles. Consequently, *Ehrlichia* was recognized as distinct from other genera of obligately intracellular bacteria of medical importance

TABLE 192.1 Anaplasmataceae Causing Medical and Veterinary Diseases

CAUSATIVE AGENT	MAMMALIAN HOST	MAJOR TARGET CELL	VECTOR, TRANSMISSION
Ehrlichia chaffeensis	Humans, deer, dogs, coyotes, marsh deer	Monocytes and macrophages	Ticks *(Amblyomma americanum, Dermacentor variabilis, Ixodes pacificus)*
Ehrlichia ewingii	Dogs, humans, deer	Granulocytes	Ticks *(A. americanum, D. variabilis)*
Ehrlichia muris subsp. *muris* and subsp. *eauclairensis*	Humans, *Apodemus* mice, voles, white-footed mice	Macrophages, endothelial cells?	Ticks *(Ixodes persulcatus, Ixodes scapularis, Haemaphysalis flava)*
Ehrlichia canis	Dogs, humans	Macrophages	Ticks *(Rhipicephalus sanguineus)*
Ehrlichia ruminantium	Cattle, sheep, wild ruminants	Endothelial cells	Ticks *(Amblyomma variegatum)*
Anaplasma phagocytophilum	Humans, white-footed mice, wood rats, bank voles, wood mice, yellow-necked mice, horses, dogs, cats, sheep, cattle, white-tailed deer, roe deer, red deer, fallow deer	Granulocytes	Ticks *(I. scapularis, I. pacificus, Ixodes ricinus, I. persulcatus), Haemaphysalis longicornis*
Anaplasma platys	Dogs	Platelets, macrophages	*Rhipicephalus* ticks
Anaplasma marginale	Cattle, wild ruminants	Erythrocytes	Ticks (e.g. *Rhipicephalus*)
Anaplasma centrale	Cattle, wild ruminants	Erythrocytes	Ticks (e.g. *Rhipicephalus*)
Anaplasma capra	Humans, goats	Unknown	Ticks (*I. persulcatus*)
Candidatus Neoehrlichia mikurensis	Wild rodents, dogs, humans	Endothelial cells, neutrophils?	Ticks *(Ixodes ovatus, I. ricinus, I. persulcatus, Haemaphysalis concinna)*
Neorickettsia sennetsu	Humans	Macrophages	Possibly ingestion of raw fish infested by digenean trematodes
Neorickettsia risticii	Horses	Macrophages, enterocytes, mast cells	Ingestion of aquatic insects (e.g., mayflies) infested by digenean trematodes
Neorickettsia helminthoeca	Dogs	Macrophages	Ingestion of trematode-infested salmon

(*Rickettsia, Coxiella,* and *Chlamydia*). In 1937 the genus name *Ehrlichia* was suggested in honor of the German bacteriologist Paul Ehrlich.[1] Subsequent phylogenetic studies have shown that two other economically important veterinary pathogens, *Anaplasma marginale* (described in 1910) and *Ehrlichia* (formerly *Cowdria*) *ruminantium* (described in 1925), are also ehrlichiae.[2] The first human disease demonstrated to have an ehrlichial cause was sennetsu neorickettsiosis, an infectious mononucleosis–like illness recognized to have occurred only in western Japan, Malaysia, and Laos. Although human infections caused by all members of the reorganized family Anaplasmataceae have been generically referred to as "ehrlichiosis," and the causative agents are referred to as "ehrlichiae," it is increasingly apparent that the clinical manifestations and causative agents are distinct. Because of the confusion arising from omitting species-specific diagnosis, more clarity in reporting the results of diagnosis is needed in order to eliminate a substantial burden of "undetermined" cases.[3]

The first diagnosed case of human ehrlichiosis in the United States occurred in a 51-year-old man who became ill in April 1986, 12 to 14 days after tick bites in rural Arkansas.[4] Although the disease was initially thought to be caused by the canine pathogen *E. canis*, the main causative agent of human monocytotropic ehrlichiosis (HME) is *Ehrlichia chaffeensis,* which was finally described in 1990.[5] In 1994, *Anaplasma phagocytophilum* was identified as the causative agent of a distinctly different infection, now called human granulocytotropic anaplasmosis (HGA).[6,7] Subsequently, human infections have been documented as caused by *E. canis,*[8] *Ehrlichia ewingii,*[9] and *Ehrlichia muris* subspecies *eauclairensis*[10,11]; by a bacterium related to *E. ruminantium* that is called Panola Mountain *Ehrlichia*[12]; by a bacterium recently identified and assigned to a new genus, *Candidatus* Neoehrlichia mikurensis[13]; by the newly recognized *Anaplasma capra*[14]; and, in a single case of infection, by *Anaplasma ovis* in a patient from Cyprus.[15]

ETIOLOGY

Members of the family Anaplasmataceae are defined predominantly by their genetic similarities and differences but also by phenotypic characteristics and host affinities (see Table 192.1). These are small (0.5 µm) gram-negative bacteria. Their clustered inclusion-like appearance of a microcolony in the host cell vacuole is called a morula, from the Latin word for mulberry (Fig. 192.1).

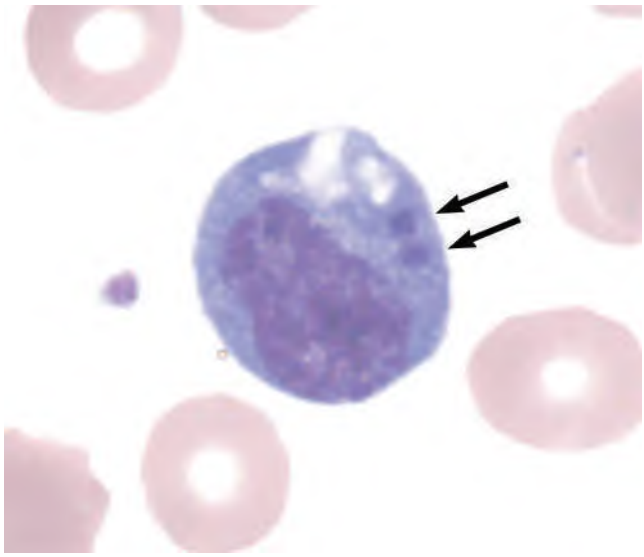

FIG. 192.1 Human monocytotropic ehrlichiosis. Peripheral blood smear (buffy coat preparation) showing intracellular inclusions *(arrows)* in mononuclear cells of a patient with human monocytotropic ehrlichiosis (Wright stain, ×400).

The taxonomic relationships of *Ehrlichia, Anaplasma, Neorickettsia, Neoehrlichia, Wolbachia, Orientia, Rickettsia, Coxiella,* and *Chlamydia* have been clarified through molecular and metabolic studies. The evolutionary relationships determined by comparison of whole genomes and the genes *rrs* (16S ribosomal RNA gene) and *groESL* indicate that *Ehrlichia, Anaplasma, Neorickettsia, Neoehrlichia, Wolbachia, Orientia,* and *Rickettsia* evolved from a common ancestor[2]; in contrast, *Coxiella* and *Chlamydia* are phylogenetically unrelated to ehrlichiae. Ehrlichiae and chlamydiae superficially resemble one another in that both reside within cytoplasmic vacuoles. Unlike chlamydiae, however, ehrlichiae are able to synthesize adenosine triphosphate through metabolism of glutamine, a metabolic characteristic shared with members of the genus *Rickettsia,* and lack the pathogen-associated molecular patterns

lipopolysaccharide and peptidoglycan. Because of the increasing use of molecular methods for genetic identification, the diversity and number of potential clades within Anaplasmataceae exceeds 323 entries within the NCBI Taxonomy database (see https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi), spanning established genera described here, but also including the genera *Candidatus* Xenohaliotis, *Candidatus* Xenolissoclinum, and *Candidatus* Cryptoplasma. Whether any of these molecular "isolates" are bacteria with the capacity to cause human disease is unknown. The family Anaplasmataceae contains at least five genera that are actually very different from one another. *E. chaffeensis* shares many antigens and genetic sequences with the predominantly canine pathogens *E. canis* and *E. ewingii*; *E. muris* and closely related species found in Japanese wild mice, voles, and ticks; and more recently in humans, a bacterium with a close genetic relationship to the ruminant pathogen *E. ruminantium*.[2,5,16–18] A second genus includes a granulocytotropic bacterium, *A. phagocytophilum*; an organism of uncertain tropism, *A. capra*; and a suspected erythrocytic bacterium, *A. ovis,* which can infect humans. All of these are related genetically to the veterinary pathogens *Anaplasma platys, Anaplasma bovis,* and *A. marginale*.[2,7,14,15] The third contains *Neorickettsia sennetsu*, which is closely related to *Neorickettsia risticii, Neorickettsia helminthoeca,* and an unnamed organism found in Japanese fish flukes.[2] The fourth genus, *Neoehrlichia,* contains two species: *Candidatus* N. mikurensis and *Candidatus* Neoehrlichia lotoris.[19,20] The former was first identified in *Ixodes ricinus* ticks in Europe[21] and then in *Ixodes ovatus* ticks and wild rats from Japan,[19] and it is the only species identified as a human pathogen in the genus. The final genus, *Wolbachia*, contains bacterial endosymbionts of invertebrates, including insects and helminths, some of which may contribute to disease in human filariasis.[22]

Ehrlichia and *Anaplasma* have two ultrastructural forms, a larger reticulate cell and a smaller, dense core cell, and the cell wall differs from that of *Rickettsia* spp., with thinner outer and inner leaflets reflecting the absence of lipopolysaccharide and lipooligosaccharide.[23] Genes coding for the enzymes required for the biosynthesis of peptidoglycan and lipopolysaccharide are not present in the *E. chaffeensis* or *A. phagocytophilum* genome.[24] The genomes of these obligately intracellular bacteria are small, ranging from 1.2 to 1.6 Mb to as low as 900 kb for *Neorickettsia*. Bacteria in the Anaplasmataceae family possess multiple genes that are members of the pfam01617.8, Surface_Ag_2 gene family, encoding major surface porin proteins responsible for antigenic variation and host cell adhesion in genera as diverse as *Neisseria, Brucella,* and *Pseudomonas. E. chaffeensis, E. canis, E. ewingii,* and *A. phagocytophilum* have gene families that encode more than 19 paralogous, surface-exposed pfam01617.8 proteins of 22 to 30 kDa (p28/Omp-1 family) for *Ehrlichia* and approximately 105 paralogous genes encoding 41 to 49 kDa (major surface protein-2 [Msp2]) for *A. phagocytophilum*.[25–27] In accordance with the pfam01617.8 predictions, roles have been shown for *A. phagocytophilum* Msp2 in antigenic variation,[28,29] and Msp2/p44 and p28/Omp-1 could function as porins in both *A. phagocytophilum* and *E. chaffeensis*.[30,31] Antigenic diversity within a single strain is based on the presence of hypervariable regions in the *Ehrlichia p28/omp1* family, where each gene is transcribed independently yet is dominated by the expression of a single protein in mammalian infection, and a different protein is expressed in ticks.[31,32] Reinfection with different *E. chaffeensis* and *A. phagocytophilum* strains has been reported, underscoring the role of antigenic diversity in immune protection.[33,34] Similarly, Msp2/p44 of *A. phagocytophilum* is characterized by conserved domains that flank a hypervariable region, but expression requires gene conversion into a single genomic site. This condition may be further complicated by segmental conversion that generates even greater antigenic complexity.[35–37] Infection in vivo yields a large number of transcriptional and antigenic variants that presumably contribute to persistence in reservoir hosts.[38]

E. chaffeensis undergoes a 72-hour developmental cycle in which the infectious dense core stage attaches, enters, and converts into the reticulate stage, which replicates numerous times by binary fission in an acidified early endosome and converts into the dense core stage, which is released from the host macrophage. The *Ehrlichia* genome encodes more than 20 outer membrane proteins (OMPs) that are porins, only one of which is expressed in humans and another in ticks, and several proteins that contain a series of tandem repeat units (TRPs). TRP120 and TRP47 are expressed exclusively on dense core ehrlichiae.[39,40] These play a role in adhesion to the target cell, in part regulated by bacterial response regulator CtrA.[41,42] Attachment of TRPs is most likely to host Wnt receptors and of EtpE to DNAX, triggering internalization via caveolae. Ehrlichiae residing in early endosomes acquire nutrients by induction of autophagy by Etp-1 and block lysosomal fusion via protein expression controlled by a two-component regulatory system. TRPs translocate to the nucleus of the infected cell, where they bind DNA at sites that are differentially transcribed with infection to reprogram host cell gene expression, downregulating interleukin (IL)-12 and IL-18 and upregulating apoptosis and cyclin inhibitors that favor ehrlichial growth. TRP120 also interacts with multiple host proteins involved in cell signaling, protein trafficking, and actin cytoskeleton organization, providing multiple opportunities to reprogram the host cell's functions.[43,44] Ehrlichiae also modulate host Wnt, Notch, and Jak/Stat signaling and SUMOylation pathways, resulting in downregulated pattern recognition receptor expression and innate proinflammatory immune responses, further creating an environment for ehrlichial survival.[45]

Both *E. chaffeensis* and *A. phagocytophilum* express functional type IV secretion systems, and at least one *A. phagocytophilum* substrate, AnkA, is translocated into the infected host cell, where it regulates bacterial entry by interacting with Abl-interactor 1 (ABI1), which influences epidermal growth factor receptor signal transduction and cytoskeletal changes.[46] AnkA eventually translocates to complex with host nuclear heterochromatin, where it binds widely throughout the genome to intergenic regions sequestered into the nuclear lamina and to gene promoters and by recruiting histone deacetylase alters histone acetylation, chromatin structure, and ultimately transcriptional activity.[47–50] Both bacteria also express two-component histidine kinase regulatory systems that influence trafficking of the parasitophorous vacuole after bacterial entry.[51]

Epidemiology and Epizootiology of Human Monocytotropic Ehrlichiosis

Human ehrlichioses are tick-borne zoonoses. Most patients give a history of tick exposure during the month before the onset of illness. The seasonality of HME, with peak incidence in May to August, reflects the tick-transmitted epidemiology.[52] Exposures are predominantly rural and suburban and involve recreational, peridomestic, occupational, and military activities. More than 60% of patients are male. Documented cases of HME have been reported in 47 states, particularly in the south-central and southeastern United States. This region conforms to the distribution of the Lone Star tick, *Amblyomma americanum,* the range of which is expanding northward and westward; along with white-tailed deer, this maintains the ehrlichiae in nature through acquisition of *E. chaffeensis* during feeding as a larva or nymph on persistently infected deer or by cofeeding with infected ticks and subsequently transmitting ehrlichiae to nonimmune deer. The pathogen is transmitted transstadially from larvae to nymphs and from nymphs to adults, but not transovarially.[53] Organisms closely related to *E. chaffeensis* and evidence of human ehrlichial infections have also been reported in South America, Africa, and eastern Asia.[54–58]

Between 1987 and 2017, 15,527 cases of HME were reported in *Morbidity and Mortality Weekly Reports* (*MMWR;* Centers for Disease Control and Prevention [CDC]). From 2008 to 2012, the incidence of HME was 3.2 cases per million population in the United States, a fourfold increase from 2000, with hospitalization in 57%, a life-threatening complication in 11%, and a case-fatality rate of 1% (4% in children <5 years and 3% in those ≥70 years of age).[52] An active, prospective, 3-year study in Cape Girardeau, Missouri, revealed 29 cases in a family practice of 7000 patients, an average annual incidence of 138 cases per 100,000 population.[59] Similarly, selected communities have prevalence rates as high as 330 cases per 100,000 population under permissive ecologic circumstances.[60]

It is presumed that most infections are not diagnosed, because cross-sectional seroprevalence in endemic regions ranges from 1.3% to 12.5% for HME.[60] Thus, subclinical seroconversion could reflect exposure to other *Ehrlichia* or *Anaplasma* species or other antigenic stimuli. *E. canis,* which induces cross-reactive serologic responses, also infects patients in South and Central America. *E. muris* subsp. *eauclairensis* and

E. ewingii are known to cause milder infections that result in serologic cross-reactions with *E. chaffeensis*.[8,9,11] A single case of infection by an *E. ruminantium*–like bacterium, called the Panola Mountain *Ehrlichia*, has been identified in a 31-year-old man in Georgia.[12] Human infection by *E. muris* subsp. *eauclairensis* has, to date, occurred only in Wisconsin and Minnesota.[11] Transfusion-transmitted HME is possible,[61] and transfusion-related *E. ewingii* infection has been reported,[62] but both are probably rare. *Candidatus* N. mikurensis is found in *Ixodes* ticks throughout Europe, Asia, and Africa. Surveillance with polymerase chain reaction (PCR) of asymptomatic forest workers in Central Europe found an infection rate of 1.6%.[63] Life-threatening transplant-associated disease occurred in both recipients of kidneys from the same deceased donor.[64]

Epidemiology and Epizootiology of Human Granulocytic Anaplasmosis

Human granulocytic anaplasmosis (HGA) also has a seasonal occurrence, peaking in June but continuing through November, in accordance with the activity of nymphal and adult stages of *Ixodes scapularis* ticks.[65] Although risk for HGA is associated with outdoor activity, a substantial proportion of cases occurs in suburban areas of northeastern and upper Midwestern cities.[65–67] HGA occurs in specific geographic locations; 92% of US cases are reported from New England and the Upper Midwest, with the highest rates in Wisconsin, Minnesota, and Rhode Island.[65] Infection rates increased from 2008 to 2012 and increased with age, and the geographic range expanded. The majority of diagnoses were made by means of PCR. The distribution is almost identical to that of Lyme disease because of the shared *Ixodes* spp. tick vectors. HGA is documented throughout Europe, particularly in Slovenia, Sweden, and Norway, and in China, Korea, Japan, and other parts of Asia.[55,68–71] Serologic studies suggest a global distribution in the Northern Hemisphere for HGA, *A. phagocytophilum,* and its tick vectors.

Between 1995 and 2017, a total of 30,759 cases of HGA were reported by the CDC in *MMWR*. The incidence of HGA nationally was 8.0 cases per million person-years in 2012, but above 50 cases per million person-years in Minnesota, Wisconsin, and Rhode Island[65]; however, active case collection has yielded an incidence of 14 to 16 cases per 100,000 population in the upper Midwest between 1990 and 1995, with rates as high as 24 to 58 cases per 100,000 population in some northwest Wisconsin counties in 1994–1995 and in Connecticut in 1997–1999.[72,73] Cross-sectional seroprevalence studies have shown that up to 15% of the population in northwestern Wisconsin, 1% of Connecticut residents and US. military personnel, 17% of Slovenians, and 12% of the population of Sweden's Koster Islands have antibodies reactive with *A. phagocytophilum* in the absence of antecedent clinical evidence for HGA.[74–78] Alternatively, in some centers in Sweden and Finland, the rate of asymptomatic seroconversion from bites of infected ticks is probably rare.[79] The demonstration of mildly affected patients who recover spontaneously, even in the absence of specific therapy, suggests that HGA could frequently be subclinical.[80] Transfusion-transmitted HGA has become an increasing threat in spite of leukoreduced blood products in the United States and in Europe.[81–88]

From 4% to 36% of patients with serologic evidence of *A. phagocytophilum* infection also have serologic evidence of *Borrelia burgdorferi* or *Babesia microti* infection, possibly representing serial infections with each agent; both agents are also transmitted by *Ixodes* spp. tick bites.[89–91] Concurrent HGA and Lyme disease, documented by isolation of both agents, has been reported,[92] although a prospective study has shown only a 2% incidence of coinfection in patients with erythema migrans and Lyme disease.[93] Coinfection with tick-borne encephalitis virus has been demonstrated in Europe and is likely in the United States with the increasing incidence of Powassan and deer tick virus infections.[94] Whether concurrent infection with these agents results in increased severity, prolonged duration of illness, or more frequent and severe sequelae has yet to be definitively determined.[91]

A. phagocytophilum is transmitted to humans by the bites of nymphal and adult *I. scapularis* ticks in the eastern United States, *Ixodes pacificus* in California, *I. ricinus* in Europe, and presumably *Ixodes persulcatus* in parts of Asia. However, *A. phagocytophilum* DNA was also found in *Haemaphysalis longicornis* ticks examined in conjunction with 62 cases of HGA in Shandong Province in China.[69] Although transstadial transmission of the infectious agent occurs, *A. phagocytophilum* is not maintained transovarially, and thus natural maintenance requires horizontal (tick-mammal-tick) transmission.[95] A major proven reservoir host is the white-footed mouse, *Peromyscus leucopus*; however, other small mammals, such as sciurids (squirrels) in the western United States, and ruminants have been found naturally infected or have serologic evidence of infection, including voles, wood rats, white-tailed deer, red deer, and roe deer.[96–98] Current serologic evidence suggests that larval ticks acquire *A. phagocytophilum* after feeding on small mammals infected earlier in the season by nymphal ticks. White-footed mice develop immunity to *A. phagocytophilum* after a period of bacteremia that may last from several days to weeks; prior immunity reduces small mammal reservoir competence and transmission.[99] Small mammals are not adversely affected by the infection, and some may become persistently infected. The contribution of persistently infected ruminants and cervids as reservoir hosts for *A. phagocytophilum* requires further investigation. In northern China, where *A. capra* infects goats, 28 of 477 (6%) persons with a history of tick bite had PCR evidence of infection, and all had a history of a nonspecific febrile illness, including 10 with rash or eschar.[14]

PATHOGENESIS AND PATHOLOGY

Human Monocytotropic Ehrlichiosis

After entering the skin by tick bite inoculation and being spread presumably through lymphatic and blood vessels, ehrlichiae invade target cells of the hematopoietic and lymphoreticular systems. Morulae of *E. chaffeensis* are observed mainly in macrophages and monocytes, less frequently in lymphocytes, and rarely in polymorphonuclear leukocytes.[100–103] Ehrlichial morulae have been identified in peripheral blood, bone marrow, hepatic sinusoids, lymph nodes, splenic cords, splenic sinusoids, splenic periarteriolar lymphoid sheaths, cerebrospinal fluid (CSF) macrophages, and macrophages in perivascular lymphohistiocytic infiltrates in organs such as the kidney, appendix, and heart.

The best studied tissue in HME is bone marrow, largely because of the frequency of leukopenia, thrombocytopenia, and anemia. Frequent findings include granulomas, myeloid hyperplasia, and megakaryocytosis.[100] Erythrophagocytosis and plasmacytosis occur in smaller proportions of patients with HME. Focal hepatocellular necrosis; hepatic granulomas, including ring granulomas; cholestasis; splenic and lymph node necrosis; diffuse mononuclear phagocyte hyperplasia in the spleen, liver, lymph nodes, and bone marrow; perivascular lymphohistiocytic infiltrates of various organs, including the kidney, heart, liver, meninges, and brain; and interstitial mononuclear cell pneumonitis are also observed.[104,105] It is worthy of emphasis that direct endothelial injury and thrombosis have not been described. The observation of erythrophagocytosis, myeloid hyperplasia, and megakaryocytosis in the bone marrow of patients with HME suggests peripheral consumption of blood elements and a compensatory response. In contrast, evidence in murine models exists for myelosuppression, which suggests either direct bacterium-related or indirect chemokine effects on bone marrow.[106]

Although *E. chaffeensis* causes a direct cytopathic effect when grown in cell culture, it appears that the host responses account for most of the clinical manifestations. The toxic shock manifestations of HME are likely to be the systemic effects of increased levels of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), IL-1α and IL-1β, and IL-6; defective production of Th1 cytokines, including interferon-γ (IFN-γ) and IL-2; the increased production of antiinflammatory IL-10; and increased levels of chemokines.[107] TNF-α is produced by natural killer T cells and CD8 T lymphocytes in murine models and possibly in humans and, together with perforin expressed by these cells, results in killing of CD4 T lymphocytes, significant reductions in IFN-γ expression, and apoptotic tissue injury.[108,109] IFN-γ stimulates macrophage killing of *E. chaffeensis* through the sequestration of iron, and opsonization with immune serum enhances the destruction of ehrlichiae by macrophages.[110,111] *E. chaffeensis* circumvents host defenses by inhibiting the fusion of infected phagosomes with lysosomes and inhibiting the signal transduction pathway of IFN-γ–mediated anti-ehrlichial activity.[112] There is evidence that *E. chaffeensis* also evades immunity through downregulation of other host defense genes of the infected macrophage and manipulation of the host cell as a niche favorable to its survival and growth.[43,44,113,114]

Human Granulocytotropic Anaplasmosis

A. phagocytophilum is observed predominantly in neutrophils in peripheral blood and tissues from infected individuals.[67,72] Dramatic histopathologic findings involve the presence of opportunistic pathogens, especially severe fungal and viral infections, which account for most fatalities. Pathologic findings in humans and animal models include normocellular or hypercellular bone marrow, erythrophagocytosis in mononuclear phagocytic organs, hepatocellular apoptoses and periportal lymphohistiocytic infiltrates, focal splenic necrosis, mild interstitial pneumonitis, and pulmonary hemorrhage.[115] Vasculitis, endothelial injury, granulomas, and meningeal inflammation have not been described.

A. phagocytophilum disseminates to blood, bone marrow, and spleen after a tick bite, likely through local infection of neutrophils attracted to the tick bite wound in the dermis.[116] In the bone marrow, progenitors of myeloid and monocytic lineages can be infected.[117,118] Endothelial cells can be infected in vitro,[119] but evidence for in vivo infection is lacking.[115,116] With neutrophil infection, the bacteria attach to the cell surface P-selectin glycoprotein ligand-1 (CD162) and perhaps other ligands,[120] enter a vacuole that is altered by secreted *A. phagocytophilum* effectors to avoid autophagy, mimic recycling endosomes by accumulating an array of Rab guanosine triphosphatases (GTPases) to the membrane, and thereby preclude lysosome fusion and replicate.[121,122]

In vitro, *A. phagocytophilum* survives by deactivation of the neutrophil antimicrobial response through AnkA-mediated silencing of granulocyte *RAC2* and *CYBB* ($gp91^{phox}$) transcription and prolonged downregulation of phagocyte oxidase activity.[47,49,123,124] Additional pathogenetic processes modified in infected granulocytes include delayed apoptosis, ineffective binding to and transmigration of activated endothelium, and inhibition of phagocytic activity.[125–131] However, infection also paradoxically stimulates an inflammatory response with neutrophil activation, chemokine secretion, and degranulation.[132–134] Increased proinflammatory activity, in part mediated by TLR2 and NALP4 inflammasome activation of macrophages[135,136] and by transcriptional upregulation of chemokine genes,[133,137,138] allows the recruitment of new neutrophil host cells and localized tissue injury that further exacerbates inflammatory stimulation when neutrophils cannot generate effective antimicrobial responses. These findings are consistent with the dissociation of bacterial burden and histopathologic evidence of tissue injury in mouse and horse models, suggesting a role for host immunity in disease.[139–142] *A. phagocytophilum* infection is initially controlled by IFN-γ, which results in macrophage activation and marked increases in inflammatory tissue injury in mouse models, paralleling evidence of macrophage activation as a mechanism of increased severity in humans[143,144]; downregulation of IFN-γ expression in animal models results in a higher bacterial burden but less inflammatory tissue injury and lessened clinical disease.[139–141] Paradoxically, infection of Stat1-deficient animals results in ample IFN-γ production with massive inflammatory consequences and marked increases in bacterial burden.[134] Although involved in inflammatory injury with infection, host innate and adaptive immune mechanisms contribute little to control of *A. phagocytophilum* infection except for CD4 T lymphocytes.[145]

CLINICAL MANIFESTATIONS

Human Monocytotropic Ehrlichiosis

Signs and Symptoms

The clinical picture of HME in immunocompetent patients is of a mild-to-severe multisystem illness, with a median duration of 23 days (Table 192.2).[52,59,146–148] Approximately 12% to 30% of infections have been reported in immunocompromised patients in whom *E. chaffeensis* acts as an opportunistic pathogen, wherein a 3.0 relative risk for life-threatening complications is documented and fatal overwhelming infections can occur.[149–151] The median incubation period is 9 days. Symptoms at onset of illness include fever, chills, headache, myalgia, and malaise. Later in the course, patients often develop nausea, anorexia, vomiting, and weight loss. Physical signs are not striking. Fewer than half of patients have a rash, which is maculopapular and can be petechial. Rash is observed more frequently in children. Adult patients with severe illness are more likely to have a cough, diarrhea, confusion, and lymphadenopathy, whereas pediatric patients may develop edema of the hands or feet. Severe complications occur in 9% to 17% of patients and include pneumonia and adult respiratory distress syndrome (18% require mechanical ventilation); disseminated intravascular coagulation (DIC)– and sepsis-like syndromes; acute renal insufficiency; central nervous system (CNS) abnormalities such as meningoencephalitis; and death.[52,107,152–154] *E. chaffeensis* infection can also lead to hemophagocytic lymphohistiocytosis or macrophage activation syndrome.[155–159] CSF pleocytosis usually shows a predominance of lymphocytes and increased protein concentration and can demonstrate the presence of infected cells.[103] Nearly half of patients with chest radiographic evaluation have infiltrates. Failure to recognize the frequency of pulmonary and CNS involvement is associated with delayed treatment and greater likelihood of intensive care unit (ICU) admission and severe complications.[149]

TABLE 192.2 Clinical and Laboratory Abnormalities in Human Monocytotropic Ehrlichiosis (HME) and Human Granulocytotropic Anaplasmosis (HGA)

SIGN, SYMPTOM, OR LABORATORY FINDING	HME MEDIAN % WITH ABNORMAL FINDING (IQR)[a]	HGA MEDIAN % WITH ABNORMAL FINDING (IQR)[b]
Fever	96 (95–99)	100 (94–100)
Headache	72 (69–72)	82 (60–96)
Myalgia	73 (63–75)	73 (61–82)
Malaise	77 (73–80)	97 (92–99)
Nausea	57 (56–59)	40 (35–52)
Vomiting	47 (37–56)	22 (17–35)
Diarrhea	25 (20–31)	20 (13–25)
Rash	26 (21–34)	5 (3–10)
Cough	28 (26–31)	27 (21–33)
Confusion/mental status changes	20 (19–22)	17 (17–18)
Leukopenia	60 (60–71)	63 (53–76)
Thrombocytopenia	79 (68–88)	80 (61–90)
Anemia	50 (38–54)	40 (14–48)
Elevated AST/ALT	88 (86–91)	80 (69–98)
Elevated creatinine	29	49 (25–71)
Elevated C-reactive protein	data not available	96 (83–100)

[a]HME meta-analysis data from references 59, 149, 151, 153, 154, 164, 217, 218.
[b]HGA meta-analysis data from references 66, 72, 68, 219, 220, 69, 177–186, 221–228.
ALT, Alanine aminotransferase; *AST,* aspartate aminotransferase; *IQR,* interquartile range.

Important laboratory features are thrombocytopenia, mild-to-moderate leukopenia, and elevations of serum hepatic aminotransferase levels (see Table 192.2). The nadir of leukopenia is usually between 1300 and 4000 cells/μL. Neutropenia, lymphopenia, or both account for the leukopenia. Thrombocytopenia occurs concurrently with leukopenia and is usually between 50,000 and 140,000 platelets/μL, although it is occasionally profound (less than 20,000 platelets/μL).

Course

The clinical course of illness ranges from mild illness to a fatal outcome. The higher incidence and risk for life-threatening complications in older patients suggests that host factors are important in disease severity. A virulent form of HME occurs in human immunodeficiency virus–infected individuals and is often associated with overwhelming infection, a toxic shock– or sepsis-like syndrome, and fatality.[107,150] Immune compromise related to corticosteroid therapy, etanercept, or immunosuppression with organ transplantation is also associated with increased severity, although prompt treatment abrogates this increased risk.[151,160,161]

The median duration of hospitalization is about 1 week. Convalescence is often prolonged. Persistent infection has been documented in only one patient with HME.[162] Fatalities occur in approximately 1% to 3%.[52] Many patients treated with doxycycline or tetracycline recover rapidly. On the other hand, most patients who receive no effective anti-ehrlichial treatment also have uncomplicated complete recovery.

Diagnosis

A diagnosis based on epidemiologic and clinical factors, including a high index of suspicion, allows prompt empirical anti-ehrlichial treatment.[149] Patients with fever, leukopenia, thrombocytopenia, elevated serum aminotransferase levels, and a history of a recent tick bite in endemic regions from May through July should be considered as possibly having HME. No absolute clinical criteria distinguish HME from Rocky Mountain spotted fever,[163] although patients with ehrlichiosis are less likely to have a rash and more likely to have leukopenia (median white blood cell count, 3500/μL). Morulae are observed in less than 7% of patients with HME, most often in immunocompromised patients with overwhelming infection.[150]

Although effectively used in one large series of cases of HME,[164] culture is only a research tool that uses special methods and unique cell lines.

Because of the presence of *E. chaffeensis* in peripheral blood or CSF mononuclear cells during active infection and before the presence of diagnostic levels of serum antibodies, methods to detect bacterial DNA, such as PCR assay, are highly sensitive and useful diagnostic tools. There are a large number of potential gene targets, with sensitivity ranging from 60% to 100%.[59,165,166]

At present, the major diagnostic confirmatory criterion for human ehrlichiosis is serologic, as determined by means of indirect immunofluorescence assay (IFA) with *E. chaffeensis*–infected cells. To be considered positive, the patient's sera must show a fourfold or greater rise in immunoglobulin G (IgG) antibody titer during the course of the disease, with a minimal peak titer of 128. IFA shows a peak geometric mean titer of 1280 at 6 weeks after onset.[167] Only 22% to 44% of the sera tested in the first week of illness have a titer of 80 or higher. Among sera from patients tested in the second week, 68% are diagnostic. Sera tested 4 or more weeks after the onset of illness should demonstrate seroconversion.

Differential Diagnosis

Early in the course of the disease, when the patient presents with fever, headache, myalgia, and malaise, the differential diagnoses may include various viral syndromes, Rocky Mountain spotted fever, upper respiratory illness, sepsis, and urinary tract infection. If nausea, vomiting, and anorexia are prominent symptoms, gastroenteritis is often suspected. With prominent cough, pneumonia is often considered. CNS signs and symptoms with CSF pleocytosis suggest viral or bacterial meningoencephalitis. On obtaining a history of recent tick bite, the physician should consider a tick-borne febrile illness, such as Rocky Mountain spotted fever, relapsing fever, tularemia, Lyme borreliosis, Colorado tick fever, babesiosis, or Powassan, Heartland, or Bourbon virus infections. Other diagnostic considerations include meningococcemia, toxic shock syndrome, leptospirosis, hepatitis, enteroviral infection, influenza, murine typhus, Q fever, typhoid fever, bacterial sepsis, endocarditis, Kawasaki disease, collagen-vascular diseases, and leukemia. *E. chaffeensis* is associated with the development of hemophagocytic lymphohistiocytosis syndrome. A comparison of the ehrlichioses and Rocky Mountain spotted fever is presented in Table 192.3.

No prospective randomized, controlled clinical studies to establish efficacy of antimicrobials for HME have been conducted. Based on retrospective clinical studies and in vitro investigations, doxycycline is the drug of choice, even in pregnant patients and children, and is administered to adults at a dose of 100 mg twice daily until the patient has become afebrile and clinically improved.[149] Courses of treatment of 5 to 10 days have yielded a favorable outcome in many patients. *E. chaffeensis* has been demonstrated to be susceptible to rifampin and resistant to fluoroquinolones in cell culture.[168,169] Patients benefit from doxycycline administration at hospital admission with significantly decreased rates of ICU transfer and mechanical ventilation, shorter hospital stays, and shorter length of illnesses.[149]

TABLE 192.3 Comparison of Human Monocytotropic Ehrlichiosis (HME) and Human Granulocytic Anaplasmosis (HGA) in the United States With Rocky Mountain Spotted Fever (Rmsf)

Similarities
History of tick attachment
Incubation period of about 1 week between tick bite and onset of symptoms
Peak incidence in late spring and summer
Acute onset with headache, fever, myalgia, and malaise
Severe cases may include coagulopathy, azotemia, and encephalopathy
White blood cell count usually not elevated, platelet count often low, serum aspartate aminotransferase level often increased
Diagnosis through acute and convalescent serology
Treatment—a tetracycline is effective
Differences
Cough, dyspnea, and vomiting present less commonly in ehrlichioses and HGA
RMSF—rash is present in 90% of patients and is petechial in about half the cases
Ehrlichiosis and anaplasmosis—rash is present in less than half of adult patients with HME and infrequently in HGA, is maculopapular and rarely petechial
Leukopenia with absolute lymphopenia and/or neutropenia common in hospitalized patients with HME and neutropenia in those with HGA, but less frequent in RMSF
Inclusions (morulae) seen infrequently in monocytes and macrophages of HME patients, occasionally in neutrophils of HGA patients but not in RMSF patients
Chloramphenicol is not likely effective for HME or HGA
Histopathologic vasculitis, hallmark of RMSF, not observed in ehrlichioses or anaplasmosis

Ehrlichiosis Caused by *Ehrlichia ewingii* and *Ehrlichia muris*

E. ewingii ehrlichiosis has been diagnosed in Missouri, Tennessee, Delaware, Maryland, Virginia, Georgia, Illinois, Kansas, Louisiana, Ohio, Oklahoma, and South Carolina and in *A. americanum* or other ticks across the same geographic regions.[170,171] *E. ewingii* has also been detected in Korea and Cameroon.[172,173] One-quarter of infections are reported in immunocompromised patients, including those with organ transplantation or history of cancer. Clinical manifestations are similar to those of HME, and despite a 77% hospitalization rate, overall the illness is not as severe, with fewer complications and no deaths reported.[52,150,151] Diagnosis requires PCR with species-specific primers or probes or with DNA sequencing of amplicons of broad-range PCR. Sera of most patients with *E. ewingii* ehrlichiosis react with *E. chaffeensis* IFA antigens, and peptides or recombinant *E. ewingii* 28-kDa proteins distinct from those of *E. chaffeensis* could allow creation of a specific serodiagnostic test.[174]

E. muris was first detected in Japan, where it was found in *Apodemus* mice and *Haemaphysalis flava* ticks; antibodies to *E. muris* were present in 1% of humans in Japan in a large serosurvey,[175] and *E. muris* or a closely related organism was also detected in *I. persulcatus* ticks in the Perm region of Russia, where 86 patients with an acute febrile illness had antibody titers of 80 to 1200 against the antigenically related *E. chaffeensis*.[176] In 2009, infection with the recently reclassified *E. muris* subsp. *eauclairensis* was identified among patients with suspected ehrlichiosis or anaplasmosis in Wisconsin and Minnesota and in *I. scapularis* ticks from the Upper Midwest United States.[10,11] Some patients had strong serologic reactivity in convalescence to *E. chaffeensis* but not *A. phagocytophilum* antigens. Among 69 human infections (including samples from Michigan, North Dakota, and Indiana), all had tick bites or exposures in Wisconsin or Minnesota during 2007 to 2013. The mean age was 63 years, and 27% were immunocompromised. The clinical features were similar to both HME and HGA, including fever (87%), headache (67%), myalgias (60%), lymphopenia (53%), and thrombocytopenia (67%). Nearly 23% of patients were hospitalized, but no deaths occurred.[11]

Human Granulocytotropic Anaplasmosis

Signs, Symptoms, and Course

Infection with *A. phagocytophilum* generally manifests with acute undifferentiated fever. Male patients comprise 60% of infections, and

the incidence rate is at least 10 times higher in patients aged 50 years or older compared with children younger than 10 years.[65] After an incubation period of approximately 1 to 2 weeks, the illness can be mild to severe, with fever, headache, malaise, myalgias and arthralgias in most patients.[66,69,72,177–186] Nausea, vomiting, diarrhea, cough, stiff neck, and confusion are present in less than half of patients, less than 10% have rash, and most of these reflect erythema migrans with concurrent Lyme disease. Most doxycycline-treated patients are well within 7 days; if individuals are untreated, the median duration of illness is 9 days (range, 1–60 days).[178,187] Approximately 31% of infections are severe enough to warrant hospitalization, and 3% to 7% of patients have life-threatening complications or require ICU admission.[65,67] Severe manifestations include respiratory insufficiency, a septic shock– or DIC-like illness, renal failure, and pneumonia or acute respiratory distress syndrome.[65] Although reported in 0.4% of cases,[65] meningoencephalitis and CSF pleocytosis are rare in documented cases of HGA; however, other neurologic sequelae can include facial diplegia, brachial plexopathy, and demyelinating polyneuropathy.[188–190] At least 18 patients died after HGA in 2008 to 2012 alone.[65] Among 62 patients with HGA in Shandong Province in China, a subset of 16 patients from one region had significantly greater disease severity, including multiorgan dysfunction; gastrointestinal, renal, and hemorrhagic manifestations; worse laboratory features; and a case-fatality rate of 16% versus 2.6% in other regions.[69] Similar but less severe findings, including hemorrhagic manifestations (ecchymosis, epistaxis) in another study of HGA in China,[185] suggest the potential for strain-related severity differences or the potential for coinfection with tick-borne hemorrhagic fever bunyaviruses known to be present in those regions.[191–193]

Laboratory features observed in a substantial portion of cases include thrombocytopenia, leukopenia, mild anemia, and increases in serum hepatic aminotransferase activities within the first 7 days of illness.[66,72,186,194] Neutropenia with a left shift and relative lymphocytosis can occur. Leukocyte, erythrocyte, and platelet counts return to normal by 14 days, but the left shift can persist.[195] Doxycycline therapy reverses the decline in leukocyte and platelet counts and blunts the degree of left shift, usually within 5 to 7 days; anemia and normalization of serum hepatic aminotransferase activities respond more slowly. Hyperferritinemia and proinflammatory cytokine response have been identified as risk factors for severity in several studies of HGA.[144,185,186]

Diagnosis

Unlike the rarity of morulae in circulating mononuclear cells in HME, 20% to 80% of patients with HGA have morulae identified in peripheral blood neutrophils (Fig. 192.2).[66,67] Culture of *A. phagocytophilum* requires 1 week or longer and is not routinely available, whereas PCR amplification of *A. phagocytophilum* nucleic acids from blood is 54% to 100% sensitive and highly specific and can be performed in a timely manner.[166,187]

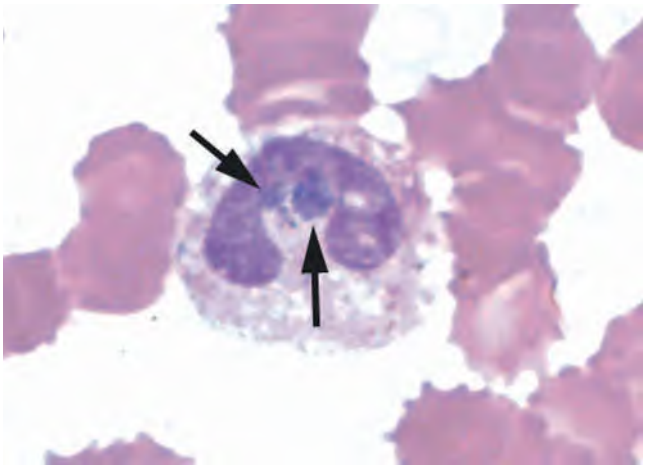

FIG. 192.2 Human granulocytic anaplasmosis. Peripheral blood smear showing intracellular inclusion within a neutrophil of a patient with human granulocytic anaplasmosis *(arrows)* (Wright stain, ×1000).

Serologic diagnosis is most often achieved retrospectively by detection of IgG antibodies reactive with *A. phagocytophilum* in infected tissue culture cells.[196,197] By current criteria, an IgG titer of at least 64 is considered supportive diagnostic information; however, because 15% to 16% of the population in the upper Midwest and New York State have preexisting serologic reactions, a fourfold rise to at least a titer of 128 provides more definitive evidence for infection.[76,78] IgM testing could be useful because reactions are demonstrated only during the first 45 to 60 days, but this test lacks sensitivity and is not generally advocated.[196] A role for Western immunoblot confirmation or use of recombinant antigens for serodiagnosis is not currently defined for humans.

TREATMENT AND PREVENTION

No prospective controlled treatment trials to determine antimicrobial efficacy for HGA have been conducted. In retrospective treatment studies, doxycycline, 100 mg twice daily, or tetracycline, 500 mg four times a day, has been used successfully. Based on *A. phagocytophilum* cell culture, doxycycline and rifamycins are bactericidal and chloramphenicol is not effective.[168,198,199] The clinical efficacy of rifampin has not yet been evaluated, but it has been used in children and during pregnancy.[200,201] Although fluoroquinolones have in vitro minimal inhibitory concentration values that predict in vivo effectiveness, relapse after use of levofloxacin unrelated to mutations in the bacterial *gyrA* gene has been documented in HGA.[202]

CANDIDATUS NEOEHRLICHIA MIKURENSIS HUMAN INFECTIONS

Documented human infections with *Candidatus* N. mikurensis are few, but increasing in number and geographic distribution. Infection has been described in humans in multiple recent cases in Europe, in 7 of 622 febrile Chinese patients, and in 5 of 316 asymptomatic foresters in Poland.[13,63,203–210] Infection was identified within peripheral blood granulocytes in 1 patient, and no isolate has been made from any patient. European patients were from Czech Republic, Germany, Sweden, and Switzerland. Eight patients had preexisting immunocompromise (chronic lymphocytic leukemia; follicular and mantle cell lymphomas; chronic inflammatory demyelinating polyneuropathy; rituximab, cyclophosphamide, or prednisolone treatment; and orthotopic liver transplantation), and several developed severe sepsis-like syndromes, sometimes protracted over weeks. Among these patients there is a high prevalence of thromboembolic complications, with an initial clinical presentation including fever, muscle and joint pain, and vascular events including deep vein thrombosis and transient ischemic attacks. The diagnosis is often delayed secondary to the similarity of the presentation to nonspecific inflammatory complications. All patients receiving doxycycline recovered. In contrast, none of the Chinese patients,[206] 2 patients from Sweden,[210] and 5 asymptomatic Polish subjects[63] were known to be immunocompromised, and all experienced only mild disease, including nausea, vomiting, myalgia, and stiff neck, or were asymptomatic. Cases were predominantly diagnosed with broad-range amplification of eubacterial *rrs* genes or with newly developed microbe-specific PCR assays. Doxycycline treatment was associated with clinical resolution in most cases; 1 patient died, and in 2 patients the infection resolved after phenoxymethylpenicillin treatment.

PREVENTION OF EHRLICHIOSIS AND ANAPLASMOSIS

At present, prevention of human ehrlichioses and anaplasmosis must rely on avoidance of tick exposure, regular careful search of the body for ticks after exposure, and prompt removal of ticks from the body. Although *A. phagocytophilum* may be transmitted within 4 hours of a tick bite, no analysis of prophylactic antibiotic therapy has been conducted.[211]

SENNETSU NEORICKETTSIOSIS

Physicians outside of Asia are unlikely to see a patient with sennetsu neorickettsiosis, which has been documented in Japan, Malaysia, and recently in Laos and is associated with consumption of raw fish and not with arthropod vectors.[212–214] Despite high seroprevalence in some

areas, disease of sufficient clinical severity to necessitate hospitalization remains rare.[215] *N. sennetsu* was isolated in 1953 from the blood, bone marrow, and lymph node of a 25-year-old man who had fever, severe headaches, myalgia, anorexia, lymphadenopathy, and an increased quantity of atypical lymphocytes in his peripheral blood.[216] Organisms isolated in mice were inoculated into human volunteers, who developed a syndrome resembling infectious mononucleosis. Neorickettsiae were recovered from their blood samples.

The average incubation period of 14 days is followed by sudden onset of chills and a fever that lasts for 2 weeks unless treated effectively. Patients also complain of headache and myalgia. Postauricular and posterior cervical lymphadenopathy appears 5 to 7 days after onset. Hepatosplenomegaly occurs in one-third to one-half of patients. Aseptic meningitis is observed only occasionally, and rash very rarely. Early in the illness leukopenia occurs; in the late febrile and convalescent phases, absolute lymphocytosis is observed, with 10% or greater atypical lymphocytes. Mild-to-moderate elevations occur in serum aminotransferase levels. Laboratory diagnosis can be made with PCR on buffy coat blood or through demonstration of specific serum antibody by IFA. Treatment with one of the tetracycline antimicrobials, including doxycycline or minocycline, results in defervescence after 1 to 2 days.

Key References

The complete reference list is available online at Expert Consult.

3. Dahlgren FS, Heitman KN, Behravesh CB. Undetermined human ehrlichiosis and anaplasmosis in the United States, 2008-2012: a catch-all for passive surveillance. *Am J Trop Med Hyg*. 2016;94:299–301.
5. Anderson BE, Dawson JE, Jones DC, et al. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. *J Clin Microbiol*. 1991;29:2838–2842.
6. Bakken JS, Dumler JS, Chen SM, et al. Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging? *JAMA*. 1994;272:212–218.
7. Chen SM, Dumler JS, Bakken JS, et al. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol*. 1994;32:589–595.
9. Buller RS, Arens M, Hmiel SP, et al. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. *N Engl J Med*. 1999;341:148–155.
10. Pritt BS, Sloan LM, Johnson DK, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. *N Engl J Med*. 2011;365:422–429.
11. Johnson DK, Schiffman EK, Davis JP, et al. Human infection with *Ehrlichia muris*-like pathogen, United States, 2007-2013(1). *Emerging Infect Dis*. 2015;21:1794–1799.
13. Welinder-Olsson C, Kjellin E, Vaht K, et al. First case of human "*Candidatus* Neoehrlichia mikurensis" infection in a febrile patient with chronic lymphocytic leukemia. *J Clin Microbiol*. 2010;48:1956–1959.
14. Li H, Zheng YC, Ma L, et al. Human infection with a novel tick-borne *Anaplasma* species in China: a surveillance study. *Lancet Infect Dis*. 2015;15:663–670.
38. Scorpio DG, Leutenegger C, Berger J, et al. Sequential analysis of *Anaplasma phagocytophilum msp2* transcription in murine and equine models of human granulocytic anaplasmosis. *Clin Vaccine Immunol*. 2008;15:418–424.
41. Popov VL, Yu X, Walker DH. The 120 kDa outer membrane protein of *Ehrlichia chaffeensis*: preferential expression on dense-core cells and gene expression in *Escherichia coli* associated with attachment and entry. *Microb Pathog*. 2000;28:71–80.
43. Luo T, Kuriakose JA, Zhu B, et al. *Ehrlichia chaffeensis* TRP120 interacts with a diverse array of eukaryotic proteins involved in transcription, signaling, and cytoskeleton organization. *Infect Immun*. 2011;79:4382–4391.
44. Zhu B, Kuriakose JA, Luo T, et al. *Ehrlichia chaffeensis* TRP120 binds a G+C-rich motif in host cell DNA and exhibits eukaryotic transcriptional activator function. *Infect Immun*. 2011;79:4370–4381.
46. Lin M, den Dulk-Ras A, Hooykaas PJ, et al. *Anaplasma phagocytophilum* AnkA secreted by type IV secretion system is tyrosine phosphorylated by Abl-1 to facilitate infection. *Cell Microbiol*. 2007;9:2644–2657.
47. Garcia-Garcia JC, Rennoll-Bankert KE, Pelly S, et al. Silencing of host cell *CYBB* gene expression by the nuclear effector AnkA of the intracellular pathogen *Anaplasma phagocytophilum*. *Infect Immun*. 2009;77:2385–2391.
48. Dumler JS, Sinclair SH, Pappas-Brown V, et al. Genome-wide *Anaplasma phagocytophilum* AnkA-DNA interactions are enriched in intergenic regions and gene promoters and correlate with infection-induced differential gene expression. *Front Cell Infect Microbiol*. 2016;6:97.
49. Rennoll-Bankert KE, Garcia-Garcia JC, Sinclair SH, et al. Chromatin-bound bacterial effector ankyrin A recruits histone deacetylase 1 and modifies host gene expression. *Cell Microbiol*. 2015;17:1640–1652.
52. Nichols Heitman K, Dahlgren FS, Drexler NA, et al. Increasing incidence of ehrlichiosis in the United States: a summary of national surveillance of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* infections in the United States, 2008-2012. *Am J Trop Med Hyg*. 2016;94:52–60.
59. Olano JP, Masters E, Hogrefe W, et al. Human monocytotropic ehrlichiosis, Missouri. *Emerg Infect Dis*. 2003;9:1579–1586.
60. Standaert SM, Dawson JE, Schaffner W, et al. Ehrlichiosis in a golf-oriented retirement community. *N Engl J Med*. 1995;333:420–425.
63. Welc-Faleciak R, Sinski E, Kowalec M, et al. Asymptomatic "*Candidatus* Neoehrlichia mikurensis" infections in immunocompetent humans. *J Clin Microbiol*. 2014;52:3072–3074.
64. Sachdev SH, Joshi V, Cox ER, et al. Severe life-threatening *Ehrlichia chaffeensis* infections transmitted through solid organ transplantation. *Transpl Infect Dis*. 2014;16:119–124.
65. Dahlgren FS, Heitman KN, Drexler NA, et al. Human granulocytic anaplasmosis in the United States from 2008 to 2012: a summary of national surveillance data. *Am J Trop Med Hyg*. 2015;93:66–72.
66. Aguero-Rosenfeld ME, Horowitz HW, Wormser GP, et al. Human granulocytic ehrlichiosis: a case series from a medical center in New York State. *Ann Intern Med*. 1996;125:904–908.
69. Zhang L, Wang G, Liu Q, et al. Molecular analysis of *Anaplasma phagocytophilum* isolated from patients with febrile diseases of unknown etiology in China. *PLoS ONE*. 2013;8:e57155.
72. Bakken JS, Krueth J, Wilson-Nordskog C, et al. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. *JAMA*. 1996;275:199–205.
79. Henningsson AJ, Wilhelmsson P, Gyllemark P, et al. Low risk of seroconversion or clinical disease in humans after a bite by an *Anaplasma phagocytophilum*-infected tick. *Ticks Tick Borne Dis*. 2015;6:787–792.
80. Petrovec M, Lotric Furlan S, Zupanc TA, et al. Human disease in Europe caused by a granulocytic *Ehrlichia* species. *J Clin Microbiol*. 1997;35:1556–1559.
84. Proctor MC, Leiby DA. Do leukoreduction filters passively reduce the transmission risk of human granulocytic anaplasmosis? *Transfusion*. 2015;55:1242–1248.
86. Annen K, Friedman K, Eshoa C, et al. Two cases of transfusion-transmitted *Anaplasma phagocytophilum*. *Am J Clin Pathol*. 2012;137:562–565.
100. Dumler JS, Dawson JE, Walker DH. Human ehrlichiosis: hematopathology and immunohistologic detection of *Ehrlichia chaffeensis*. *Hum Pathol*. 1993;24:391–396.
109. Dierberg KL, Dumler JS. Lymph node hemophagocytosis in rickettsial diseases: a pathogenetic role for CD8 T lymphocytes in human monocytic ehrlichiosis (HME). *BMC Infect Dis*. 2006;6:121.
123. Banerjee R, Anguita J, Roos D, et al. Cutting edge: infection by the agent of human granulocytic ehrlichiosis prevents the respiratory burst by down-regulating gp91phox. *J Immunol*. 2000;164:3946–3949.
124. Carlyon JA, Chan WT, Galan J, et al. Repression of *rac2* mRNA expression by *Anaplasma phagocytophila* is essential to the inhibition of superoxide production and bacterial proliferation. *J Immunol*. 2002;169:7009–7018.
139. Martin ME, Caspersen K, Dumler JS. Immunopathology and ehrlichial propagation are regulated by interferon-gamma and interleukin-10 in a murine model of human granulocytic ehrlichiosis. *Am J Pathol*. 2001;158:1881–1888.
143. Dumler JS. The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. *FEMS Immunol Med Microbiol*. 2012;64:13–20, 3256277.
144. Dumler JS, Barat NC, Barat CE, et al. Human granulocytic anaplasmosis and macrophage activation. *Clin Infect Dis*. 2007;45:199–204.
149. Hamburg BJ, Storch GA, Micek ST, et al. The importance of early treatment with doxycycline in human ehrlichiosis. *Medicine (Baltimore)*. 2008;87:53–60.
150. Paddock CD, Folk SM, Shore GM, et al. Infections with *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in persons coinfected with human immunodeficiency virus. *Clin Infect Dis*. 2001;33:1586–1594.
151. Thomas LD, Hongo I, Bloch KC, et al. Human ehrlichiosis in transplant recipients. *Am J Transplant*. 2007;7:1641–1647.
153. Fishbein DB, Dawson JE, Robinson LE. Human ehrlichiosis in the United States, 1985 to 1990. *Ann Intern Med*. 1994;120:736–743.
166. Bell CA, Patel R. A real-time combined polymerase chain reaction assay for the rapid detection and differentiation of *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii*. *Diagn Microbiol Infect Dis*. 2005;53:301–306.
180. Bakken JS, Dumler S. Human granulocytic anaplasmosis. *Infect Dis Clin North Am*. 2008;22:433–448.
185. Jiao XY, Fan ZC, Li YZ, et al. Clinical and laboratory features parameters of human granulocytic anaplasmosis (HGA) in patients admitted to hospital in Guangdong Province, China. *Trop Doct*. 2015;45:209–213.
186. Schotthoefer AM, Schrodi SJ, Meece JK, et al. Pro-inflammatory immune responses are associated with clinical signs and symptoms of human anaplasmosis. *PLoS ONE*. 2017;12:e0179655.
200. Buitrago MI, Ijdo JW, Rinaudo P, et al. Human granulocytic ehrlichiosis during pregnancy treated successfully with rifampin. *Clin Infect Dis*. 1998;27:213–215.
201. Krause PJ, Corrow CL, Bakken JS. Successful treatment of human granulocytic ehrlichiosis in children using rifampin. *Pediatrics*. 2003;112:e252–e253.
203. Pekova S, Vydra J, Kabickova H, et al. *Candidatus* Neoehrlichia mikurensis infection identified in 2 hematooncologic patients: benefit of molecular techniques for rare pathogen detection. *Diagn Microbiol Infect Dis*. 2011;69:266–270.
206. Li H, Jiang JF, Liu W, et al. Human infection with *Candidatus* Neoehrlichia mikurensis, China. *Emerg Infect Dis*. 2012;18:1636–1639.
213. Newton PN, Rolain JM, Rasachak B, et al. Sennetsu neorickettsiosis: a probable fish-borne cause of fever rediscovered in Laos. *Am J Trop Med Hyg*. 2009;81:190–194.
215. Dittrich S, Phuklia W, Turner GD, et al. *Neorickettsia sennetsu* as a neglected cause of fever in South-East Asia. *PLoS Negl Trop Dis*. 2015;9:e0003908.

References

1. Silverstein AM. On the naming of rickettsiae after Paul Ehrlich. *Bull Hist Med*. 1998;72:731–733.
2. Dumler JS, Barbet AF, Bekker CP, et al. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia with Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *Int J Syst Evol Microbiol*. 2001;51:2145–2165.
3. Dahlgren FS, Heitman KN, Behravesh CB. Undetermined human ehrlichiosis and anaplasmosis in the United States, 2008-2012: a catch-all for passive surveillance. *Am J Trop Med Hyg*. 2016;94:299–301.
4. Maeda K, Markowitz N, Hawley RC, et al. Human infection with *Ehrlichia canis*, a leukocytic rickettsia. *N Eng J Med*. 1987;316:853–856.
5. Anderson BE, Dawson JE, Jones DC, et al. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. *J Clin Microbiol*. 1991;29:2838–2842.
6. Bakken JS, Dumler JS, Chen SM, et al. Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging? *JAMA*. 1994;272:212–218.
7. Chen SM, Dumler JS, Bakken JS, et al. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol*. 1994;32:589–595.
8. Perez M, Rikihisa Y, Wen B. *Ehrlichia canis*-like agent isolated from a man in Venezuela: antigenic and genetic characterization. *J Clin Microbiol*. 1996;34:2133–2139.
9. Buller RS, Arens M, Hmiel SP, et al. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. *N Engl J Med*. 1999;341:148–155.
10. Pritt BS, Sloan LM, Johnson DK, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. *N Engl J Med*. 2011;365:422–429.
11. Johnson DK, Schiffman EK, Davis JP, et al. Human infection with *Ehrlichia muris*-like pathogen, United States, 2007-2013(1). *Emer Infect Dis*. 2015;21:1794–1799.
12. Reeves WK, Loftis AD, Nicholson WL, et al. The first report of human illness associated with the Panola Mountain *Ehrlichia* species: a case report. *J Med Case Rep*. 2008;2:139.
13. Welinder-Olsson C, Kjellin E, Vaht K, et al. First case of human "*Candidatus* Neoehrlichia mikurensis" infection in a febrile patient with chronic lymphocytic leukemia. *J Clin Microbiol*. 2010;48:1956–1959.
14. Li H, Zheng YC, Ma L, et al. Human infection with a novel tick-borne *Anaplasma* species in China: a surveillance study. *Lancet Infect Dis*. 2015;15:663–670.
15. Chochlakis D, Ioannou I, Tselentis Y, et al. Human anaplasmosis and *Anaplasma ovis* variant. *Emerg Infect Dis*. 2010;16:1031–1032.
16. Anderson BE, Greene CE, Jones DC, et al. *Ehrlichia ewingii* sp. nov., the etiologic agent of canine granulocytic ehrlichiosis. *Int J Syst Bacteriol*. 1992;42:299–302.
17. Wen B, Rikihisa Y, Mott J, et al. *Ehrlichia muris* sp. nov., identified on the basis of 16S rRNA base sequences and serological, morphological, and biological characteristics. *Int J Syst Bacteriol*. 1995;45:250–254.
18. Pritt BS, Allerdice MEJ, Sloan LM, et al. Proposal to reclassify *Ehrlichia muris* as *Ehrlichia muris* subsp. *muris* subsp. nov. and description of *Ehrlichia muris* subsp. *eauclairensis* subsp. nov., a newly recognized tick-borne pathogen of humans. *Int J Syst Evol Microbiol*. 2017;67:2121–2126.
19. Kawahara M, Rikihisa Y, Isogai E, et al. Ultrastructure and phylogenetic analysis of '*Candidatus* Neoehrlichia mikurensis' in the family *Anaplasmataceae*, isolated from wild rats and found in *Ixodes ovatus* ticks. *Int J Syst Evol Microbiol*. 2004;54:1837–1843.
20. Yabsley MJ, Murphy SM, Luttrell MP, et al. Characterization of '*Candidatus* Neoehrlichia lotoris' (family Anaplasmataceae) from raccoons *(Procyon lotor)*. *Int J Syst Evol Microbiol*. 2008;58:2794–2798.
21. Schouls LM, Van De Pol I, Rijpkema SG, et al. Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. *J Clin Microbiol*. 1999;37:2215–2222.
22. Nutman TB. Insights into the pathogenesis of disease in human lymphatic filariasis. *Lymphat Res Biol*. 2013;11:144–148.
23. Popov VL, Han VC, Chen SM, et al. Ultrastructural differentiation of the genogroups in the genus *Ehrlichia*. *J Med Microbiol*. 1998;47:235–251.
24. Dunning Hotopp JC, Lin M, Madupu R, et al. Comparative genomics of emerging human ehrlichiosis agents. *PLoS Genet*. 2006;2:e21.
25. Ohashi N, Zhi N, Zhang Y, et al. Immunodominant major outer membrane proteins of *Ehrlichia chaffeensis* are encoded by a polymorphic multigene family. *Infect Immun*. 1998;66:132–139.
26. Yu X, McBride JW, Zhang X, et al. Characterization of the complete transcriptionally active *Ehrlichia chaffeensis* 28 kDa outer membrane protein multigene family. *Gene*. 2000;248:59–68.
27. Asanovich KM, Bakken JS, Madigan JE, et al. Antigenic diversity of granulocytic *Ehrlichia* isolates from humans in Wisconsin and New York and a horse in California. *J Infect Dis*. 1997;176:1029–1034.
28. IJdo JW, Wu C, Telford SR 3rd, et al. Differential expression of the p44 gene family in the agent of human granulocytic ehrlichiosis. *Infect Immun*. 2002;70:5295–5298.
29. Rejmanek D, Foley P, Barbet A, et al. Antigen variability in *Anaplasma phagocytophilum* during chronic infection of a reservoir host. *Microbiology*. 2012;158:2632–2641, PMC4083626.
30. Huang H, Wang X, Kikuchi T, et al. Porin activity of *Anaplasma phagocytophilum* outer membrane fraction and purified P44. *J Bacteriol*. 2007;189:1998–2006.
31. Zhang JZ, Guo H, Winslow GM, et al. Expression of members of the 28-kilodalton major outer membrane protein family of *Ehrlichia chaffeensis* during persistent infection. *Infect Immun*. 2004;72:4336–4343.
32. Singu V, Peddireddi L, Sirigireddy KR, et al. Unique macrophage and tick cell-specific protein expression from the p28/p30-outer membrane protein multigene locus in *Ehrlichia chaffeensis* and *Ehrlichia canis*. *Cell Microbiol*. 2006;8:1475–1487.
33. Liddell AM, Sumner JW, Paddock CD, et al. Reinfection with *Ehrlichia chaffeensis* in a liver transplant recipient. *Clin Infect Dis*. 2002;34:1644–1647.
34. Horowitz HW, Aguero-Rosenfeld M, Dumler JS, et al. Reinfection with the agent of human granulocytic ehrlichiosis. *Ann Intern Med*. 1998;129:461–463.
35. Caspersen K, Park JH, Patil S, et al. Genetic variability and stability of *Anaplasma phagocytophila msp2* (*p44*). *Infect Immun*. 2002;70:1230–1234.
36. Zhi N, Ohashi N, Rikihisa Y. Multiple p44 genes encoding major outer membrane proteins are expressed in the human granulocytic ehrlichiosis agent. *J Biol Chem*. 1999;274.
37. Barbet AF, Lundgren AM, Alleman AR, et al. Structure of the expression site reveals global diversity in MSP2 (P44) variants in *Anaplasma phagocytophilum*. *Infect Immun*. 2006;74:6429–6437.
38. Scorpio DG, Leutenegger C, Berger J, et al. Sequential analysis of *Anaplasma phagocytophilum msp2* transcription in murine and equine models of human granulocytic anaplasmosis. *Clin Vaccine Immunol*. 2008;15:418–424.
39. Wakeel A, den Dulk-Ras A, Hooykaas PJ, et al. *Ehrlichia chaffeensis* tandem repeat proteins and Ank200 are type 1 secretion system substrates related to the repeats-in-toxin exoprotein family. *Front Cell Infect Microbiol*. 2011;1:22.
40. McBride JW, Zhang X, Wakeel A, et al. Tyrosine-phosphorylated *Ehrlichia chaffeensis* and *Ehrlichia canis* tandem repeat orthologs contain a major continuous cross-reactive antibody epitope in lysine-rich repeats. *Infect Immun*. 2011;79:3178–3187.
41. Popov VL, Yu X, Walker DH. The 120 kDa outer membrane protein of *Ehrlichia chaffeensis*: preferential expression on dense-core cells and gene expression in *Escherichia coli* associated with attachment and entry. *Microb Pathog*. 2000;28:71–80.
42. Cheng Z, Miura K, Popov VL, et al. Insights into the CtrA regulon in development of stress resistance in obligatory intracellular pathogen *Ehrlichia chaffeensis*. *Mol Microbiol*. 2011;82:1217–1234.
43. Luo T, Kuriakose JA, Zhu B, et al. *Ehrlichia chaffeensis* TRP120 interacts with a diverse array of eukaryotic proteins involved in transcription, signaling, and cytoskeleton organization. *Infect Immun*. 2011;79:4382–4391.
44. Zhu B, Kuriakose JA, Luo T, et al. *Ehrlichia chaffeensis* TRP120 binds a G+C-rich motif in host cell DNA and exhibits eukaryotic transcriptional activator function. *Infect Immun*. 2011;79:4370–4381.
45. Lina TT, Farris T, Luo T, et al. Hacker within! *Ehrlichia chaffeensis* effector driven phagocyte reprogramming strategy. *Front Cell Infect Microbiol*. 2016;6:58.
46. Lin M, den Dulk-Ras A, Hooykaas PJ, et al. *Anaplasma phagocytophilum* AnkA secreted by type IV secretion system is tyrosine phosphorylated by Abl-1 to facilitate infection. *Cell Microbiol*. 2007;9:2644–2657.
47. Garcia-Garcia JC, Rennoll-Bankert KE, Pelly S, et al. Silencing of host cell *CYBB* gene expression by the nuclear effector AnkA of the intracellular pathogen *Anaplasma phagocytophilum*. *Infect Immun*. 2009;77:2385–2391.
48. Dumler JS, Sinclair SH, Pappas-Brown V, et al. Genome-wide *Anaplasma phagocytophilum* AnkA-DNA interactions are enriched in intergenic regions and gene promoters and correlate with infection-induced differential gene expression. *Front Cell Infect Microbiol*. 2016;6:97.
49. Rennoll-Bankert KE, Garcia-Garcia JC, Sinclair SH, et al. Chromatin-bound bacterial effector ankyrin A recruits histone deacetylase 1 and modifies host gene expression. *Cell Microbiol*. 2015;17:1640–1652.
50. Garcia-Garcia JC, Barat NC, Trembley SJ, et al. Epigenetic silencing of host cell defense genes enhances intracellular survival of the rickettsial pathogen *Anaplasma phagocytophilum*. *PLoS Pathog*. 2009;5:e1000488.
51. Cheng Z, Kumagai Y, Lin M, et al. Intra-leukocyte expression of two-component systems in *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* and effects of the histidine kinase inhibitor closantel. *Cell Microbiol*. 2006;8:1241–1252.
52. Nichols Heitman K, Dahlgren FS, Drexler NA, et al. Increasing incidence of ehrlichiosis in the United States: a summary of national surveillance of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* infections in the United States, 2008-2012. *Am J Trop Med Hyg*. 2016;94:52–60.
53. Long SW, Zhang X, Zhang J, et al. Evaluation of transovarial transmission and transmissibility of *Ehrlichia chaffeensis* (Rickettsiales: Anaplasmataceae) in *Amblyomma americanum* (Acari: Ixodidae). *J Med Entomol*. 2003;40:1000–1004.
54. Calic SB, Galvao MA, Bacellar F, et al. Human ehrlichioses in Brazil: first suspect cases. *Braz J Infect Dis*. 2004;8:259–262.
55. Heo EJ, Park JH, Koo JR, et al. Serologic and molecular detection of *Ehrlichia chaffeensis* and *Anaplasma phagocytophila* (human granulocytic ehrlichiosis agent) in Korean patients. *J Clin Microbiol*. 2002;40:3082–3085.
56. Brouqui P, Le Cam C, Kelly PJ, et al. Serologic evidence for human ehrlichiosis in Africa. *Eur J Epidemiol*. 1994;10:695–698.
57. Ndip LM, Ndip RN, Ndive VE, et al. *Ehrlichia* species in *Rhipicephalus sanguineus* ticks in Cameroon. *Vector Borne Zoonotic Dis*. 2007;7:221–227.
58. Machado RZ, Duarte JM, Dagnone AS, et al. Detection of *Ehrlichia chaffeensis* in Brazilian marsh deer *(Blastocerus dichotomus)*. *Vet Parasitol*. 2006;139:262–266.
59. Olano JP, Masters E, Hogrefe W, et al. Human monocytotropic ehrlichiosis, Missouri. *Emerg Infect Dis*. 2003;9:1579–1586.
60. Standaert SM, Dawson JE, Schaffner W, et al. Ehrlichiosis in a golf-oriented retirement community. *N Engl J Med*. 1995;333:420–425.
61. McKechnie DB, Slater KS, Childs JE, et al. Survival of *Ehrlichia chaffeensis* in refrigerated, ADSOL-treated RBCs. *Transfusion*. 2000;40:1041–1047.
62. Regan J, Matthias J, Green-Murphy A, et al. A confirmed *Ehrlichia ewingii* infection likely acquired through platelet transfusion. *Clin Infect Dis*. 2013;56:e105–e107.
63. Welc-Faleciak R, Sinski E, Kowalec M, et al. Asymptomatic "*Candidatus* Neoehrlichia mikurensis" infections in immunocompetent humans. *J Clin Microbiol*. 2014;52:3072–3074.
64. Sachdev SH, Joshi V, Cox ER, et al. Severe life-threatening *Ehrlichia chaffeensis* infections transmitted through solid organ transplantation. *Transpl Infect Dis*. 2014;16:119–124.
65. Dahlgren FS, Heitman KN, Drexler NA, et al. Human granulocytic anaplasmosis in the United States from 2008 to 2012: a summary of national surveillance data. *Am J Trop Med Hyg*. 2015;93:66–72.
66. Aguero-Rosenfeld ME, Horowitz HW, Wormser GP, et al. Human granulocytic ehrlichiosis: a case series from a medical center in New York State. *Ann Intern Med*. 1996;125:904–908.
67. Bakken JS, Dumler JS. Human granulocytic anaplasmosis. *Infect Dis Clin North Am*. 2015;29:341–355.
68. Blanco JR, Oteo JA. Human granulocytic ehrlichiosis in Europe. *Clin Microbiol Infect*. 2002;8:763–772.
69. Zhang L, Wang G, Liu Q, et al. Molecular analysis of *Anaplasma phagocytophilum* isolated from patients with febrile diseases of unknown etiology in China. *PLoS ONE*. 2013;8:e57155.
70. Strle F. Human granulocytic ehrlichiosis in Europe. *Int J Med Microbiol*. 2004;293(suppl 37):27–35.
71. Ohashi N, et al. Human granulocytic anaplasmosis, Japan. *Emerg Infect Dis*. 2013;19:289–292.
72. Bakken JS, Krueth J, Wilson-Nordskog C, et al. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. *JAMA*. 1996;275:199–205.
73. IJdo JW, Meek JI, Cartter ML, et al. The emergence of another tickborne infection in the 12-town area around Lyme, Connecticut: human granulocytic ehrlichiosis. *J Infect Dis*. 2000;181:1388–1393.
74. Bunnell JE, Magnarelli LA, Dumler JS. Infection of laboratory mice with the human granulocytic ehrlichiosis

agent does not induce antibodies to diagnostically significant *Borrelia burgdorferi* antigens. *J Clin Microbiol*. 1999;37:2077–2079.

75. Dumler JS, Dotevall L, Gustafson R, et al. A population-based seroepidemiologic study of human granulocytic ehrlichiosis and Lyme borreliosis on the west coast of Sweden. *J Infect Dis*. 1997;175:720–722.
76. Bakken JS, Goellner P, Van Etten M, et al. Seroprevalence of human granulocytic ehrlichiosis among permanent residents of northwestern Wisconsin. *Clin Infect Dis*. 1998;27:1491–1496.
77. Graf PC, Chretien JP, Ung L, et al. Prevalence of seropositivity to spotted fever group rickettsiae and *Anaplasma phagocytophilum* in a large, demographically diverse US sample. *Clin Infect Dis*. 2008;46:70–77.
78. Aguero-Rosenfeld ME, Donnarumma L, Zentmaier L, et al. Seroprevalence of antibodies that react with *Anaplasma phagocytophila*, the agent of human granulocytic ehrlichiosis, in different populations in Westchester County, New York. *J Clin Microbiol*. 2002;40:2612–2615.
79. Henningsson AJ, Wilhelmsson P, Gyllemark P, et al. Low risk of seroconversion or clinical disease in humans after a bite by an *Anaplasma phagocytophilum*-infected tick. *Ticks Tick Borne Dis*. 2015;6:787–792.
80. Petrovec M, Lotric Furlan S, Zupanc TA, et al. Human disease in Europe caused by a granulocytic *Ehrlichia* species. *J Clin Microbiol*. 1997;35:1556–1559.
81. Shields K, Cumming M, Rios J, et al. Transfusion-associated *Anaplasma phagocytophilum* infection in a pregnant patient with thalassemia trait: a case report. *Transfusion*. 2015;55:719–725.
82. Jereb M, Pecaver B, Tomazic J, et al. Severe human granulocytic anaplasmosis transmitted by blood transfusion. *Emerg Infect Dis*. 2012;18:1354–1357.
83. Townsend RL, Moritz ED, Fialkow LB, et al. Probable transfusion-transmission of *Anaplasma phagocytophilum* by leukoreduced platelets. *Transfusion*. 2014;54:2828–2832.
84. Proctor MC, Leiby DA. Do leukoreduction filters passively reduce the transmission risk of human granulocytic anaplasmosis? *Transfusion*. 2015;55:1242–1248.
85. Centers for Disease Control and Prevention. *Anaplasma phagocytophilum* transmitted through blood transfusion–Minnesota, 2007. *MMWR Morb Mortal Wkly Rep*. 2008;57:1145–1148.
86. Annen K, Friedman K, Eshoa C, et al. Two cases of transfusion-transmitted *Anaplasma phagocytophilum*. *Am J Clin Pathol*. 2012;137:562–565.
87. Fine AB, Sweeney JD, Nixon CP, et al. Transfusion-transmitted anaplasmosis from a leukoreduced platelet pool. *Transfusion*. 2016;56:699–704.
88. Alhumaidan H, Westley B, Esteva C, et al. Transfusion-transmitted anaplasmosis from leukoreduced red blood cells. *Transfusion*. 2013;53:181–186.
89. Magnarelli LA, Dumler JS, Anderson JF, et al. Coexistence of antibodies to tick-borne pathogens of babesiosis, ehrlichiosis, and Lyme borreliosis in human sera. *J Clin Microbiol*. 1995;33:3054–3057.
90. Nieto NC, Foley JE. Meta-analysis of coinfection and coexposure with *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in humans, domestic animals, wildlife, and *Ixodes ricinus*-complex ticks. *Vector Borne Zoonotic Dis*. 2009;9:93–102.
91. Diuk-Wasser MA, Vannier E, Krause PJ. Coinfection by *Ixodes* tick-borne pathogens: ecological, epidemiological, and clinical consequences. *Trends Parasitol*. 2016;32:30–42.
92. Nadelman RB, Horowitz HW, Hsieh TC, et al. Simultaneous human granulocytic ehrlichiosis and Lyme borreliosis. *N Engl J Med*. 1997;337:27–30.
93. Steere AC, McHugh G, Suarez C, et al. Prospective study of coinfection in patients with erythema migrans. *Clin Infect Dis*. 2003;36:1078–1081.
94. Aliota MT, Dupuis AP 2nd, Wilczek MP, et al. The prevalence of zoonotic tick-borne pathogens in *Ixodes scapularis* collected in the Hudson Valley, New York State. *Vector Borne Zoonotic Dis*. 2014;14:245–250.
95. Telford SR 3rd, Dawson JE, Katavolos P, et al. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. *Proc Natl Acad Sci USA*. 1996;93:6209–6214.
96. Nicholson WL, Muir S, Sumner JW, et al. Serologic evidence of infection with *Ehrlichia* spp. in wild rodents (Muridae: Sigmodontinae) in the United States. *J Clin Microbiol*. 1998;36:695–700.
97. Liz JS, Sumner JW, Pfister K, et al. PCR detection and serological evidence of granulocytic ehrlichial infection in roe deer *(Capreolus capreolus)* and chamois *(Rupicapra rupicapra)*. *J Clin Microbiol*. 2002;40:892–897.
98. Magnarelli LA, Ijdo JW, Stafford KC 3rd, et al. Infections of granulocytic ehrlichiae and *Borrelia burgdorferi* in white-tailed deer in Connecticut. *J Wildl Dis*. 1999;35:266–274.
99. Levin ML, Fish D. Immunity reduces reservoir host competence of *Peromyscus leucopus* for *Ehrlichia phagocytophila*. *Infect Immun*. 2000;68:1514–1518.
100. Dumler JS, Dawson JE, Walker DH. Human ehrlichiosis: hematopathology and immunohistologic detection of *Ehrlichia chaffeensis*. *Hum Pathol*. 1993;24:391–396.
101. Dumler JS, Brouqui P, Aronson J, et al. Identification of *Ehrlichia* in human tissue. *N Engl J Med*. 1991;325:1109–1110.
102. Sehdev AE, Dumler JS. Hepatic pathology in human monocytic ehrlichiosis. *Ehrlichia chaffeensis* infection. *Am J Clin Pathol*. 2003;119:859–865.
103. Dunn BE, Monson TP, Dumler JS, et al. Identification of *Ehrlichia chaffeensis* morulae in cerebrospinal fluid mononuclear cells. *J Clin Microbiol*. 1992;30:2207–2210.
104. Paddock CD, Suchard DP, Grumbach KL, et al. Brief report: fatal seronegative ehrlichiosis in a patient with HIV infection. *N Engl J Med*. 1993;329:1164–1167.
105. Walker DH, Dumler JS. Human monocytic and granulocytic ehrlichioses. Discovery and diagnosis of emerging tick-borne infections and the critical role of the pathologist. *Arch Pathol Lab Med*. 1997;121:785–791.
106. Borjesson D, Macnamara K, Johns J, et al. *Anaplasma phagocytophilum* and *Ehrlichia muris* induce cytopenias and global defects in hematopoiesis. *Clin Microbiol Infect*. 2009;15(suppl 2):66–67.
107. Ismail N, Walker DH, Ghose P, et al. Immune mediators of protective and pathogenic immune responses in patients with mild and fatal human monocytotropic ehrlichiosis. *BMC Immunol*. 2012;13:26.
108. Ismail N, Crossley EC, Stevenson HL, et al. Relative importance of T-cell subsets in monocytotropic ehrlichiosis: a novel effector mechanism involved in *Ehrlichia*-induced immunopathology in murine ehrlichiosis. *Infect Immun*. 2007;75:4608–4620.
109. Dierberg KL, Dumler JS. Lymph node hemophagocytosis in rickettsial diseases: a pathogenetic role for CD8 T lymphocytes in human monocytic ehrlichiosis (HME). *BMC Infect Dis*. 2006;6:121.
110. Barnewall RE, Rikihisa Y. Abrogation of gamma interferon-induced inhibition of *Ehrlichia chaffeensis* infection in human monocytes with iron-transferrin. *Infect Immun*. 1994;62:4804–4810.
111. Winslow GM, Yager E, Shilo K, et al. Antibody-mediated elimination of the obligate intracellular bacterial pathogen *Ehrlichia chaffeensis* during active infection. *Infect Immun*. 2000;68:2187–2195.
112. Lee EH, Rikihisa Y. Protein kinase A-mediated inhibition of gamma interferon-induced tyrosine phosphorylation of Janus kinases and latent cytoplasmic transcription factors in human monocytes by *Ehrlichia chaffeensis*. *Infect Immun*. 1998;66:2514–2520.
113. Zhang JZ, McBride JW, Yu XJ. L-selectin and E-selectin expressed on monocytes mediating *Ehrlichia chaffeensis* attachment onto host cells. *FEMS Microbiol Lett*. 2003;227:303–309.
114. Lin M, Rikihisa Y. *Ehrlichia chaffeensis* downregulates surface Toll-like receptors 2/4, CD14 and transcription factors PU.1 and inhibits lipopolysaccharide activation of NF-kappa B, ERK 1/2 and p38 MAPK in host monocytes. *Cell Microbiol*. 2004;6:175–186.
115. Lepidi H, Bunnell JE, Martin ME, et al. Comparative pathology, and immunohistology associated with clinical illness after *Ehrlichia phagocytophila*-group infections. *Am J Trop Med Hyg*. 2000;62:29–37.
116. Granquist EG, Aleksandersen M, Bergstrom K, et al. A morphological and molecular study of *Anaplasma phagocytophilum* transmission events at the time of *Ixodes ricinus* tick bite. *Acta Vet Scand*. 2010;52:43.
117. Klein MB, Miller JS, Nelson CM, et al. Primary bone marrow progenitors of both granulocytic and monocytic lineages are susceptible to infection with the agent of human granulocytic ehrlichiosis. *J Infect Dis*. 1997;176:1405–1409.
118. Bunnell JE, Trigiani ER, Srinivas SR, et al. Development and distribution of pathologic lesions are related to immune status and tissue deposition of human granulocytic ehrlichiosis agent-infected cells in a murine model system. *J Infect Dis*. 1999;180:546–550.
119. Wang J, Dyachenko V, Munderloh UG, et al. Transmission of *Anaplasma phagocytophilum* from endothelial cells to peripheral granulocytes in vitro under shear flow conditions. *Med Microbiol Immunol*. 2015;204:593–603.
120. Goodman JL, Nelson CM, Klein MB, et al. Leukocyte infection by the granulocytic ehrlichiosis agent is linked to expression of a selectin ligand. *J Clin Invest*. 1999;103:407–412.
121. Truchan HK, Seidman D, Carlyon JA. Breaking in and grabbing a meal: *Anaplasma phagocytophilum* cellular invasion, nutrient acquisition, and promising tools for their study. *Microbes Infect*. 2013;15:1017–1025.
122. Niu H, Yamaguchi M, Rikihisa Y. Subversion of cellular autophagy by *Anaplasma phagocytophilum*. *Cell Microbiol*. 2008;10:593–605.
123. Banerjee R, Anguita J, Roos D, et al. Cutting edge: infection by the agent of human granulocytic ehrlichiosis prevents the respiratory burst by down-regulating gp91phox. *J Immunol*. 2000;164:3946–3949.
124. Carlyon JA, Chan WT, Galan J, et al. Repression of *rac2* mRNA expression by *Anaplasma phagocytophila* is essential to the inhibition of superoxide production and bacterial proliferation. *J Immunol*. 2002;169:7009–7018.
125. Garyu JW, Dumler JS. *Anaplasma phagocytophilum* infection reduces expression of phagocytosis-related receptors on neutrophils. *Ann N Y Acad Sci*. 2005;1063:416–419.
126. Garyu JW, Choi KS, Grab DJ, et al. Defective phagocytosis in *Anaplasma phagocytophilum*-infected neutrophils. *Infect Immun*. 2005;73:1187–1190.
127. Choi KS, Park JT, Dumler JS. *Anaplasma phagocytophilum* delay of neutrophil apoptosis through the p38 mitogen-activated protein kinase signal pathway. *Infect Immun*. 2005;73:8209–8218.
128. Choi KS, Garyu J, Park J, et al. Diminished adhesion of *Anaplasma phagocytophilum*-infected neutrophils to endothelial cells is associated with reduced expression of leukocyte surface selectin. *Infect Immun*. 2003;71:4586–4594.
129. Park J, Choi KS, Grab DJ, et al. Divergent interactions of *Ehrlichia chaffeensis*- and *Anaplasma phagocytophilum*-infected leukocytes with endothelial cell barriers. *Infect Immun*. 2003;71:6728–6733.
130. Ge Y, Yoshiie K, Kuribayashi F, et al. *Anaplasma phagocytophilum* inhibits human neutrophil apoptosis via upregulation of bfl-1, maintenance of mitochondrial membrane potential and prevention of caspase 3 activation. *Cell Microbiol*. 2005;7:29–38.
131. Schaff UY, Trott KA, Chase S, et al. Neutrophils exposed to *A. phagocytophilum* under shear stress fail to fully activate, polarize, and transmigrate across inflamed endothelium. *Am J Physiol Cell Physiol*. 2010;299:C87–C96.
132. Choi KS, Grab DJ, Dumler JS. *Anaplasma phagocytophilum* infection induces protracted neutrophil degranulation. *Infect Immun*. 2004;72:3680–3683.
133. Borjesson DL, Kobayashi SD, Whitney AR, et al. Insights into pathogen immune evasion mechanisms: *Anaplasma phagocytophilum* fails to induce an apoptosis differentiation program in human neutrophils. *J Immunol*. 2005;174:6364–6372.
134. Choi KS, Scorpio DG, Dumler JS. Stat1 negatively regulates immune-mediated injury with Anaplasma phagocytophilum infection. *J Immunol*. 2014;193:5088–5098.
135. Choi KS, Scorpio DG, Dumler JS. *Anaplasma phagocytophilum* ligation to toll-like receptor (TLR) 2, but not to TLR4, activates macrophages for nuclear factor-kappa B nuclear translocation. *J Infect Dis*. 2004;189:1921–1925.
136. Wang X, Shaw DK, Hammond HL, et al. The prostaglandin E2-EP3 receptor axis regulates *Anaplasma phagocytophilum*-mediated NLRC4 inflammasome activation. *PLoS Pathog*. 2016;12:e1005803.
137. Scorpio DG, Akkoyunlu M, Fikrig E, et al. CXCR2 blockade influences *Anaplasma phagocytophilum* propagation but not histopathology in the mouse model of human granulocytic anaplasmosis. *Clin Diagn Lab Immunol*. 2004;11:963–968.
138. Akkoyunlu M, Malawista SE, Anguita J, et al. Exploitation of interleukin-8-induced neutrophil chemotaxis by the agent of human granulocytic ehrlichiosis. *Infect Immun*. 2001;69:5577–5588.
139. Martin ME, Caspersen K, Dumler JS. Immunopathology and ehrlichial propagation are regulated by interferon-gamma and interleukin-10 in a murine model of human granulocytic ehrlichiosis. *Am J Pathol*. 2001;158:1881–1888.
140. Davies RS, Madigan JE, Hodzic E, et al. Dexamethasone-induced cytokine changes associated with diminished disease severity in horses infected with *Anaplasma phagocytophilum*. *Clin Vaccine Immunol*. 2011;18:1962–1968.
141. Scorpio DG, von Loewenich FD, Gobel H, et al. Innate immune response to *Anaplasma phagocytophilum* contributes to hepatic injury. *Clin Vaccine Immunol*. 2006;13:806–809.
142. Choi KS, Webb T, Oelke M, et al. Differential innate immune cell activation and proinflammatory response in *Anaplasma phagocytophilum* infection. *Infect Immun*. 2007;75:3124–3130.

143. Dumler JS. The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. *FEMS Immunol Med Microbiol*. 2012;64:13–20, 3256277.
144. Dumler JS, Barat NC, Barat CE, et al. Human granulocytic anaplasmosis and macrophage activation. *Clin Infect Dis*. 2007;45:199–204.
145. Birkner K, Steiner B, Rinkler C, et al. The elimination of *Anaplasma phagocytophilum* requires CD4+ T cells, but is independent of Th1 cytokines and a wide spectrum of effector mechanisms. *Eur J Immunol*. 2008;38:3395–3410.
146. Fishbein DB, Kemp A, Dawson JE, et al. Human ehrlichiosis: prospective active surveillance in febrile hospitalized patients. *J Infect Dis*. 1989;160:803–809.
147. Harkess JR, Ewing SA, Crutcher JM, et al. Human ehrlichiosis in Oklahoma. *J Infect Dis*. 1989;159:576–579.
148. Eng TR, Fishbein DB, Dawson JE, et al. Surveillance of human ehrlichiosis in the United States: 1988. *Ann N Y Acad Sci*. 1990;590:306–307.
149. Hamburg BJ, Storch GA, Micek ST, et al. The importance of early treatment with doxycycline in human ehrlichiosis. *Medicine (Baltimore)*. 2008;87:53–60.
150. Paddock CD, Folk SM, Shore GM, et al. Infections with *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in persons coinfected with human immunodeficiency virus. *Clin Infect Dis*. 2001;33:1586–1594.
151. Thomas LD, Hongo I, Bloch KC, et al. Human ehrlichiosis in transplant recipients. *Am J Transplant*. 2007;7:1641–1647.
152. Dumler JS, Madigan JE, Pusterla N, et al. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. *Clin Infect Dis*. 2007;45(suppl 1):S45–S51.
153. Fishbein DB, Dawson JE, Robinson LE. Human ehrlichiosis in the United States, 1985 to 1990. *Ann Intern Med*. 1994;120:736–743.
154. Schutze GE, Buckingham SC, Marshall GS, et al. Human monocytic ehrlichiosis in children. *Pediatr Infect Dis J*. 2007;26:475–479.
155. Kumar N, Goyal J, Goel A, et al. Macrophage activation syndrome secondary to human monocytic ehrlichiosis. *Indian J Hematol Blood Transfus*. 2014;30:145–147.
156. Abbott KC, Vukelja SJ, Smith CE, et al. Hemophagocytic syndrome: a cause of pancytopenia in human ehrlichiosis. *Am J Hematol*. 1991;38:230–234.
157. Burns S, Saylors R, Mian A. Hemophagocytic lymphohistiocytosis secondary to *Ehrlichia chaffeensis* infection: a case report. *J Pediatr Hematol Oncol*. 2010;32:e142–e143.
158. Cheng A, Williams F, Fortenberry J, et al. Use of extracorporeal support in hemophagocytic lymphohistiocytosis secondary to ehrlichiosis. *Pediatrics*. 2016;138.
159. Hanson D, Walter AW, Powell J. Ehrlichia-induced hemophagocytic lymphohistiocytosis in two children. *Pediatr Blood Cancer*. 2011;56:661–663.
160. Marty AM, Dumler JS, Imes G, et al. Ehrlichiosis mimicking thrombotic thrombocytopenic purpura. Case report and pathological correlation. *Hum Pathol*. 1995;26:920–925.
161. Stone JH, Dierberg K, Aram G, et al. Human monocytic ehrlichiosis. *JAMA*. 2004;292:2263–2270.
162. Dumler JS, Sutker WL, Walker DH. Persistent infection with *Ehrlichia chaffeensis*. *Clin Infect Dis*. 1993;17:903–905.
163. Carpenter CF, Gandhi TK, Kong LK, et al. The incidence of ehrlichial and rickettsial infection in patients with unexplained fever and recent history of tick bite in central North Carolina. *J Infect Dis*. 1999;180:900–903.
164. Standaert SM, Yu T, Scott MA, et al. Primary isolation of *Ehrlichia chaffeensis* from patients with febrile illnesses: clinical and molecular characteristics. *J Infect Dis*. 2000;181:1082–1088.
165. Childs JE, Sumner JW, Nicholson WL, et al. Outcome of diagnostic tests using samples from patients with culture-proven human monocytic ehrlichiosis: implications for surveillance. *J Clin Microbiol*. 1999;37:2997–3000.
166. Bell CA, Patel R. A real-time combined polymerase chain reaction assay for the rapid detection and differentiation of *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii*. *Diagn Microbiol Infect Dis*. 2005;53:301–306.
167. Dawson JE, Fishbein DB, Eng TR, et al. Diagnosis of human ehrlichiosis with the indirect fluorescent antibody test: kinetics and specificity. *J Infect Dis*. 1990;162:91–95.
168. Branger S, Rolain JM, Raoult D. Evaluation of antibiotic susceptibilities of *Ehrlichia canis*, *Ehrlichia chaffeensis*, and *Anaplasma phagocytophilum* by real-time PCR. *Antimicrob Agents Chemother*. 2004;48:4822–4828.
169. Brouqui P, Raoult D. In vitro antibiotic susceptibility of the newly recognized agent of ehrlichiosis in humans, *Ehrlichia chaffeensis*. *Antimicrob Agents Chemother*. 1992;36:2799–2803.
170. Arens MQ, Liddell AM, Buening G, et al. Detection of *Ehrlichia* spp. in the blood of wild white-tailed deer in Missouri by PCR assay and serologic analysis. *J Clin Microbiol*. 2003;41:1263–1265.
171. Yabsley MJ, Varela AS, Tate CM, et al. *Ehrlichia ewingii* infection in white-tailed deer *(Odocoileus virginianus)*. *Emerg Infect Dis*. 2002;8:668–671.
172. Kim CM, Yi YH, Yu DH, et al. Tick-borne rickettsial pathogens in ticks and small mammals in Korea. *Appl Environ Microbiol*. 2006;72:5766–5776.
173. Ndip LM, Ndip RN, Esemu SN, et al. Ehrlichial infection in Cameroonian canines by *Ehrlichia canis* and *Ehrlichia ewingii*. *Vet Microbiol*. 2005;111:59–66.
174. O'Connor TP, Saucier JM, Daniluk D, et al. Evaluation of peptide- and recombinant protein-based assays for detection of anti-*Ehrlichia ewingii* antibodies in experimentally and naturally infected dogs. [corrected]. *Am J Vet Res*. 2010;71:1195–1200.
175. Kawahara M, Ito T, Suto C, et al. Comparison of *Ehrlichia muris* strains isolated from wild mice and ticks and serologic survey of humans and animals with *E. muris* as antigen. *J Clin Microbiol*. 1999;37:1123–1129.
176. Ravyn MD, Korenberg EI, Oeding JA, et al. Monocytic *Ehrlichia* in *Ixodes persulcatus* ticks from Perm, Russia. *Lancet*. 1999;353:722–723.
177. Wallace BJ, Brady G, Ackman DM, et al. Human granulocytic ehrlichiosis in New York. *Arch Intern Med*. 1998;158:769–773.
178. Lotric-Furlan S, Petrovec M, Avsic-Zupanc T, et al. Comparison of patients fulfilling criteria for confirmed and probable human granulocytic ehrlichiosis. *Scand J Infect Dis*. 2004;36:817–822.
179. Lotric-Furlan S, Rojko T, Petrovec M, et al. Epidemiological, clinical and laboratory characteristics of patients with human granulocytic anaplasmosis in Slovenia. *Wien Klin Wochenschr*. 2006;118:708–713.
180. Bakken JS, Dumler S. Human granulocytic anaplasmosis. *Infect Dis Clin North Am*. 2008;22:433–448.
181. Weil AA, Baron EL, Brown CM, et al. Clinical findings and diagnosis in human granulocytic anaplasmosis: a case series from Massachusetts. *Mayo Clin Proc*. 2012;87:233–239, PMC3498394.
182. Li H, Zhou Y, Wang W, et al. The clinical characteristics and outcomes of patients with human granulocytic anaplasmosis in China. *Int J Infect Dis*. 2011;15: e859–e866.
183. Wormser GP, Aguero-Rosenfeld ME, Cox ME, et al. Differences and similarities between culture-confirmed human granulocytic anaplasmosis and early Lyme disease. *J Clin Microbiol*. 2013;51:954–958.
184. Lotric-Furlan S, Rojko T, Jelovsek M, et al. Comparison of clinical and laboratory characteristics of patients fulfilling criteria for proven and probable human granulocytic anaplasmosis. *Microbes Infect*. 2015;17: 829–833.
185. Jiao XY, Fan ZC, Li YZ, et al. Clinical and laboratory features parameters of human granulocytic anaplasmosis (HGA) in patients admitted to hospital in Guangdong Province, China. *Trop Doct*. 2015;45:209–213.
186. Schotthoefer AM, Schrodi SJ, Meece JK, et al. Pro-inflammatory immune responses are associated with clinical signs and symptoms of human anaplasmosis. *PLoS ONE*. 2017;12:e0179655.
187. Horowitz HW, Aguero-Rosenfeld ME, McKenna DF, et al. Clinical and laboratory spectrum of culture-proven human granulocytic ehrlichiosis: comparison with culture-negative cases. *Clin Infect Dis*. 1998;27: 1314–1317.
188. Horowitz HW, Marks SJ, Weintraub M, et al. Brachial plexopathy associated with human granulocytic ehrlichiosis. *Neurology*. 1996;46:1026–1029.
189. Bakken JS, Erlemeyer SA, Kanoff RJ, et al. Demyelinating polyneuropathy associated with human granulocytic ehrlichiosis. *Clin Infect Dis*. 1998;27:1323–1324.
190. Lee FS, Chu FK, Tackley M, et al. Human granulocytic ehrlichiosis presenting as facial diplegia in a 42-year-old woman. *Clin Infect Dis*. 2000;31:1288–1291.
191. Liu Y, Yu XJ. Human granulocytic anaplasmosis and Lyme Disease–Reply. *JAMA*. 2016;316:99.
192. Zhang L, Liu Y, Ni D, et al. Nosocomial transmission of human granulocytic anaplasmosis in China. *JAMA*. 2008;300:2263–2270.
193. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. *N Engl J Med*. 2011;364:1523–1532.
194. Lotric-Furlan S, Petrovec M, Avsic-Zupanc T, et al. Epidemiological, clinical and laboratory distinction between human granulocytic ehrlichiosis and the initial phase of tick-borne encephalitis. *Wien Klin Wochenschr*. 2002;114:636–640.
195. Bakken JS, Aguero-Rosenfeld ME, Tilden RL, et al. Serial measurements of hematologic counts during the active phase of human granulocytic ehrlichiosis. *Clin Infect Dis*. 2001;32:862–870.
196. Walls JJ, Aguero-Rosenfeld M, Bakken JS, et al. Inter- and intralaboratory comparison of *Ehrlichia equi* and human granulocytic ehrlichiosis (HGE) agent strains for serodiagnosis of HGE by the immunofluorescent-antibody test. *J Clin Microbiol*. 1999;37:2968–2973.
197. Bakken JS, Haller I, Riddell D, et al. The serological response of patients infected with the agent of human granulocytic ehrlichiosis. *Clin Infect Dis*. 2002;34:22–27.
198. Maurin M, Bakken JS, Dumler JS. Antibiotic susceptibilities of *Anaplasma* (*Ehrlichia*) *phagocytophilum* strains from various geographic areas in the United States. *Antimicrob Agents Chemother*. 2003;47:413–415.
199. Horowitz HW, Hsieh TC, Aguero-Rosenfeld ME, et al. Antimicrobial susceptibility of *Ehrlichia phagocytophila*. *Antimicrob Agents Chemother*. 2001;45:786–788.
200. Buitrago MI, Ijdo JW, Rinaudo P, et al. Human granulocytic ehrlichiosis during pregnancy treated successfully with rifampin. *Clin Infect Dis*. 1998;27: 213–215.
201. Krause PJ, Corrow CL, Bakken JS. Successful treatment of human granulocytic ehrlichiosis in children using rifampin. *Pediatrics*. 2003;112:e252–e253.
202. Wormser GP, Filozov A, Telford SR 3rd, et al. Dissociation between inhibition and killing by levofloxacin in human granulocytic anaplasmosis. *Vector Borne Zoonotic Dis*. 2006;6:388–394.
203. Pekova S, Vydra J, Kabickova H, et al. *Candidatus* Neoehrlichia mikurensis infection identified in 2 hematooncologic patients: benefit of molecular techniques for rare pathogen detection. *Diagn Microbiol Infect Dis*. 2011;69:266–270.
204. Fehr JS, Bloemberg GV, Ritter C, et al. Septicemia caused by tick-borne bacterial pathogen *Candidatus* Neoehrlichia mikurensis. *Emerg Infect Dis*. 2010;16:1127–1129.
205. von Loewenich FD, Geissdorfer W, Disque C, et al. Detection of "*Candidatus* Neoehrlichia mikurensis" in two patients with severe febrile illnesses: evidence for a European sequence variant. *J Clin Microbiol*. 2010;48:2630–2635.
206. Li H, Jiang JF, Liu W, et al. Human infection with *Candidatus* Neoehrlichia mikurensis, China. *Emerg Infect Dis*. 2012;18:1636–1639.
207. Dadgar A, Grankvist A, Wernbro L, et al. Oklar feber hos patient med MS och rituximabbehandling var neoehrlichios - Ny fästingburen infektion som är svår att diagnostisera (Swedish). *Lakartidningen*. 2017;114.
208. Andreasson K, Jonsson G, Lindell P, et al. Recurrent fever caused by *Candidatus* Neoehrlichia mikurensis in a rheumatoid arthritis patient treated with rituximab. *Rheumatology*. 2015;54:369–371.
209. Grankvist A, Andersson PO, Mattsson M, et al. Infections with the tick-borne bacterium "*Candidatus* Neoehrlichia mikurensis" mimic noninfectious conditions in patients with B cell malignancies or autoimmune diseases. *Clin Infect Dis*. 2014;58:1716–1722.
210. Grankvist A, Sandelin LL, Andersson J, et al. Infections with *Candidatus* Neoehrlichia mikurensis and cytokine responses in 2 persons bitten by ticks, Sweden. *Emerg Infect Dis*. 2015;21:1462–1465.
211. Des Vignes F, Levin ML, Fish D. Comparative vector competence of *Dermacentor variabilis* and *Ixodes scapularis* (Acari: Ixodidae) for the agent of human granulocytic ehrlichiosis. *J Med Entomol*. 1999;36:182–185.
212. Tachibana N. Sennetsu fever: the disease, diagnosis, and treatment. In: Lieve L, ed. *Microbiology 1986*. Washington, D.C.: ASM Press; 1986:205–208.
213. Newton PN, Rolain JM, Rasachak B, et al. Sennetsu neorickettsiosis: a probable fish-borne cause of fever rediscovered in Laos. *Am J Trop Med Hyg*. 2009;81:190–194.
214. Bhengsri S, Baggett HC, Edouard S, et al. Sennetsu neorickettsiosis, Spotted Fever group, and Typhus group rickettsioses in three provinces in Thailand. *Am J Trop Med Hyg*. 2016;95:43–49.
215. Dittrich S, Phuklia W, Turner GD, et al. *Neorickettsia sennetsu* as a neglected cause of fever in South-East Asia. *PLoS Negl Trop Dis*. 2015;9:e0003908.
216. Misao T, Kobayashi Y. Studies on infectious mononucleosis (glandular fever). I. Isolation of etiologic agent from blood, bone marrow, and lymph node of a patient with infectious mononucleosis by using mice. *Kyushu J Med Sci*. 1955;6:145–152.
217. Olano JP, Hogrefe W, Seaton B, et al. Clinical manifestations, epidemiology, and laboratory diagnosis of human monocytotropic ehrlichiosis in a commercial laboratory setting. *Clin Diagn Lab Immunol*. 2003;10:891–896.
218. Eng TR, Harkess JR, Fishbein DB, et al. Epidemiologic, clinical, and laboratory findings of human ehrlichiosis in the United States, 1988. *JAMA*. 1990;264:2251–2258.

219. Lotric-Furlan S, Petrovec M, Avsic-Zupanc T, et al. Human granulocytic ehrlichiosis in Slovenia. *Ann N Y Acad Sci*. 2003;990:279–284.
220. Bjoersdorff A, Wittesjo B, Berglun J, et al. Human granulocytic ehrlichiosis as a common cause of tick-associated fever in Southeast Sweden: report from a prospective clinical study. *Scand J Infect Dis*. 2002;34:187–191.
221. Belongia EA, Gale CM, Reed KD, et al. Population-based incidence of human granulocytic ehrlichiosis in northwestern Wisconsin, 1997-1999. *J Infect Dis*. 2001;184:1470–1474.
222. Belongia EA, Reed KD, Mitchell PD, et al. Clinical and epidemiological features of early Lyme disease and human granulocytic ehrlichiosis in Wisconsin. *Clin Infect Dis*. 1999;29:1472–1477.
223. Lotric-Furlan S, Petrovec M, Avsic-Zupanc T, et al. Concomitant tickborne encephalitis and human granulocytic ehrlichiosis. *Emerg Infect Dis*. 2005;11:485–488.
224. Bakken JS, Dumler JS. Clinical diagnosis and treatment of human granulocytotropic anaplasmosis. *Ann N Y Acad Sci*. 2006;1078:236–247.
225. Walder G, Fuchs D, Sarcletti M, et al. Human granulocytic anaplasmosis in Austria: epidemiological, clinical, and laboratory findings in five consecutive patients from Tyrol, Austria. *Int J Med Microbiol*. 2006;296(suppl 40):297–301.
226. Grzeszczuk A, Ziarko S, Kovalchuk O, et al. Etiology of tick-borne febrile illnesses in adult residents of North-Eastern Poland: report from a prospective clinical study. *Int J Med Microbiol*. 2006;296(suppl 40):242–249.
227. Koebel C, Kern A, Edouard S, et al. Human granulocytic anaplasmosis in eastern France: clinical presentation and laboratory diagnosis. *Diagn Microbiol Infect Dis*. 2012;72:214–218.
228. Yi J, Kim KH, Ko MK, et al. Human granulocytic anaplasmosis as a cause of febrile illness in Korea since at least 2006. *Am J Trop Med Hyg*. 2017;96:777–782.

F Bacterial Diseases

193 Introduction to Bacteria and Bacterial Diseases

Jennifer A. Philips and Martin J. Blaser

Bacteria, the oldest forms of life on earth, are remarkably diverse and exist in astounding numbers. Diseases caused by bacteria include some of the most common infections in the world and some of the most important human scourges, past, present, and probably future. At the same time, each of us is colonized by as many bacterial cells as we have human cells in our bodies. In general, this is a peaceful and even productive (symbiotic) relationship, but occasionally even these well-tolerated residents of the human biosphere cause disease.[1–3]

We are surrounded by and always exposed to bacteria, including those that evolved to live well with us (e.g., *Bacteroides* species) and those whose evolution has promoted the tendency to cause disease (e.g., *Mycobacterium tuberculosis*) and death (e.g., *Bacillus anthracis*). In consequence, and not surprisingly, many of the presently recognized infectious diseases are caused by bacteria. It also may be safely predicted that many important illnesses not yet recognized or widespread ("emerging" infectious diseases) will be found to be caused by bacteria,[4] as will some chronic inflammatory diseases of unknown cause and malignancies (see later). Therefore knowledge of pathogenic bacteria, the diseases to which they lead, and current preventive and therapeutic strategies are critical for all health care providers, especially specialists in infectious diseases. An emerging concept is that our residential bacteria, part of human physiology and protective against introduced pathogens, are changing, with important health consequences.[4–8]

CLASSIFICATION OF BACTERIA

Bacteria have been classified according to phenotype, including size, shape, staining properties, and biochemical properties, since the beginning of microbiology. In recent years classification has been dominated by genotype, especially relying on conserved molecules, such as 16S ribosomal RNA.[9] Although there is a considerable degree of overlap between phenotype and genotype, as would be expected, dichotomies occur. In the future, taxonomy, understanding of pathogenesis, and diagnostics will be increasingly based on genotype and gene expression. Even in resource-limited settings, rapid genotypic identification of drug-resistant *M. tuberculosis* is close at hand.[9] Proteins expressed under a given condition can be determined by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, which generates proteomic mass patterns or fingerprints. As a method for identification of microorganisms, this is rapidly being integrated into the routine analytic pipeline of clinical microbiology laboratories. More advanced "proteotyping" will provide strain-level characterization, including virulence and antibiotic resistance factor expression.[10] Thus physicians and other students of infectious diseases must broaden their knowledge of molecular biology and taxonomy. As bacteriology advances in its differentiation of genera and species, as subspecies diversity is increasingly appreciated,[11] as variation within individual hosts is better understood, and as the evolution of pathogens is better outlined,[12,13] a grounding in evolutionary biology and ecology also will become more critical. The recent National Institutes of Health Human Microbiome Project already has provided a new scientific basis for the biomedical community toward understanding strain and species variation among the indigenous organisms in human hosts.[14] Over time, this information will be translated into clinical advances in prevention, diagnosis, and treatment of infectious diseases; studies of the human microbiome are already suggesting bacterial roles in diseases that were not previously suspected to have a microbial cause.[2–4]

VARIATION IN BACTERIAL INFECTIONS

Because all organs of the body are subject to bacterial infection, a recitation of these sites would be exhaustive and thus not useful. However, at the least, bacterial infections may be considered as varying in cause, mechanisms, and time frame. Infections may be caused by gram-positive or gram-negative bacilli or cocci; these were the first recognized bacterial agents of disease. However, this simple taxonomy does not fully account for other causative bacterial agents, including *Mycobacterium* species, treponemes, mycoplasmas, rickettsiae, chlamydiae, and actinomyces, all of which are Eubacteria. Each of these types of organisms has particular stereotypic features that characterize its interactions with hosts, but exceptions and variations abound. In recent years Archaea, a widespread and ancient group of prokaryotes, most closely resembling bacteria, has been isolated from human specimens[15]; the extent to which they play roles in human diseases is not yet known.

The mechanisms whereby bacteria cause disease are quite varied (Table 193.1). There is no universal mechanism or principle; the causative organism need not even be present in the human body. For example, food poisoning is commonly caused by the ingestion of preformed toxin produced by *Clostridium botulinum* or *Staphylococcus aureus* when they are growing in food, not in the host. The scope of bacterial infections includes interactions across time frames that vary from minutes to decades, or longer (Table 193.2). The descendants of the organisms that we each acquire from our mother[16] as a newborn can be the cause of our death in old age (e.g., caused by a perforated diverticulum) to carry the argument to the farthest extreme. Each bacterial infection is unique and reflects the underlying virulence properties of the bacteria and the predisposition of the host. For example, commensals or generally nonpathogenic environmental organisms can cause devastating disease in certain hosts because of genetic inborn errors[17,18]; acquired immunodeficiencies from diverse causes, such as human immunodeficiency virus, autoantibodies,[19] or immunomodulatory drugs; or from anatomic defects, such as perforated viscera, indwelling lines, or traumatic injuries. This complexity increases the difficulty in grasping the underlying concepts but also makes the practice of infectious diseases so intellectually satisfying.

POLYMORPHISM AND BACTERIAL INFECTION

From the earliest days of microbiology, when it was recognized that some organisms of the same species were encapsulated and many were not, it has been clear that pathogenic bacteria are polymorphic. With the development of antisera came the recognition that apparently identical organisms showed variation; this information became the basis for typing

schemes based on capsular, somatic O-antigen, or flagellar antigenic differences. Such polymorphisms have enabled better classification of virulent (and avirulent) meningococci, pneumococci, and *Escherichia coli*, for example, and have led to diagnostics, therapeutic antisera, and vaccines. However, in recent years has come the understanding that once bacteria begin to multiply in a host, their own populations become polymorphic.[20–21] Antigenic variation is one subtheme of that phenomenon and has been known since the studies of *Borrelia recurrentis*, the cause of relapsing fever. Increasingly, with the tools provided by the sequencing of whole bacterial genomes and single cell transcriptomics, we are learning just how polymorphic are bacteria, often considered as clonal organisms, and how dynamic their changes in relation to individual hosts. Even the most highly clonal bacteria, such as *M. tuberculosis*, exhibit extensive phenotypic variation, as well as genetic variation at particular loci, often those involving interaction with hosts.[21–22]

In parallel, we have been learning more about human genetic polymorphisms and their relationship to bacterial diseases. Medical science is rapidly advancing from phenotypes (e.g., blood groups) to genotypes (e.g., alleles of the interleukin-1β promoter). The genetic composition of each individual helps determine its response to bacterial infections and thus the outcome. Increasingly, these host characteristics will become the focus of the information that clinicians will need when considering prevention, differential diagnosis, and therapy of bacterial infections.[18,23]

TABLE 193.1 Disease Mechanisms Involved in Bacterial Infections

MECHANISM	EXAMPLES
Pyogenic infection	Pneumococcal pneumonia, staphylococcal abscess
Granulomatous infection	Pulmonary tuberculosis, brucellosis, syphilis
Intoxication (augmentation of host physiology)	Cholera *(Vibrio cholerae)*
Intoxication (tissue destruction)	Gas gangrene *(Clostridium perfringens)*, diphtheria *(Corynebacterium diphtheriae)*
Immunologic mediation	Guillain-Barré syndrome after *Campylobacter jejuni* infection; reactive arthritis after shigellosis; acute rheumatic fever after pharyngitis due to *Streptococcus pyogenes*
Neoplasia	Adenocarcinoma of the stomach as a consequence of *Helicobacter pylori* persistence; adenocarcinoma of the esophagus as a consequence of physiologic and microbiologic changes induced by absence of *Helicobacter pylori*

BACTERIA AS "NEW" CAUSES FOR "OLD" DISEASES

The finding that an indigenous bacterium, *Helicobacter pylori*, plays pathogenetic roles in two important illnesses, peptic ulcer disease and gastric cancer,[24] has advanced a new paradigm—that many of the diseases that we consider diseases of unknown cause may in fact be infectious diseases.[4] This idea, which gained prominence with the knowledge about the relationship of streptococcal pharyngitis to rheumatic fever, has been growing since the 1930s, and other examples have been recognized. For example, after an episode of enteritis caused by *Campylobacter jejuni*, the Guillain-Barré syndrome may develop.[25] Similarly, more than 30 years ago it was recognized that acute infection with enterohemorrhagic *E. coli* may lead to the hemolytic uremic syndrome.[26] Thus acute, transient, and self-limited infections of the respiratory or gastrointestinal tracts may trigger cardiac, neurologic, or systemic diseases, with consequences lasting months or permanently. Of importance, the disease locus (e.g., kidney, peripheral nervous system, heart) may be distant from the original infection at a mucosal surface. How many other examples of parallel phenomena may be present? The uncovering of clinical and epidemiologic associations has led to research identifying pathogenetic mechanisms that provide new paradigms for autoimmunity.[27] Could a more persistent bacterial pathogen trigger multiple sclerosis or Graves disease? Conversely, does exposure to diverse environmental microbes modulate development of the immune system and protect against atopic conditions?[28]

Diseases such as sarcoidosis, ulcerative colitis, Crohn disease, Wegener granulomatosis, and thyroiditis are chronic inflammatory diseases for which a bacterial role in causation is not improbable. In addition, there is a growing appreciation for the role of our microbiome and its metabolites in modulating human development, physiology, and immunity.[2] Our metagenome and metatranscriptome may become useful indices for interpreting human physiology.[29] Thus unnecessary use of antibiotics may have significant unforeseen immediate health consequences, including enhanced susceptibility to viral infections[30] and long-term sequelae. For example, antibiotic use in early life in both farm and experimental animals produces major shifts in microbiota characteristics, host developmental phenotypes, and adiposity.[31–33] Whether such precedents are applicable to human children is unknown, but it seems likely. New studies suggest connections between the bacteria we carry and seemingly unrelated diseases, such as diabetes and hypertension.[34–35]

BACTERIAL EVOLUTION

The study of infectious diseases is a dynamic field, at least in part because of the evolving nature of the pathogens we consider. An obvious and absolutely critical aspect of bacterial evolution is the acquisition of resistance to antimicrobial agents. As the prescribing of antimicrobial therapies flourishes—whether they address important, controversial,

TABLE 193.2 Variation in Time Courses for Representative Bacterial Infections

TIME FRAME	DISEASE	REPRESENTATIVE CAUSATIVE ORGANISM	CLINICAL MANIFESTATIONS	MECHANISMS
Minutes	Food poisoning	*Clostridium perfringens*	Vomiting, diarrhea	Preformed enterotoxin
Hours	Necrotizing fasciitis	*Streptococcus pyogenes*	Devitalization of muscle, sepsis	Bacterial spread across tissue planes
Days	Anthrax	*Bacillus anthracis*	Cough, chest pain, fever, dyspnea	Resistance to macrophage killing
Weeks	Lung abscess	Oral anaerobes	Cough, fever, chest pain	Necrotizing pyogenic process
Months	Subacute bacterial endocarditis	α-Hemolytic streptococci	Fever, anemia, stroke, heart failure, uremia	Infection of immunologically privileged site
Years	Whipple disease	*Tropheryma whipplei*	Fever, diarrhea, weight loss	Resistance to macrophage killing
Decades (persistence)	Osteomyelitis	*Staphylococcus aureus*	Fever, wound discharge, pain	Pyogenic infection of devitalized tissue (±foreign body)
Decades (latency)	Pulmonary tuberculosis	*Mycobacterium tuberculosis*	Cough, fever, weight loss	Reactivation of latent focus into active granulomatous process
Decades (oncogenesis)	Gastric cancer	*Helicobacter pylori*	Cachexia, abdominal pain	Persistent inflammation leading to progressive metaplastic and dysplastic conditions

or even trivial indications—antimicrobial resistance by pathogens and indigenous organisms continues to grow.[36–38] Understanding resistance patterns is pivotal to understanding proper therapeutic approaches.[36] Also important is that understanding the biology, epidemiology, and mechanisms for resistance will lead to the methods needed to prevent and curtail resistance in the populations of microbes that infect and colonize humans, which may provide reservoirs for resistance.[38,39] Physicians, especially those who are specialists in infectious diseases, must be at the forefront of efforts to reduce the development of resistance.

Of interest, considerations of resistance are useful for understanding other important aspects in the evolution of infectious diseases; we live in a world of natural selection. Resistance is among the easiest phenotypes to detect and understand, but bacteria continue to be selected on the basis of differences in the soaps used (also a function of resistance), sizes of families and other social groups, presence of daycare centers and jet planes, and changing dietary habits (functions of transmission).[40] As the connectivity of human populations increases, there may be greater selection for virulence.[13] We may already be witnessing progressively increasing virulence of *M. tuberculosis*,[22] in addition to its progressively increasing antibiotic resistance.[41] The spontaneous and widespread emergence of hypervirulent *Clostridioides difficile* (formerly *Clostridium difficile*) strains of different genotypes but similar phenotypes (hypertoxigenic because of deletions involving regulatory elements) indicates the power of microbes to adapt to strong selective forces.[42,43] Study of bacterial infections provides a rich school, not only for the health care provider but also for the student of human evolutionary biology.[22,44,45] The lessons learned will also be critical in clinical medicine and epidemiology.

BACTERIA AS THERAPEUTICS

Since Metchnikoff's time and earlier, physicians have sought ways to harness bacteria to fight disease. At present, the highly lethal exotoxin of *C. botulinum* is being used as a therapeutic agent for medical and cosmetic purposes. The bacillus Calmette-Guérin vaccine, an attenuated form of *Mycobacterium bovis*, is used as adjuvant therapy for bladder cancer. Although at first glance such harnessing of bacteria for useful purposes seems extraordinary, it actually makes great sense. The bacteria that live with us, or that attack us, often know us well; their evolution has selected for organisms that exploit chinks in our armor.[13,46–48] They are skilled cell biologists, immunologists, and physiologists and are great competitors with one another. Bacteria have potential usefulness as probiotics to treat disease.[49] Perhaps the best example is the least defined—the utility of fecal transplantation to cure recurrent *C. difficile* infection, which has become the standard of care.[50] This therapy is a proof of principle that using a bacterial mixture that normalizes the gut microbiome can cure an infectious disease. Defined probiotic strains and prebiotics, nutrients that provide a substrate for particular bacteria or biochemical processes, extend the concept of harnessing bacteria for treatment by another step.[51] Engineering bacteria to do our work will be a further development.[52] Understanding the clinical manifestations and pathogenesis of bacterial infections will lead to new approaches to medicine—new therapeutics[53] and new preventives.[7] Predictably, each of the new treatments will lead to new complications. A thorough grounding in knowledge about bacterial infections will enable physicians and researchers to develop these new therapeutic modalities and to predict and treat the expected complications.

Key References

The complete reference list is available online at Expert Consult.

1. Relman DA. The human body as microbial observatory. *Nat Genet*. 2002;30:131–133.
2. Costello EK, Stagaman K, Dethlefsen L, et al. The application of ecological theory toward an understanding of the human microbiome. *Science*. 2012;336:1255–1262.
3. Ballal SA, Gallini CA, Segata N, et al. Host and gut microbiota symbiotic factors: lessons from inflammatory bowel disease and successful symbionts. *Cell Microbiol*. 2011;13:508–517.
4. Blaser MJ. Bacteria and diseases of unknown cause. *Ann Intern Med*. 1994;121:144–145.
5. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486:207–214.
6. Chen Y, Blaser MJ. *Helicobacter pylori* colonization is inversely associated with childhood asthma. *J Infect Dis*. 2008;198:553–560.
7. Blaser MJ. Disappearing microbiota: *Helicobacter pylori* protection against esophageal adenocarcinoma. *Cancer Prev Res (Phila)*. 2008;1:308–311.
9. Xie YL, Chakravorty S, Armstrong DT, et al. Evaluation of a rapid molecular drug-susceptibility test for tuberculosis. *N Engl J Med*. 2017;377:1043–1054.
10. Karlsson R, Gonzales-Siles L, Boulund F, et al. Proteotyping: proteomic characterization, classification and identification of microorganisms–a prospectus. *Syst Appl Microbiol*. 2015;38:246–257.
11. Caldwell HD, Wood H, Crane D, et al. Polymorphisms in *Chlamydia trachomatis* tryptophan synthase genes differentiate between genital and ocular isolates. *J Clin Invest*. 2003;111:1757–1769.
12. Walder MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science*. 1996;272:1910–1914.
13. Blaser MJ, Kirschner D. The equilibria that permit bacterial persistence in human hosts. *Nature*. 2007;449:843–849.
14. Lloyd-Price J, Mahurkar A, Rahnavard G, et al. Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature*. 2017;550:61–66.
16. Palmer C, Bik EM, Digiulio DB, et al. Development of the human infant intestinal microbiota. *PLoS Biol*. 2007;5:e177.
17. Davila S, Wright VJ, Khor CC, et al. Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nat Genet*. 2010;42:772–776.
18. Casanova JL. Severe infectious diseases of childhood as monogenic inborn errors of immunity. *Proc Natl Acad Sci USA*. 2015;112:E7128–E7137.
19. Browne SK, Burbelo PD, Chetchotisakd P, et al. Adult-onset immunodeficiency in Thailand and Taiwan. *N Engl J Med*. 2012;367:725–734.
20. Avraham R, Haseley N, Brown D, et al. Pathogen cell-to-cell variability drives heterogeneity in host immune responses. *Cell*. 2015;162:1309–1321.
22. Brites D, Gagneux S. The nature and evolution of genomic diversity in the *Mycobacterium tuberculosis* complex. *Adv Exp Med Biol*. 2017;1019:1–26.
23. Cobat A, Barrera LF, Henao H, et al. Tuberculin skin test reactivity is dependent on host genetic background in Colombian tuberculosis household contacts. *Clin Infect Dis*. 2012;54:968.
24. Blaser MJ. The changing relationships of *Helicobacter pylori* and humans: implications for health and disease. *J Infect Dis*. 1999;179:1523–1530.
25. Rees JH, Soudain SE, Gregson NA, et al. *Campylobacter jejuni* infection and Guillain-Barré syndrome. *N Engl J Med*. 1995;333:1374–1379.
26. Karmali MA, Petric MA, Lim C, et al. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis*. 1985;151:775–782.
27. Ang CW, Noordzij PG, de Klerk MA, et al. Ganglioside mimicry of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity in rabbits. *Infect Immun*. 2002;70:5081–5085.
28. Ege MJ, Mayer M, Normand AC, et al. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med*. 2011;364:701–709.
29. Franzosa EA, Huang K, Meadow JF, et al. Identifying personal microbiomes using metagenomic codes. *Proc Natl Acad Sci USA*. 2015;112:E2930–E2938.
31. Jukes TH. Antibiotics in feeds. *Science*. 1979;204:8.
32. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature*. 2012;488:621–626.
33. Wang Y, Kuang Z, Yu X, et al. The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. *Science*. 2017;357:912–916.
34. Suez J, Korem T, Zeevi D, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*. 2014;514:181–186.
35. Wilck N, Matus MG, Kearney SM, et al. Salt-responsive gut commensal modulates TH17 axis and disease. *Nature*. 2017;551:585–589.
36. Lonks JR, Garau J, Gomez L, et al. Failure of macrolide antibiotic treatment in patients with bacteremia due to erythromycin resistant *Streptococcus pneumoniae*. *Clin Infect Dis*. 2002;35:556–564.
37. Fenton KA, Ison C, Johnson AP, et al. Ciprofloxacin resistance in *Neisseria gonorrhoeae* in England and Wales in 2002. *Lancet*. 2003;361:1867–1868.
38. Sjölund M, Andersson DI, Blaser MJ, et al. Long-term persistence of resistant *Enterococcus* species after antibiotic treatment to eliminate *Helicobacter pylori*. *Ann Intern Med*. 2003;139:483–487.
39. Sjölund M, Tano E, Blaser MJ, et al. Persistence of resistant *Staphylococcus epidermidis* after single course of clarithromycin. *Emerg Infect Dis*. 2005;11:1389–1393.
41. Gandhi NR, Nunn P, Dheda K, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet*. 2010;375:1830–1843.
42. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med*. 2005;353:2433–2441.
43. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med*. 2005;353:2442–2449.
45. Linz B, Balloux F, Moodley Y, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature*. 2007;445:915–918.
46. Collin M, Olsen A. Extracellular enzymes with immunomodulating activities: variations on a theme in *Streptococcus pyogenes*. *Infect Immun*. 2003;71:2983–2992.
48. Mehra A, Zahra A, Thompson V, et al. Mycobacterium tuberculosis Type VII Secreted Effector EsxH Targets Host ESCRT to Impair Trafficking. *PLoS Pathog*. 2013;9:e1003734.
49. Kalliomaki M, Salminen S, Poussa T, et al. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*. 2003;361:1869–1870.
50. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med*. 2013;368:407–415.
52. Lim B, Zimmerman M, Barry NA, et al. Engineered regulatory systems modulate gene expression of human commensals in the gut. *Cell*. 2017;169:547–558.
53. Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLoS ONE*. 2012;7:e34938.

References

1. Relman DA. The human body as microbial observatory. *Nat Genet*. 2002;30:131–133.
2. Costello EK, Stagaman K, Dethlefsen L, et al. The application of ecological theory toward an understanding of the human microbiome. *Science*. 2012;336:1255–1262.
3. Ballal SA, Gallini CA, Segata N, et al. Host and gut microbiota symbiotic factors: lessons from inflammatory bowel disease and successful symbionts. *Cell Microbiol*. 2011;13:508–517.
4. Blaser MJ. Bacteria and diseases of unknown cause. *Ann Intern Med*. 1994;121:144–145.
5. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486:207–214.
6. Chen Y, Blaser MJ. *Helicobacter pylori* colonization is inversely associated with childhood asthma. *J Infect Dis*. 2008;198:553–560.
7. Blaser MJ. Disappearing microbiota: *Helicobacter pylori* protection against esophageal adenocarcinoma. *Cancer Prev Res (Phila)*. 2008;1:308–311.
8. Woese C. Microbiology in transition. *Proc Natl Acad Sci USA*. 1994;91:1601–1603.
9. Xie YL, Chakravorty S, Armstrong DT, et al. Evaluation of a rapid molecular drug-susceptibility test for tuberculosis. *N Engl J Med*. 2017;377:1043–1054.
10. Karlsson R, Gonzales-Siles L, Boulund F, et al. Proteotyping: proteomic characterization, classification and identification of microorganisms–a prospectus. *Syst Appl Microbiol*. 2015;38:246–257.
11. Caldwell HD, Wood H, Crane D, et al. Polymorphisms in *Chlamydia trachomatis* tryptophan synthase genes differentiate between genital and ocular isolates. *J Clin Invest*. 2003;111:1757–1769.
12. Walder MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science*. 1996;272:1910–1914.
13. Blaser MJ, Kirschner D. The equilibria that permit bacterial persistence in human hosts. *Nature*. 2007;449:843–849.
14. Lloyd-Price J, Mahurkar A, Rahnavard G, et al. Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature*. 2017;550:61–66.
15. Koskinen K, Pausan MR, Perras AK, et al. First insights into the diverse human Archaeome: specific detection of Archaea in the gastrointestinal tract, lung, and nose and on skin. *MBio*. 2017;8:e00824–17.
16. Palmer C, Bik EM, Digiulio DB, et al. Development of the human infant intestinal microbiota. *PLoS Biol*. 2007;5:e177.
17. Davila S, Wright VJ, Khor CC, et al. Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nat Genet*. 2010;42:772–776.
18. Casanova JL. Severe infectious diseases of childhood as monogenic inborn errors of immunity. *Proc Natl Acad Sci USA*. 2015;112:E7128–E7137.
19. Browne SK, Burbelo PD, Chetchotisakd P, et al. Adult-onset immunodeficiency in Thailand and Taiwan. *N Engl J Med*. 2012;367:725–734.
20. Avraham R, Haseley N, Brown D, et al. Pathogen cell-to-cell variability drives heterogeneity in host immune responses. *Cell*. 2015;162:1309–1321.
21. Martin CJ, Cadena AM, Leung VW, et al. Digitally barcoding *Mycobacterium tuberculosis* reveals in vivo infection dynamics in the macaque model of tuberculosis. *MBio*. 2017;8:e312–e317.
22. Brites D, Gagneux S. The nature and evolution of genomic diversity in the *Mycobacterium tuberculosis* complex. *Adv Exp Med Biol*. 2017;1019:1–26.
23. Cobat A, Barrera LF, Henao H, et al. Tuberculin skin test reactivity is dependent on host genetic background in Colombian tuberculosis household contacts. *Clin Infect Dis*. 2012;54:968.
24. Blaser MJ. The changing relationships of *Helicobacter pylori* and humans: implications for health and disease. *J Infect Dis*. 1999;179:1523–1530.
25. Rees JH, Soudain SE, Gregson NA, et al. *Campylobacter jejuni* infection and Guillain-Barré syndrome. *N Engl J Med*. 1995;333:1374–1379.
26. Karmali MA, Petric MA, Lim C, et al. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis*. 1985;151:775–782.
27. Ang CW, Noordzij PG, de Klerk MA, et al. Ganglioside mimicry of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity in rabbits. *Infect Immun*. 2002;70:5081–5085.
28. Ege MJ, Mayer M, Normand AC, et al. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med*. 2011;364:701–709.
29. Franzosa EA, Huang K, Meadow JF, et al. Identifying personal microbiomes using metagenomic codes. *Proc Natl Acad Sci USA*. 2015;112:E2930–E2938.
30. Steed AL, Christophi GP, Kaiko GE, et al. The microbial metabolite desaminotyrosine protects from influenza through type I interferon. *Science*. 2017;357:498–502.
31. Jukes TH. Antibiotics in feeds. *Science*. 1979;204:8.
32. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature*. 2012;488:621–626.
33. Wang Y, Kuang Z, Yu X, et al. The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. *Science*. 2017;357:912–916.
34. Suez J, Korem T, Zeevi D, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*. 2014;514:181–186.
35. Wilck N, Matus MG, Kearney SM, et al. Salt-responsive gut commensal modulates TH17 axis and disease. *Nature*. 2017;551:585–589.
36. Lonks JR, Garau J, Gomez L, et al. Failure of macrolide antibiotic treatment in patients with bacteremia due to erythromycin resistant *Streptococcus pneumoniae*. *Clin Infect Dis*. 2002;35:556–564.
37. Fenton KA, Ison C, Johnson AP, et al. Ciprofloxacin resistance in *Neisseria gonorrhoeae* in England and Wales in 2002. *Lancet*. 2003;361:1867–1868.
38. Sjölund M, Andersson DI, Blaser MJ, et al. Long-term persistence of resistant *Enterococcus* species after antibiotic treatment to eliminate *Helicobacter pylori*. *Ann Intern Med*. 2003;139:483–487.
39. Sjölund M, Tano E, Blaser MJ, et al. Persistence of resistant *Staphylococcus epidermidis* after single course of clarithromycin. *Emerg Infect Dis*. 2005;11:1389–1393.
40. Wilson ME. Infectious diseases: an ecological perspective. *BMJ*. 1995;331:1681–1684.
41. Gandhi NR, Nunn P, Dheda K, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet*. 2010;375:1830–1843.
42. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med*. 2005;353:2433–2441.
43. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med*. 2005;353:2442–2449.
44. Ghose C, Perez-Perez GI, Dominguez-Bello MG, et al. East Asian genotypes of *Helicobacter pylori:* strains in Amerindians provide evidence for its ancient human carriage. *Proc Natl Acad Sci USA*. 2002;99:15107–15111.
45. Linz B, Balloux F, Moodley Y, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature*. 2007;445:915–918.
46. Collin M, Olsen A. Extracellular enzymes with immunomodulating activities: variations on a theme in *Streptococcus pyogenes*. *Infect Immun*. 2003;71:2983–2992.
47. Ouimet M, Koster S, Sakowski E, et al. *Mycobacterium tuberculosis* induces the miR-33 locus to reprogram autophagy and host lipid metabolism. *Nat Immunol*. 2016;17:677–686.
48. Mehra A, Zahra A, Thompson V, et al. *Mycobacterium tuberculosis* Type VII Secreted Effector EsxH Targets Host ESCRT to Impair Trafficking. *PLoS Pathog*. 2013;9:e1003734.
49. Kalliomaki M, Salminen S, Poussa T, et al. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*. 2003;361:1869–1870.
50. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med*. 2013;368:407–415.
51. Tannock G, ed. *Probiotics and Prebiotics: Where Are We Going*? Wymondham, UK: Caister Academic Press; 2002.
52. Lim B, Zimmerman M, Barry NA, et al. Engineered regulatory systems modulate gene expression of human commensals in the gut. *Cell*. 2017;169:547–558.
53. Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLoS ONE*. 2012;7:e34938.

194 *Staphylococcus aureus* (Including Staphylococcal Toxic Shock Syndrome)

Yok-Ai Que and Philippe Moreillon

SHORT VIEW SUMMARY

Definition
- *Staphylococcus aureus* is a gram-positive pathogen that is responsible for superficial and deep-seated infections.
- It is a frequent colonizer of asymptomatic carriers.
- The organism is responsible for both pyogenic and toxin-related diseases.
- It is the primary cause of community- and hospital-acquired bloodstream infections and the first cause of invasive infections including infective endocarditis and osteomyelitis.
- Frequently, *S. aureus* is resistant to methicillin (methicillin-resistant *S. aureus* [MRSA]) and almost all β-lactam drugs (in up to 50% of hospital isolates).
- Often it is coresistant to many clinically available antibiotics.

Epidemiology
- *S. aureus* is a colonizer of the anterior nostrils in 20% to 40% of the normal population.
- With regard to drug-resistant nosocomial infections, it is the number one public health problem.
- Clonal spread of MRSA occurs from health care settings (health care–associated [HCA] MRSA) and other permissive environments (see Fig. 194.2).
- Polyclonal dispersion of susceptible strains occurs in the community, but successful clones of community-acquired (CA) MRSA may spread worldwide (e.g., USA300).
- The organism is a superantigen producer responsible for toxic shock syndrome and food poisoning.

Microbiology
- *S. aureus* is the most virulent species of the more than 40 *Staphylococcus* spp. taxa.
- Its conserved core genome consists of approximately 2.8 million base pairs.
- It has multiple mobile genetic elements (MGEs): pathogenic and genomic islands, transposons, and prophages encoding virulence and antibiotic-resistance genes (see Tables 194.1 and 194.3).
- Multiple immune-evasion strategies are used by the organism, which impedes vaccine development (see Table 194.4).
- Evolution of successful clones occurs by means of mutations and acquisition of MGEs.
- Methicillin resistance is conferred by a polymorph family of SCC*mec* cassettes.

Diagnosis
- Conventional cultures are mandatory and critical for the detection of new resistance phenotypes.
- Molecular tests are useful for rapid identification of known drug-resistance genes.
- Molecular typing is critical for management of MRSA epidemics and infection control.

Therapy (See Tables 194.9 and 194.10)
- The first choice for methicillin-susceptible *S. aureus* is a penicillinase-resistant β-lactam or first-generation cephalosporin—for instance, cephalexin or dicloxacillin orally; nafcillin or oxacillin intravenously; or, outside the United States, flucloxacillin. Alternatives include clindamycin or trimethoprim-sulfamethoxazole (TMP-SMX) orally or intravenously.
- The first choice for MRSA would be vancomycin or daptomycin intravenously, or, if susceptible, TMP-SMX or clindamycin given orally or intravenously. Alternatives include ceftaroline, linezolid, or telavancin or, for acute bacterial skin and skin structure infections, dalbavancin, oritavancin, or tedizolid.
- A partner drug for combinations is rifampin.
- The benefit of aminoglycosides not well demonstrated and no longer recommended for native valve endocarditis.

Prevention
- Prevention measures include the following:
  - Decolonization of staphylococcal carriers (see Table 194.8)
  - Detection of HCA-MRSA and epidemic CA-MRSA
  - Hand hygiene measures
  - Vaccines in development

Staphylococcus aureus is a highly successful opportunistic pathogen. It is a frequent colonizer of the skin and mucosa of humans and animals (it is present in the anterior nares of up to 30% of the healthy human population) and can produce a wide variety of diseases. These diseases encompass relatively benign skin infections, such as folliculitis and furunculosis, and life-threatening conditions, including erysipelas, deep-seated abscesses, osteomyelitis, pneumonia, sepsis, and endocarditis.[1] In addition to infections in which the organism is physically present at the infected site, *S. aureus* is also capable of producing "distant" diseases, which are mediated by the secretion of toxins.[2] The toxins can be produced *directly* by bacteria that colonize the skin or mucosa or *indirectly* by microorganisms that colonize food or beverages. The former is exemplified by staphylococcal scalded skin syndrome (SSSS),[3,4] which is the result of skin, mucosal, or wound colonization by *S. aureus*–producing exfoliative toxin A or B (ETA or ETB) and by staphylococcal toxic shock syndrome (TSS),[2,5] which is the result of the production of toxic shock syndrome toxin 1 (TSST-1) or exotoxins B or C. The latter is exemplified by *S. aureus* food intoxication, in which the toxin is ingested with the contaminated dish, and disease follows shortly thereafter in the form of vomiting and diarrhea. Food intoxication is the result of staphylococcal toxins called *enterotoxins*.[2,6] These toxins are heat stable. Cooking may kill the contaminants but does not denature the toxins. Hence, subsequent culture of the dish may fail to grow the culprit bacterium.

S. aureus has an extraordinary capacity to adapt and survive in a great variety of environments. During the past decades, molecular and genetic dissection of *S. aureus* has revealed a great number of surface adhesins, which mediate adherence to and colonization of target tissues, and secreted enzymes, toxins, superantigens (SAgs) and immune evasion determinants that are responsible for invasion and distant disease

(Table 194.1).[1,7–9] The availability of now several thousands of *S. aureus* genome assemblies and annotation reports (www.ncbi.nlm.nih.gov/genome/genomes/154) has helped complete this portrait. *S. aureus* is part of the Firmicutes phylum and shares approximately 50% of orthologue genes with notoriously nonpathogenic *Bacillus subtilis,* which indicates that the two organisms have evolved from a common ancestor.[10–12] Homology searches on the chromosome revealed numerous new surface-attached and secreted factors that represent additional pathogenic factors. *S. aureus* harbors a large number of mobile genetic elements (MGEs) from exogenous origin, including insertion sequences, transposons, bacteriophages, pathogenicity islands, and genomic islands, which contain specific determinants responsible for disease and antibiotic resistance.[7,8,10,13,14] The presence of these exogenous elements attests to the high capacity of *S. aureus* to undergo horizontal gene transfer and exchange genetic elements with other organisms, including staphylococcal and nonstaphylococcal genera. Because gene exchange is a key player of evolution, this peculiar genetic plasticity is a likely explanation for the success of *S. aureus,* both as a colonizer and a disease-producing microbe. In the case of SAgs (see later discussion), one of the trading partner is suspected to be *Streptococcus pyogenes*.[2]

THE MICROORGANISM

Members of the *Staphylococcus* genus are gram-positive cocci (0.5–1.5 µm in diameter) that occur singly and in pairs, tetrads, short chains, and irregular grapelike clusters. Ogston[15] introduced the name *Staphylococcus* (Greek *staphylé,* "a bunch of grapes") to describe micrococci responsible for inflammation and suppuration. Staphylococci are nonmotile, non–spore forming, and usually catalase positive, and they are often unencapsulated or have a limited capsule (Fig. 194.1). Most species are facultative anaerobes.[11,12]

The genus *Staphylococcus* contains up to 40 taxa, 16 of which are commonly found in humans (Table 194.2). Only a few are pathogenic in the absence of predisposing immunosuppression or implanted foreign material. The most virulent ones include *S. aureus, Staphylococcus lugdunensis,* and *Staphylococcus schleiferi* in humans, and *S. aureus* and *Staphylococcus intermedius* in animals. Although *Staphylococcus epidermidis, Staphylococcus haemolyticus,* and *Staphylococcus saprophyticus* are commonly responsible for device-related and urinary tract infections, they produce substantially less devastating disease syndromes than *S. aureus.*

S. aureus harbors some unique features when compared with its less–disease-producing congeners. These include coagulase and clumping factors (or fibrinogen-binding proteins), which have laboratory diagnostic value because they help rapidly discriminate between coagulase-positive (i.e., *S. aureus*) and coagulase-negative staphylococci (CoNS; see Table 194.2). Moreover, *S. aureus* carries between more than 20 and more than 30 adhesin and toxin genes, respectively, as compared with 10 or fewer adhesin genes and virtually no toxin genes for the CoNS mentioned previously.[7,16–18] Thus, *S. aureus* is a distinct pathogen within the *Staphylococcus* genus.

Habitat

Staphylococci are ubiquitous colonizers of the skin and mucosa of virtually all animals, including mammals and birds.[11,12] Some species have preferential niches as indicated by their names (see Table 194.2). *S. epidermidis* and *Staphylococcus capitis* are constant colonizers of the skin and scalp, respectively. *Staphylococcus pseudintermedius* is a colonizer of cats and dogs and may be misidentified as *S. aureus* (tube coagulase positive, slide coagulase negative) when transmitted by animal bites.[19]

In animals, *S. aureus* is a major cause of livestock infection, including mastitis in bovine and ovine herds. In humans, *S. aureus* has a niche

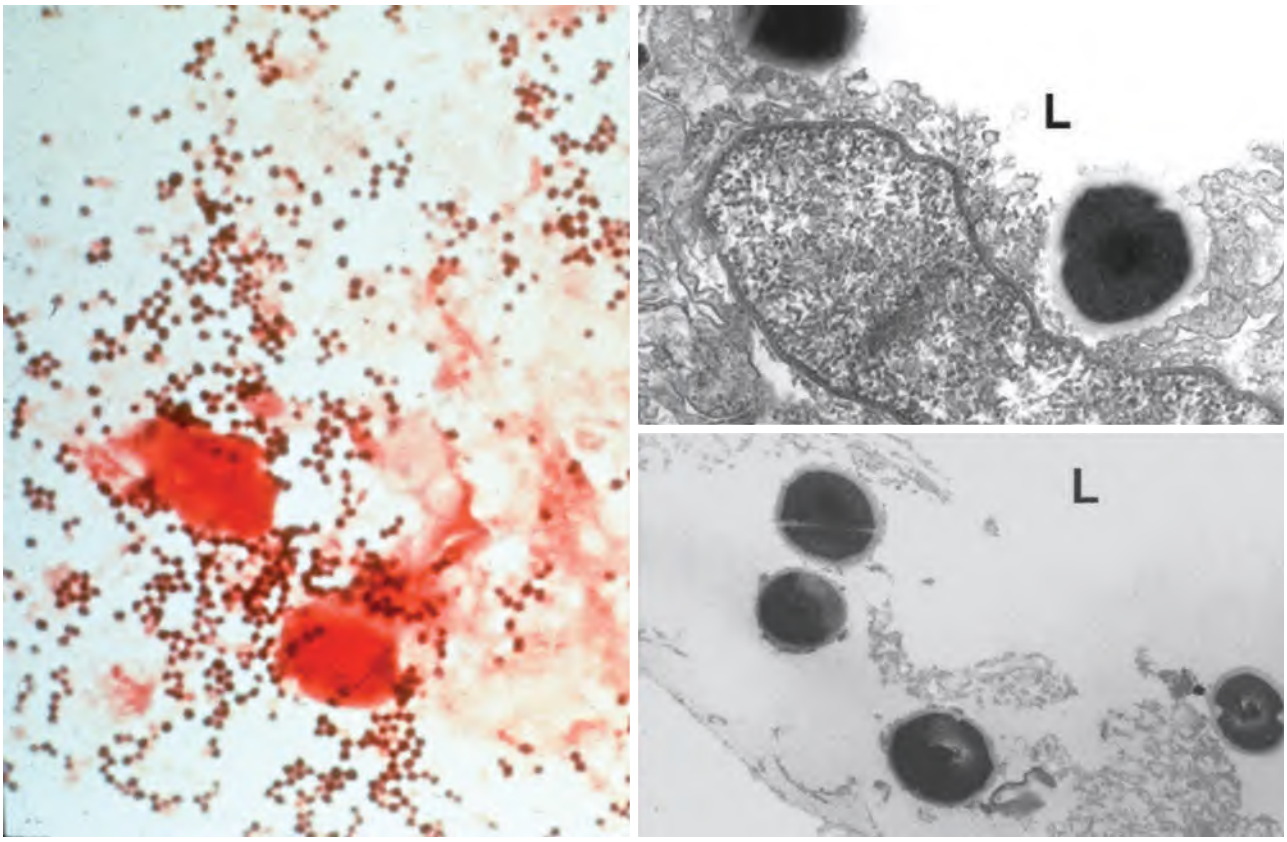

FIG. 194.1 Gram staining and transmission electron microscopy of clinical samples of *Staphylococcus aureus.* *Left,* Gram-stained sputum specimen from 20-year-old patient with fulminant hemorrhagic *S. aureus* pneumonia (see also Fig. 194.8). Grapelike clusters of bacteria and eukaryotic cells are visible. *Right,* Electron microscopy images from rat with experimental aortic endocarditis caused by *S. aureus. Upper part* depicts *S. aureus* in process of invading valve endothelial cell. *Lower part* depicts endothelial cell that has been lysed by invading bacteria, probably as a result of *S. aureus* hemolysin secretion. *L,* Lumen side of endothelium.

TABLE 194.1 Some of the Major *Staphylococcus aureus* Extracellular Factors Involved in Pathogenesis and Response to Global Regulatory Elements During Bacterial Growth

GENE	LOCATION	PRODUCT	ACTIVITY OR FUNCTION	TIMING[a]	ACTION OF REGULATORY GENES[b]			
					agr	*saeRS*	*rot*	*sarA*
Surface Proteins								
spa	Chromosome	Protein A	Blocks IgGs, binds von Willebrand factor	Exp	–	See footnote[c]	+	
cna	Chromosome	Collagen BP	Collagen binding	Exp	–			
fnbA	Chromosome	Fibronectin BPA	Fibronectin binding	Exp	–			-
fnbB	Chromosome	Fibronectin BPB	Fibronectin binding	Exp	–			+
clfA	Chromosome	Clumping factor A	Fibrinogen binding	Exp	0			
clfB	Chromosome	Clumping factor B	Fibrinogen binding	Exp	0		+	0
sdrC	Chromosome	Serine-aspartate repeat protein	Fibrinogen binding Cytokeratin binding	Exp				+
Capsular Polysaccharides								
cap5	Chromosome	Polysaccharide capsule type 5	Antiphagocytosis	Pxp	+			+
cap8	Chromosome	Polysaccharide capsule type 8	Antiphagocytosis	Pxp	+			
Cytotoxins								
hla	Chromosome	α-Hemolysin	Hemolysin, cytotoxin	Pxp	+	+	–	See footnote[c]
hlb	Chromosome	β-Hemolysin	Hemolysin, cytotoxin	Pxp	+	+	–	See footnote[c]
hld	Chromosome	δ-Hemolysin	Hemolysin, cytotoxin	Pxp	+	0		+
hlg	Chromosome	γ-Hemolysin	Hemolysin, cytotoxin	Pxp	+		–	See footnote[c]
lukS/F	PVL phage	PVL	Leucocidin	Pxp	+		–	
Superantigens								
sea	Bacteriophage	Enterotoxin A	Food poisoning, TSS	Xp	0			
seb	SaPI3[d]	Enterotoxin B	Food poisoning, TSS	Pxp	+			See footnote[c]
sec	SaPI4[d]	Enterotoxin C	Food poisoning, TSS	Pxp	+			
sed	Plasmid	Enterotoxin D	Food poisoning, TSS	Pxp	+			
eta	ETA phage	Exfoliatin A	Scalded skin syndrome	Pxp	+			
etb	Plasmid	Exfoliatin B	Scalded skin syndrome	Pxp	+			
tst	SaPI1,2, bov1[d]	Toxic shock toxin 1	TSS	Pxp	+			See footnote[c]
Enzymes								
SplA-F	Chromosome	Serine protease-like	Putative protease		+		–	
ssp	Chromosome	V8 protease	Spreading factor	Pxp	+	0		–
aur	Chromosome	Metalloprotease (aureolysin)	Processing enzyme?	Pxp	+			–
sspB	Chromosome	Cysteine protease	Processing enzyme?	?			–	
scp	Chromosome	Staphopain (protease II)	Spreading, nutrition	Pxp	+			–
geh	Chromosome	Glycerol ester hydrolase	Spreading, nutrition	Pxp	+	0	–	See footnote[c]
lip	Chromosome	Lipase (butyryl esterase)	Spreading, nutrition	Pxp	+	0		See footnote[c]
fme	Chromosome	FAME	Fatty acid esterification	Pxp	+			See footnote[c]
plc	Chromosome	PI-phospholipase C		Pxp	+			
nuc	Chromosome	Nuclease	Nutrition	Pxp	+	+		
has	Chromosome	Hyaluronidase	Spreading factor	Xp	See footnote[c]			
coa	Chromosome	Coagulase	Clotting, clot digestion	Exp		+	+	+
sak	Bacteriophage	Staphylokinase	Plasminogen activator	Pxp	+	0		

[a]Timing: Xp, throughout exponential phase; Exp, early exponential phase only; Pxp, postexponential phase; 0, no effect of gene on. Expression: +, upregulated; –, downregulated.
[b]*agr,* Accessory gene regulator; PVL, Panton-Valentine leukocidin; *saeRS, S. aureus* exoproteins; *rot,* repressor of toxins; *sarA, Staphylococcus* accessory regulator.
[c]Controversial.
[d]*SaPI, S. aureus* pathogenic island.
BP, Binding protein; *FAME,* fatty acid modifying enzyme; *TSS,* toxic shock syndrome.
Modified from Cheung AL, Projan SJ, Gresham H. The genomic aspect of virulence, sepsis, and resistance to killing mechanisms in Staphylococcus aureus. Curr Infect Dis Rep. *2002;4:400–410; and Novick RP, Geisinger E. Quorum sensing in staphylococci.* Ann Rev Genet. *2008;42:541–564.*

TABLE 194.2 Some Staphylococcal Species From Mammals and Relationship Between Production of Coagulase and Clumping Factor (Fibrinogen-Binding Protein A) and Potential Virulence

HOST	SPECIES	COAGULASE[a]	CLUMPING FACTOR[a]	VIRULENCE[a]
Human and other primates	*S. aureus*	++	++	+++
	S. epidermidis	–	–	+
	S. capitis	–	–	±
	S. caprae	–	–	±
	S. saccharolyticus	±	–	–
	S. warneri	–	–	–
	S. pasteuri	–	–	–
	S. haemolyticus	–	–	+
	S. hominis	–	–	±
	S. lugdunensis	–	±	+
	S. auricularis	–	–	±
	S. saprophyticus	–	–	+
	S. cohnii	–	–	–
	S. xilosus	–	–	–
	S. simulans	–	–	–
	S. schleiferi	±	+	+
Carnivores	*S. intermedius*	+	–	++
	S. felis	–	–	++

[a]Semiquantitative estimate of production of coagulase and clumping factor and relation to virulence.
Modified from Kloos WE, Schleifer KH, Goetz F. The genus Staphylococcus. *In: Balows A, Trüper HG, Dworkin M, et al, eds.* The Prokaryotes. *2nd ed. New York: Springer-Verlag; 1992:1369–1420; and Kloos WE, Bannerman TL.* Staphylococcus *and* Micrococcus. *In: Murray PR, Baron EJ, Pfaller MA, et al, eds.* Manual of Clinical Microbiology. *6th ed. Washington, DC: ASM Press; 1995:282–298.*

preference for the anterior nares, especially in adults,[20–22] and is shed onto healthy skin, including axilla and perineum. However, certain clones may have preferences for more hidden niches, as was shown in the case of a peculiar epidemic hospital methicillin-resistant *S. aureus* (MRSA) clone that colonized the groin and rectum.[23] *S. aureus* can exist as a resident or a transient member of the normal flora. Nasal carrier rate varies from 10% to 40% in both the community and the hospital environment. *S. aureus* carriage in various anatomic sites may put certain populations at an increased risk for infection, such as patients with recurring furunculosis and patients who are subject to medical procedures, including hemodialysis, peritoneal dialysis, and surgery (see later section "Carriage of *Staphylococcus aureus*").[24–26]

S. aureus carriage has also become a way of persistence and spread of multiresistant staphylococci, especially MRSA.[20,21,23,26] Because MRSA can resist many of the antibiotics in common use, it has risen to the level of a public health threat in the hospital for 3 decades and in the community since the beginning of this century.[25,27]

Culture and Identification

Live organisms obtained by means of culture are critical for phenotypic diagnosis and revealing emerging antibiotic resistant phenotypes from as yet unknown mechanisms. In addition, molecular diagnosis helps speed up the results, which take a few hours instead of 1 to 3 days with bacterial subculturing. Molecular methods also help detect the presence of nonculturable microbes, mostly when patients have taken antibiotics before sample collection.

Techniques for culture and identification of staphylococci have been described.[11] Specimens should be inoculated both on blood agar and into rich liquid media such as Mueller-Hinton broth. With *S. aureus,* abundant growth occurs normally within 18 to 24 hours. However, morphologic variants (see subsequent discussion) may require prolonged growth periods, and plates should be kept 2 to 3 days in order to detect them. Colonies should be Gram stained, subcultured, and tested for genus, species, and antibiotic susceptibility when appropriate. Phenotypic tests for species identification include coagulase tests and agglutination tests, which detect the presence of surface determinants, including clumping factor, protein A, and polysaccharides.[28] Phenotypic antibiotic susceptibility tests vary from agar-diffusion methods (e.g., Kirby-Bauer and Etests) to automated measurement of metabolic activity or growth rates. Macro broth or agar dilution methods are precise but are not routinely performed in the laboratory.[29]

Molecular specification may be necessary in case of unclear phenotype, such as, for instance, in the case of morphologic variants (see next section).

Morphologic Variants

Prolonged incubation is particularly important for the detection of morphology variants such as *small colony variants* (SCVs). SCVs grow into tiny colonies that are difficult to distinguish and may be mistakenly disregarded as contaminants.[30] They are usually recovered from protracted, difficult-to-treat infections such as chronic osteomyelitis and infected osteosynthetic prostheses, and have also been described in patients with cystic fibrosis.[31]

The most classic types of SCVs are selected during aminoglycoside therapy and result from alterations in the respiratory chain. Such SCVs have a lower transmembrane potential, which impedes the intake of the drug.[32] Interesting to note, switching from normal colonies to SCVs occurs naturally in the absence of antibiotic at a high rate (about 10^{-6}), and switching back from SCVs to normal colonies also occurs.[33] Hence, SCVs are proposed to result from an intrinsic capacity of the bacterium to survive in unfavorable conditions rather than fortuitous mutations.

SCVs are also selected by other antimicrobial agents, including triclosan.[34] They were recovered from the sputa of up to 25% of children with cystic fibrosis and were statistically significantly associated with previous trimethoprim-sulfamethoxazole (TMP-SMX) therapy.[31] Such SCVs carry mutations in the thymidylate synthase gene *(thyA)* and are dependent on exogenous thymidine to grow.[35] *S. aureus* synthesizes thymidine by using *thyA* plus tetrahydrofolate to convert uridine monophosphate into thymidine monophosphate. TMP inhibits the synthesis of tetrahydrofolate, thus making *thyA* useless. By mutating the *thyA* gene, *S. aureus* forces itself to rely on exogenous vital thymidine by importing it. This makes the bacterium resistant to TMP. Thymidine is available in DNA-rich lung secretions of patients with cystic fibrosis and in abscesses. However, the rate of thymidine import is limiting, which results in slow growth and SCV phenotype.

In spite of slow growth, SCVs are equally as or more infective than their fast-growing parents in experimental infections such as osteoarthritis[36] and endocarditis.[37] Moreover, SCVs are particularly prone to invade eukaryotic cells and persist in them,[30,38] which may explain their occurrence in latent infections. SCVs are cross-resistant to drug-induced killing by most antibiotics,[32] and their eradication necessitates prolonged antibiotic therapy including drug combinations with rifampin.

MOLECULAR DIAGNOSIS

Molecular diagnosis plays an increasing role in rapid detection of microbial pathogens and identification of drug-resistance determinants. Techniques based on molecular probing have been reviewed.[39] One of

these techniques relies on fluorescent detection of 16S rRNA with a peptide nucleic acid probe (peptide nucleic acid fluorescence in situ hybridization [PNA-FISH]). Such a technique has been shown to be highly specific and to help in discriminating *S. aureus* from CoNS in blood culture within 4 hours.[40] In addition, its usefulness in clinical therapeutic decision making has also been demonstrated.[41]

Multiplex real-time polymerase chain reaction (PCR) assays are being developed to quantify organisms directly in clinical samples. Genes representative of both species and resistance mechanisms are amplified simultaneously. For MRSA, the resistance gene sought is *mecA,* which encodes low-affinity penicillin-binding protein A (PBP2A).

However, *mecA* is also present in methicillin-resistant CoNS and thus detects simultaneously both MRSA and commensal methicillin-resistant CoNS, which may result in false diagnosis. One way to bypass this limit is to extend the *mecA* amplification product to *orfX*. *orfX* is an open reading frame that is specific for *S. aureus,* and its amplification ensures the correct diagnosis.[42] Another way is to choose additional *S. aureus* or CoNS specific genes. These include typical *S. aureus* gene versions such as *femA,*[42] protein A *(spa)*, coagulase *(coa)*, and nuclease *(nuc)*.[43] Other genes or gene combinations were also successfully used to discriminate between *S. aureus* and CoNS in clinical samples.[42,44,45]

Limits may occur with PCR amplification techniques. In some cases, proprietary DNA targets, known only to the manufacturer, can make it difficult to assess the vulnerability of commercial molecular assays to changes in the DNA sequence of isolates being tested. In a large multicenter US study of one molecular platform for identifying MRSA, 3 of 93 MRSA isolates were called methicillin-susceptible *S. aureus* (MSSA), and 8 of 102 MSSA isolates were called MRSA, indicating that although molecular typing may be useful for rapid screening of carriers, it may carry the risk of misdiagnosis in clinical care.[46] Likewise, PCR amplification using standard primers failed to amplify a new version of the *mecA* gene, renamed *mecC,* that emerged in livestock MRSA.[47] In this very case, methicillin resistance was detected with phenotypic tests.[48] Moreover, MSSA isolates have been found that had PCR-detected *mecA* elements but later reverted to methicillin resistance under therapy. The loss of a transposon that interrupted *mecA* and replication errors accounted for this conversion.[49]

Currently, more rapid identification by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is being developed, allowing MRSA identification from a colony within 5 minutes as compared with several hours with PCR.[50]

However, although more rapid than phenotypic tests, these molecular techniques still require prior growth of the organisms, which may take 12 to 24 hours, and do not test all possible antibiotic resistance genes. Whole-genome sequencing may become an option to screen for species and resistance genes, but the bioinformatic workload remains a limiting factor. One emerging technique relies on metagenomic analysis of DNA amplified directly from clinical samples, without prior culturing, through use of next-generation sequencing (NGS). Here, identification of genus, species, and known resistance genes will rely on comparison with large databases,[51] but unknown resistance genes will be missed. Thus, phenotypic testing must always be kept in mind.

Molecular Typing

There is a dual interest in studying the genealogy of life. One is academic and aims at solving the evolutionary journey of peculiar organisms. The other is epidemiologic and aims at tracing a peculiar pathogen responsible for clinical problems. *S. aureus* is a common pathogen both in the hospital and in the community.[27,52] Although the prevalence of MRSA has been slowly decreasing over the last decade in the United States and Europe,[53] this trend is not global and the proportion of MRSA in health care–related infections remains over 50% in other geographic locations.[54]

MRSA is highly clonal, and a few highly successful clones, named according to the place where they were described, can be recovered at multiple locations both nationwide and worldwide (i.e., the Iberian, Brazilian, Hungarian, New York/Japan, Pediatric, and EMRSA-16 pandemic clones).[55,56] The main molecular typing methods underlying this comprehension are briefly presented later. More complete total genome sequencing may not be required for routine tracing of epidemic strains.

Pulsed-Field Gel Electrophoresis

The seminal method is a restriction-fragment length technique based on large chromosomal fragments generated by digestion with the low-frequency cutting enzyme *Sma*I. The fragments are separated with pulsed-field gel electrophoresis (PFGE) and yield banding patterns specific for particular clones. Banding comparison allowed identification of the major epidemic clones listed earlier, which represented 70% of more than 3000 MRSA isolates recovered worldwide.[56]

One limitation of PFGE is that it does not provide accurate information on the genealogy of the organism. Indeed, the length of chromosomal fragments, and thus the clone-specific banding, may be modified with acquisition or loss of mobile DNA (MGEs) such as transposons, prophages, or pathogenicity islands. The new banding pattern may identify a different clone, which is in fact the same bacterium that has gained or lost MGEs. This is exemplified by the fact that several PFGE major MRSA epidemic clones belonged to the same multilocus sequence typing (MLST; see later) group.[55] Thus, PFGE is useful to follow epidemic clones, but not to build the parental staphylococcal genealogy.

Multilocus Sequence Typing

In contrast to PFGE, MLST is a sequence-based method that allows the unambiguous assignment of the ancestral phylogeny of the staphylococcal population.[57] It consists of sequencing seven housekeeping genes (i.e., *arcC, aroE, glpF, gmk, pta, tpi,* and *yqiL*) and comparing them with the sequences of other isolates collected in a central database (www.mlst.net). It compares allelic diversity based on approximately 500-bp internal gene fragments. Thousands of sequences have been submitted, generating numerous sequence types (STs). Organisms that share all seven alleles are defined as *clones,* those that share five of seven identical alleles are defined as *clonal complexes* (CC), and those that share less than five alleles are defined as *unrelated*. Within such arborescence, STs can be considered as founders of further evolutionary groups such as CCs.

Because housekeeping genes are independent of acquired MGEs, MLST traces staphylococci back to their latest common ancestor. Of the seven pandemic clones mentioned previously, six could be traced back to three ancestral MSSA types (i.e., CC5, CC8, and CC30; Fig. 194.2).[58] Thus, a few ancestral clones of MSSA took the lead and successfully colonized humans and animals before antibiotic resistance developed. Later acquisition of MGEs carrying drug-resistance or virulence genes helped further adaptation to new conditions (e.g., antibiotic use in hospitals), generating a new PFGE makeup on similar ancestral parents (see "Comparative Genomics and Evolution"). Moreover, genomics now shows that acquisition of antibiotic resistance genes is reversible and that the contemporary decrease in MRSA prevalence is associated with the loss of the methicillin-resistance determinants.[53]

Spa Typing and Double-Locus *Spa-ClfB* Typing

Spa typing and double-locus *spa-clfB* typing rely on PCR amplification of strain-specific regions within hypervariable segments of the *spa* (protein A) or *clfB* (clumping factor B) genes.[59] The variable regions are made of 24 nucleotide repeats in *spa* and serine-aspartate repeats in *clfB,* the length of which may vary from duplication or accidental loss of DNA material. Single PFGE or MLST types can evolve into different *spa* or *clfB* sublineages. Hence, combining these techniques generates unambiguous data sets that can be compared in multicenter studies.

Typing is critical in order to understand the *S. aureus* epidemiology. On the other hand, although a handful of founding ST types appear to be prevalent in MRSA[55] and PVL-positive strains,[60] no specific types could be attributed to disease-producing versus mere colonizing strains.[10]

PATHOGENESIS

Regulation and Virulence Determinants

S. aureus is extremely well equipped in surface factors and secreted proteins that mediate host colonization and disease (see Table 194.1).[1]

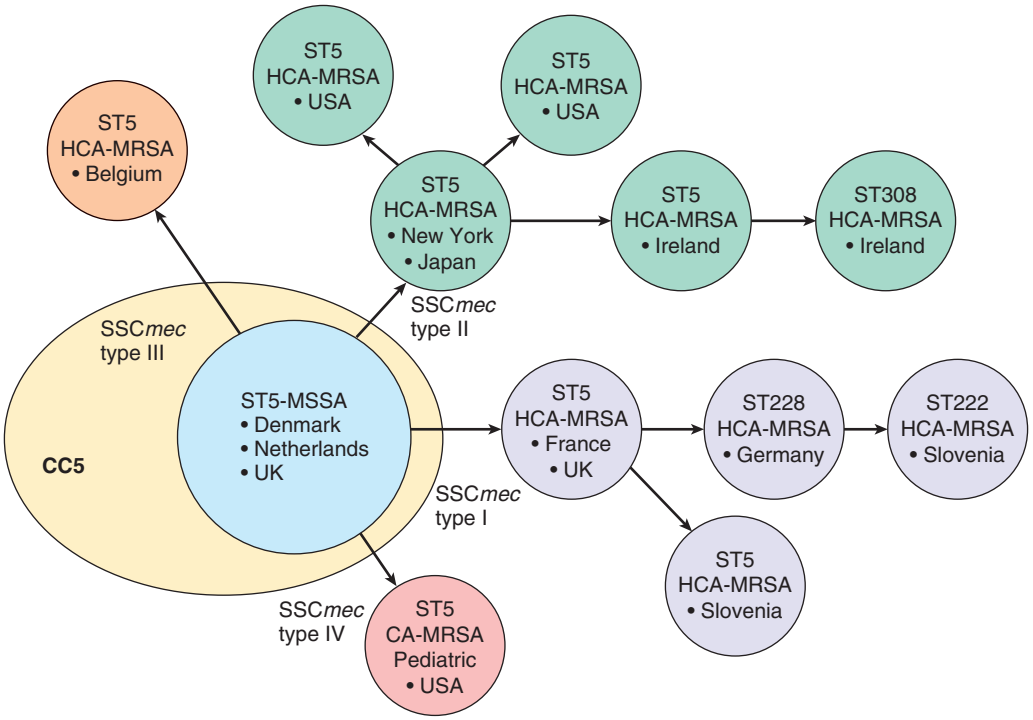

FIG. 194.2 Evolution of methicillin-susceptible *Staphylococcus aureus (MSSA)* into methicillin-resistant *S. aureus (MRSA)* as exemplified by sequence type 5 *(ST5).* ST5 belongs to clonal cluster 5 *(CC5),* which gathers *S. aureus* isolates sharing homologies in five of the seven genes (*arcC, aroE, glpF, gmk, pta, tpi,* and *yqiL*) compared with method of multilocus sequence typing (MLST). Parental ST5 is an MSSA that has been isolated in several countries, including Denmark, the Netherlands, and the United Kingdom. It acquired various types of SCC*mec* at several independent occasions, probably from coagulase-negative staphylococci (CoNS) donor strains. After SCC*mec* acquisition, new MRSA clones followed their own geographic and genetic evolution, spreading either as HCA-MRSA (SCC*mec* I, II, or III) or CA-MRSA clones (SCC*mec* IV) and sometimes evolving into new ST types (e.g., ST222, ST228, and ST308). Three clonal clusters (CC5, CC8, and CC30) generated six of the seven major pandemic MRSA clones described over the past 3 decades. *(Modified from Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant* Staphylococcus aureus. Antimicrob Agents Chemother. *2003;47:3926–3934.)*

In addition to these features, *S. aureus* is equipped with regulatory systems that sense environmental conditions and respond by fine-tuning the expression of given metabolic and virulence determinants (for review, see Novick and Geisinger,[61] Pragman and Schlievert,[62] and Balasubramanian and colleagues[63]). Some aspects of this subtle adaptation machinery are described subsequently.

Regulation

At least three families of regulatory elements intertwine to adjust gene expression to specific environmental conditions: first, two-component regulatory systems, of which *agr* (for accessory gene regulator) is a paradigm; second, DNA-binding proteins, largely represented by the Sar (for staphylococcal accessory regulator) family of proteins; and third, small regulatory RNAs.

agr and Other Two-Component Regulatory Systems

The paradigm of two-component regulatory systems virulence gene regulation is *agr,* which is schematized in Fig. 194.3.[64] *agr* functions as a quorum sensing control that reacts to bacterial density, allowing the preferential expression of surface adhesins during the exponential phase of growth (low cell density) and switching to the expression of exoproteins during the postexponential and stationary growth phases (high cell density).[61,64,65] The switch is composed of two divergent operons (see Fig. 194.3). On the left hand, promoter P2 drives the transcription of a series of components that comprises (1) a transmembrane protein (AgrB); (2) an autoinducing peptide precursor (AgrD), which is processed and exported by membrane-spanning AgrB; (3) a transmembrane sensor (AgrC), which is the cognate receptor of the AgrD-derived autoinducing peptide; and (4) a transcription regulator (AgrA) that can be activated by AgrC. At low cell density (exponential growth phase), the P2 promoter is off and the operon is transcribed at a low level. As cell growth proceeds, the concentrations of both bacteria and extracellular autoinducing peptide increase in the milieu, thereby augmenting the chance of the autoinducing peptide to make contact with its cognate AgrC receptor. On contact, AgrC activates the response regulator AgrA, a process that may involve AgrA dephosphorylation.[61,64]

Activated AgrA is a DNA-binding protein that turns on the transcription from both promoter P2, generating a positive feedback on the system, and promoter P3, which drives the transcription of δ-hemolysin and of a peculiar effector called RNAIII. RNAIII has a reciprocal effect and activates the expression of several secreted proteins while downregulating the expression of surface-bound factors (see Table 194.1). RNAIII has a complex three-dimensional structure and a long half-life (up to 15 minutes). It regulates gene expression in several ways, including at the translational level by blocking the messenger RNA (mRNA) ribosome-binding site (RBS) of the target genes, or by prolonging the half-life of mRNA of downstream pleiotropic transcriptional regulators such as MgrA.[66]

The *S. aureus* chromosome encodes for up to 16 two-component regulatory systems involved in both metabolic environmental control and virulence gene regulation.[61,64,65] Important two-component regulatory systems regarding virulence genes include *saeR/S* (for *S. aureus* exoproteins),[67] *srrAB* (for staphylococcal respiratory response),[68] and *arlS* (for autolysis-related locus sensor).[69] *saeR/S* was identified with transposon mutation in a pleiotropic mutant defective in exoprotein synthesis other than that regulated by *agr* (e.g., coagulase and nuclease; see Table 194.1).[70] *saeR/S* acts independently of *agr* and responds to environmental stimuli such as high salt, low pH, glucose, and subinhibitory antibiotic concentrations. *srrAB* and *arlS* interfere with growth in microaerobic conditions and autolysis, respectively. *srrAB* represses the expression of TSST-1 and protein A in microaerobic conditions,[68] an observation that may be relevant for the pathogenesis of tampon-related

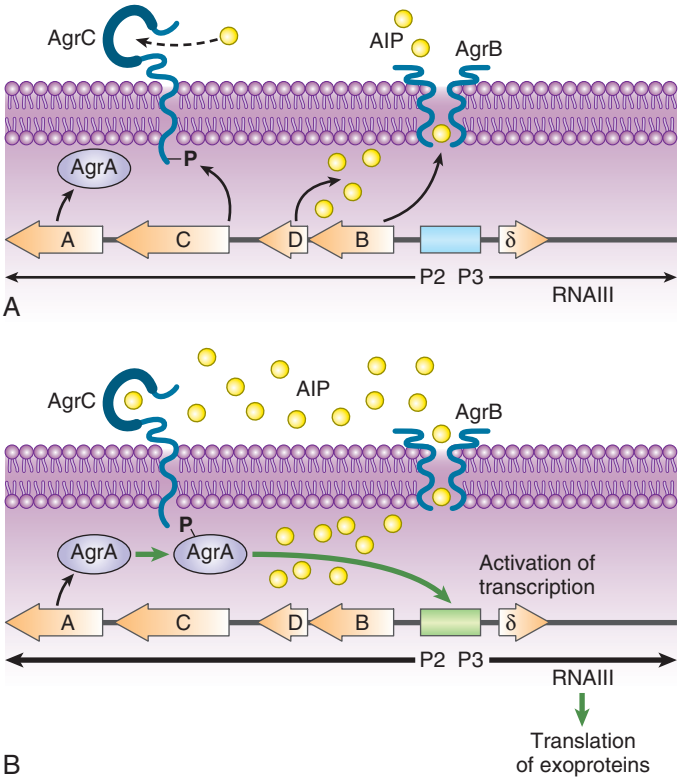

FIG. 194.3 Schematic representation of *Staphylococcus aureus* global regulatory system *agr* (accessory gene regulator). (A) System at rest. It consists of two divergent operons, transcribed from promoters P2 and P3. Promoter P2 encodes putative membrane protein AgrB, precursor of autoinducing peptide *(AIP)* AgrD, which is processed by AgrB; transmembrane receptor AgrC; and response regulator AgrA. Promoter P3 encodes δ-hemolysin and RNAIII. At low bacterial density, P2 and P3 are off and only a small amount of AIP is secreted because of promoter leakiness *(A).* As bacterial growth proceeds and bacterial density increases *(B),* chance of AIP encountering its cognate receptor AgrC increases. On contact, AgrC undergoes conformational change and phosphorylates (or dephosphorylates) response regulator AgrA. (B) Activated AgrA activates transcription from both P2 and P3, resulting in positive feedback. δ-Hemolysin is membrane-active protein toxic for eukaryotic cells. RNAIII is an intracellular regulator that acts in *trans* and regulates expression of many virulence genes, including numerous toxins (see Table 194.1). Although *agr* is pivotal in quorum-sensing regulation of gene expression, it is not the only regulator of pathogenic determinants in *S. aureus. sar, saeRS, rot,* and other systems may affect the expression of *agr* itself or affect virulence genes directly (e.g., *sar*) or both (see Table 194.1).

TSS (see later discussion).[5] Both *srrAB* and *arlS* interact reciprocally with *agr.*[62,65]

DNA-Binding Proteins

sar is an important locus that encodes the DNA-binding protein SarA, which positively controls *agr* (Fig. 194.4), and maybe also *sae* and *arlS.*[61,65] In addition, *sar* directly regulates adhesin genes (see Table 194.1). The *sarA* transcripts peak at the end of the logarithmic phase of growth, thus promoting *agr* expression. Moreover, *sarA* itself is transcribed downstream of three alternate promoters, which can themselves be regulated by as yet incompletely solved factors.

SarA is the prototype of a growing family of DNA-binding proteins that may drive a number of transcriptional activities, including the expression of housekeeping genes and phage-related genes. *sarA* homologues include *sarR, sarS, sarT, sarV, sarU, sarY, rot,* and *mgrA.*[62,65,71] *rot* stands for "repressor of toxins" and counters toxin expression by repressing *agr.*[72] Inactivation of *rot* partially restored the *agr* phenotype in *agr*-negative mutants, probably by alleviating a repressing effect on the downstream P3 cascade of the *agr.* This downstream cascade might be the target of several additional regulators that also affect the *agr* phenotype (see Fig. 194.4). *mgrA* stands for multiple gene regulator.[71]

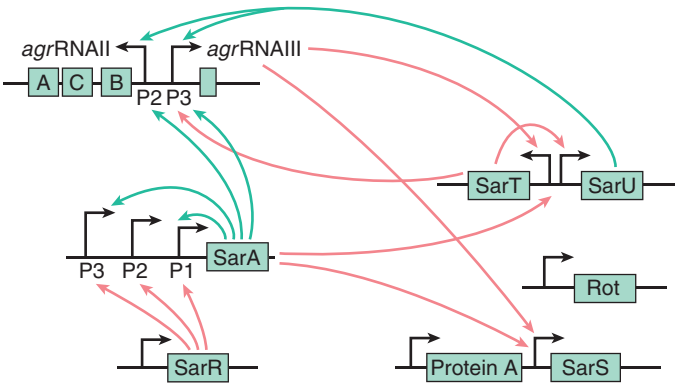

FIG. 194.4 Regulatory network of *agr* and Sar family of DNA-binding proteins. Intertwining of activation *(green arrows)* and repression *(red arrows)* underlines complexity of system. Gene expression is further modulated by additional factors, including alternative σ^B, *arlS, sae,* and *srrAB,* which can act on *agr* promoters or directly on specific genes. Gene promoters are denominated P1, P2, and P3 and represented by *black lines. (Modified from Pragman AA, Schlievert PM. Virulence regulation in* Staphylococcus aureus*: the need for in vivo analysis of virulence factor regulation.* FEMS Immunol Med Microbiol. *2004;42:147–154.)*

It controls the transcription of up to 355 genes (175 upregulated and 180 downregulated), including capsule, and protein A and α-hemolysin genes in an *agr*-dependent way.[66,71]

Sigma factors (σ) are another major mechanism of response to environmental stimuli. In bacteria, σ factors combine with and activate RNA polymerase to transcribe specific sets of genes. *S. aureus* contains one σ^A and two alternative σ^B and σ^C. Alternative σ^B is important for the microbial response to a variety of stresses, including temperature, energy depletion, and chemical stimuli.[73] It acts mostly via the global regulatory network and affects the expression of up to 251 genes (198 positively, 53 negatively),[74] but also has some direct effect by activating the expression of coagulase and fibronectin-binding proteins at the early growth phase, and downregulating certain secreted proteins in the stationary phase. Mutants overexpressing σ^B were more virulent in experimental endocarditis, probably by increasing the expression of surface adhesins.[75] Conversely σ^B defective mutants were less infective in a model of catheter-related systemic infection.[76]

Small RNAs and Endoribonuclease III

Small RNAs (sRNAs) are increasingly recognized as major players in regulation of gene expression. They act mainly at the translational level via antisense hybridization with mRNA, where they can alter mRNA stability, hide RBSs from ribosome recognition, or conversely reveal RBSs that are hidden in secondary mRNA structures by unfolding these very structures. Alternatively, sRNA can also bind regulatory DNA-binding proteins, thus sequestering them from their original gene regulatory function. A genome-wide analysis generated a "Staphylococcal Regulatory RNA Database" (SRD; http://srd.genouest.org/) and identified at least 550 potential regulatory sRNAs. The best functionally characterized of them are RNAIII, which orchestrates the *agr* response, and RNAI, which regulates the replication of multiresistance plasmid pSK41.

In symmetry, posttranscriptional expression is also modulated by direct RNA alteration via endoribonuclease III (RNase III). This RNA double-stranded endonuclease plays a critical role in RNA processing and decay. It has been shown to modulate posttranscriptional expression through various mechanisms, including turnover of transcribed and nontranscribed RNAs, and by maturating the 5′ untranslated region (5′UTR) of the mRNAs of the cold-shock protein *cpsA* and maybe the protein A *spa* genes, to increase their stability and translation.[77]

The regulatory network must be considered as a metabolic hub that integrates both external and internal information and responds in the most appropriate way. The observed phenotypes result from complex interplays among sometimes contradicting signals of sensors and transcriptional and posttranscriptional regulators, the understanding of which will require a systems biology approach.[78] Moreover,

experimentally interrupting one of these circuitries may cause compensation by others, thus introducing biases in the observed phenotype. In this complex system, *agr* appears to be a central switch toward which many other regulators converge (see Fig. 194.4).

Role in Pathogenesis

The intuitive *agr*-based model suggests that scattered growing bacteria produce primarily adhesins, promoting tissue colonization, whereas installed organisms that form dense populations switch to the production of hydrolytic enzymes and toxins for the purpose of feeding and escaping host defenses.[61,65] Accordingly, inactivation of the function of *agr* alone decreased pathogenicity in experimental models of tissue destruction (e.g., subcutaneous abscesses), where exoprotein production is likely to be important.[79] On the other hand, *agr* inactivation did not much influence the course of experimental endocarditis, where bacterial surface adhesins are critical for valve colonization.[80] Indeed, although *agr*-negative mutants are hampered in exoprotein production, they are still fully equipped with surface-bound colonizing determinants (see Table 194.1). In contrast, inactivation of *sar* decreased infectivity in experimental endocarditis[80] because in addition to its effect on *agr* expression (see Table 194.1 and Fig. 194.4), *sar* also acts directly on expression of surface-bound fibronectin-binding protein A (FnBPA), which promotes experimental endocarditis.[81,82]

In addition, in vivo gene expression revealed a further level of complexity.[62] For instance, although *sar* transcripts were detected in infected vegetations during experimental endocarditis, they were expressed from both P1 and P2 promoters, rather than only from the P1 promoter as observed in vitro.[83] Likewise, in vivo expression of several genes appeared dissociated from their control regulator as described in vitro. Although *agr* positively regulates TSST-1 in vitro (see Table 194.1), the toxin was still expressed by an *agr*-negative mutant in a rabbit model of TSS in vivo.[84] This may result from alternative regulation by other regulators that act either downstream of the *agr* locus or directly on the *tss* gene promoter. Eventually, *agr*-negative mutants can be recovered from clinical samples as in cystic fibrosis[85] and in carrier and bacteremic patients.[86] Such *agr*-negative clinical isolates, and *agr*-negative laboratory mutants, have increased surface adhesins and an increased ability to form biofilms, and are found in chronic infections such as osteomyelitis and device infections.[87]

Hence, the pathogenic implication of regulatory circuitries cannot be drawn merely from in vitro observations. In vivo experimentation reveals the plurality of *S. aureus* infection forms, which may be variously altered by novel antivirulence therapies. For instance, inhibition of the *agr* loop by action on the autoinducing peptide impedes acute tissue destruction[79] but might promote biofilm formation and chronic infection.[87,88]

Ecologic and Epidemiologic Implication of *agr*

Genetic and functional experiments revealed the existence of at least four *agr* groups in *S. aureus,* which were characterized by specific variations in all three AgrB, AgrD, and AgrC proteins (see Fig. 194.3).[89] Whereas the autoinducing peptide of a given *agr* group stimulated signaling in other strains sharing the same *agr* group, it either cross-inhibited (e.g., group I and group IV) or cross-activated (e.g., group I and group II) members of other groups. This suggests that certain antagonistic *agr* groups could be mutually exclusive with attempts to simultaneously colonize the same niche. However, studies regarding this hypothesis gave conflicting results. In particular, patients with cystic fibrosis colonized with *S. aureus* can successfully harbor organisms from two antagonistic *agr* groups.[90]

Although *agr* and other global regulators control the timely expression of pathogenic genes, they are not bona fide pathogenic factors themselves. The *agr* locus has homologues in numerous nonpathogenic staphylococci. A phylogenic study of nonpathogenic CoNS indicated that variations in *agr* genes followed parallel variations in species-specific rRNA genes.[91] In fact, *agr* groups diverged very early during the evolution of staphylococci (see "Comparative Genomics and Evolution") and represent a lineage marker of strains that evolve in distinct environments rather than a strategy to exclude potential competitors. Thus, global regulators were originally meant to control the expression of useful metabolic genes. How exogenous virulent genes, which were acquired later, succeeded in taking advantage of such systems remains a fascinating question of evolutionary genetics.

The Journey to Invasive Disease

Although *S. aureus* is an innocuous resident of the skin and mucosal flora in up to 30% of the human population,[20–22] healthy carriers are notoriously more prone than noncarriers to develop invasive *S. aureus* infections. This is exemplified by recurrent skin and wound or bloodstream infections (BSIs), which are due to the patient's own carriage strain in up to 80% of the cases.[21,24]

Colonization of the anterior nares is ideal for microbial dissemination. Outward dissemination is illustrated in Fig. 194.5. A few drops of fluorescein were instilled intranasally in a volunteer, followed by ultraviolet imaging. Two hours after instillation, fluorescein was all over the hands and clothes, ensuring both sneezing-induced and contact dissemination.

Inward dissemination by host invasion is an opportunity for ample bacterial proliferation. Host invasion is often considered a bacterial dead end, because invading microorganisms may be destroyed by the immune system. However, this only holds true if the immune system can eliminate the invading organisms, which is mostly not the case with *S. aureus* (see "Immune Evasion" later). Alternatively, invading microbes can kill the host, but then incur the risk of disappearing with the decaying corpse. Nevertheless, although this is expected in humans wherein dead bodies are eliminated through burial or cremation, it is different in the wild, where scavengers eat corpses, thus contributing to further dissemination. As a result, *S. aureus* has little evolutionary pressure to dampen its invasive lifestyle—which comes in addition to commensalism—whereas it has ample reasons to withstand host defenses, including resistance to antibiotics.

Mucosal and Skin Colonization

Persistent mucosal and skin colonization is critical. Factors involved in attachment to nasal epithelia involve teichoic acids,[92] which may attach

FIG. 194.5 Example of environmental spread of bacteria colonizing the nose. A few drops of fluorescein were instilled in the anterior nares of a volunteer, who was left to go about his routine activities and was photographed under ultraviolet light 2 hours later. Fluorescein is found all over face, hands, and clothes, exemplifying the appropriateness of colonizing the nose to ensure rapid microbial spread.

to lectin glycoproteins on the surface of mucosal cells, fibrinogen-binding protein B (clumping factor B or ClfB), serine-aspartate rich proteins C and D (SdrC and SdrD), SasG, and IsdA (Table 194.3).[93–95] ClfB, SdrC, SdrD, SasG, and IsdA are members of a family of *S. aureus* surface-bound proteins referred to as MSCRAMMs (for matrix surface components recognizing matrix molecules).[96] MSCRAMMs are covalently attached to the *S. aureus* peptidoglycan via the membrane-bound transpeptidase sortase A (SortA) (Fig. 194.6). SortA-attached proteins include at least 21 members,[97] some of them having functions different than host-matrix adherence (see Table 194.3). However, all to them are recognized by SortA at an LPXTG signature motif, cleaved by SortA between LPXT and G and covalently attached to the last G (glycine) residue of the peptidoglycan pentaglycine side chain (see Fig. 194.6).[98,99]

ClfB, SdrC, and SdrD are able to bind fibrinogen and keratin in vitro. SdrD was also shown to bind desmoglein 1, a desmosomal transmembrane protein that binds epidermal cells to keratin,[100] which is likely to facilitate *S. aureus* persistence in deeper layer of keratinized epithelia. The epithelial ligand of SasG is not known, but SasG is involved

TABLE 194.3 *Staphylococcus aureus* MSCRAMMs Belonging to Sortase-Mediated Cell Wall–Associated Proteins

GENE	PROTEIN	AA	SORTASE	MOTIF	LIGAND SPECIFICITY	POTENTIAL IMPLICATION IN DISEASE
Spa	Protein A	508	SrtA	LPETG	Antibody Fc fragment (IgG, IgM) von Willebrand factor, TNFR1, platelets	Experimental sepsis, experimental osteoarthritis
clfA	Clumping factor A	933	SrtA	LPDTG	Fibrinogen, platelets	Experimental endocarditis
clfB	Clumping factor B	913	SrtA	LPETG	Fibrinogen, cytokeratin 10, platelets	Colonization of nasal mucosa
cna	Collagen-binding protein	1183	SrtA	LPKTG	Collagen	Experimental osteomyelitis, septic arthritis
fnA	Fibronectin-binding protein A	1018	SrtA	LPETG	Fibronectin, fibrinogen, elastin Platelets	Experimental endocarditis Cell invasion, experimental mastitis
fnB	Fibronectin-binding protein B	914	SrtA	LPETG	Fibronectin, fibrinogen, elastin, platelets	Experimental mastitis
sdrC	Serine-aspartate repeat protein	947	SrtA	LPETG	Fibrinogen, cytokeratin of nasal epithelia	Nasal colonization
sdrD	Serine-aspartate repeat protein	1315	SrtA	LPETG	Fibrinogen, desmosomal desmoglein	Nasal, deep skin colonization, biofilm
sdrE	Serine-aspartate repeat protein	1166	SrtA	LPETG	Bridges fibrinogen and complement factor H on the *S. aureus* surface	Immune evasion
pls	Plasmin-sensitive protein	1637	SrtA	LPDTG	Cellular lipids, ganglioside M3; nasal epithelial cells	Colonization of nasal mucosa
sraP (or sasA)	Serine-rich adhesin for platelets	2261	SrtA	LPDTG	Platelets	Experimental endocarditis
IsdA	Iron-regulated surface determinant A	354	SrtA	LPKTG	Fibrinogen, fibronectin	Nasal colonization
(sasE)					Hemoglobin/transferrin	
IsdB	Iron-regulated surface determinant B	645	SrtA	LPQTG	Hemoglobin/hemin	Experimental bacteremia and renal abscesses
(sasJ)						
isdC	Iron-regulated surface determinant C	227	SrtB	NPQTN	Hemin	Experimental bacteremia and renal abscesses
isdH (or haR)	Iron-regulated surface determinant H	895	SrtA	LPKTG	Haptoglobin/hemoglobin complex	Nasal colonization
sasI	Putative *S. aureus* surface protein I				Undetermined	Associated with bovine gangrenous mastitis strains
sasB	*S. aureus* surface protein B	937	SrtA	LPDTG	Undetermined	—
sasC	*S. aureus* surface protein C	2186	SrtA	LPNTG	Intercellular adhesion	Involved in biofilm
sasD	*S. aureus* surface protein D	241	SrtA	LPAAG	Undetermined	Involved in biofilm
sasF	*S. aureus* surface protein F	637	SrtA	LPKAG	Undetermined	—
sasG	*S. aureus* surface protein G	1117	SrtA	LPKTG	Nasal epithelial cells	Associated to invasive disease
Sas (or adsA)	*S. aureus* surface protein H	308	SrtA	LPKTG	Cell wall associated adenosine synthase	Escape phagocyte-induced killing
sasK	*S. aureus* surface protein K	211	SrtA	LPKTG	Undetermined	—
fmtB	Formyl transferase B		SrtA	LPXTG	Cell wall synthesis, β-lactam resistance	Antibiotic resistance

AA, Protein length in amino acids; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *MSCRAMMs,* microbial surface components recognizing adhesive matrix molecules; *Srt,* sortase; *TNFR1,* tumor necrosis factor receptor 1.
Modified from Roche FM, Massey R, Peacock SJ, et al. Characterization of novel LPXTG-containing proteins of Staphylococcus aureus *identified from genome sequences.* Microbiology. *2003;149:643–654; Clarke S, Foster S. Surface adhesins of* Staphylococcus aureus. Adv Microb Physiol. *2006;51:187–224; and Dedent A, Marraffini L, Schneewind O. Staphylococcal sortases and surface proteins. In: Fischetti V, Novick RP, Ferretti J, et al, eds.* Gram-Positive Pathogens. *2nd ed. Washington, DC: ASM Press; 2006:486–495.*

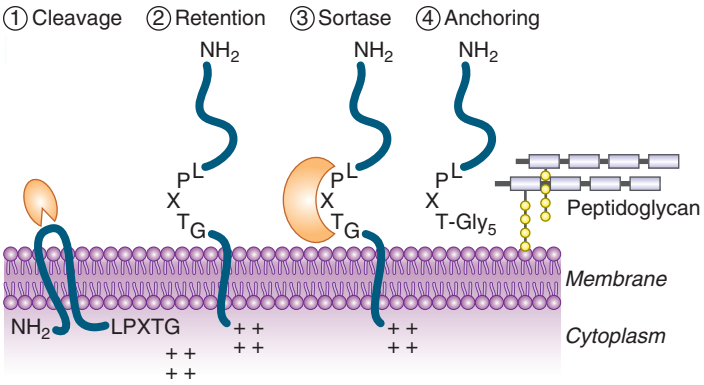

FIG. 194.6 Anchoring of gram-positive surface proteins to peptidoglycan through sortase-mediated processing of LPXTG consensus motif. During membrane translocation, amino-terminal leader sequence is clipped off. Protein is then transiently retained on cell surface through membrane-anchor domain, rich in positively charged amino acids at its intracellular carboxyl-terminal portion. LPXTG consensus region is then processed by sortase that clips between threonine and glycine (T-G) and transfers covalent bond to glycine acceptor in peptidoglycan meshwork. *(Modified from Fischetti VA, Pancholi V, Schneewind O. Conservation of a hexapeptide sequence in the anchor region of surface proteins from gram-positive cocci.* Mol Microbiol. *1990;4:1603–1605; Mazmanian SK, Ton-That H, Schneewind O. Sortase-catalysed anchoring of surface proteins to the cell wall of* Staphylococcus aureus. Mol Microbiol. *2001;40:1049–1057; and Bradshaw WJ et al. Molecular features of the sortase enzyme family.* FEBS J. *2015;282:2097–2114.)*

in biofilm formation, which is an ingredient of local persistence.[101] Likewise, the exact role of IsdA in nasal colonization is unclear. However, because IsdA is a heme-binding protein,[102] it might help acquire essential iron for the colonizing bacteria.[95] Finally, *S. aureus* can also survive in a dormant state inside nasal epithelial cells.[103] This is one reason, in addition to biofilm, that explains why it is difficult to eradicate chronic carriage, especially with antimicrobials that do not penetrate inside eukaryotes cells—for instance, β-lactams.

Whether the fibrinogen-binding capacity of ClfB, SdrC, and SdrD may facilitate further invasion in case of mucosal or skin breaches is not known. On the other hand, SasG (among few others) elicited an antibody response in patients with invasive *S. aureus* diseases,[97] a finding that supports its involvement in deep-seated infections.

Most interestingly, there is an as yet unexplained privileged liaison between *S. aureus* and chronic nasal carriers. In one study, *S. aureus* carrier and noncarrier volunteers had the nose disinfected and reinoculated with a mixture of four *S. aureus* strains, including the carriage strain in case of chronic carriers. Over a few days, noncarriers tended to eliminate all inoculated strains, whereas chronic carriers eliminated all foreign strains and reselected their own.[104]

In certain occurrences, mucosal or wound colonization with *S. aureus* may produce distant diseases such as SSSS or TSS. These issues are discussed in dedicated sections later.

Taken together, although the subtle relation between *S. aureus* and its host remains incompletely solved, it is critical to detect and eliminate *S. aureus* carriage in groups at risk of severe infection, such as patients undergoing operation,[26] dialysis patients,[105] and possibly patients with prosthetic heart valves, in whom the 1-year mortality of *S. aureus* valve infection is up to 50%.[106]

Host Invasion

Because *S. aureus* is nonmotile, invasion takes advantage of mucosal or skin breaches, where the microorganism engages with constituents of deeper tissue and blood compartments. The first encounters are constituents of microthrombi, which occur as a normal healing process of tissue breaches. *S. aureus* avidly binds to soluble fibrinogen and clotted fibrin via clumping factor A (ClfA)[107] and the fibrinogen-binding domain of fibronectin-binding protein A (FnBPA).[108] ClfA and FnBPA are SortA-LPXTG wall-associated MSCRAMMs (see Fig. 194.4 and Table 194.3), that serve at least two purposes. On one hand, they encourage *S. aureus* attachment at the place of preexisting lesions.[81] On the other hand, they also act as immune camouflage factors against complement-induced phagocytosis by surrounding the bacterium with a shield of soluble fibrinogen and fibronectin (see Table 194.3).[109]

Once attached to microthrombi, *S. aureus* may encourage further thrombus formation via the action of two secreted coagulases—coagulase (Coa) and von Willebrand–binding protein (vWbp). Coa and vWbp bind to prothrombin and induce a conformational change that converts it into active staphylothrombin.[110] Staphylothrombin is unique in that it polymerizes fibrinogen into fibrin and activates platelets even in blood anticoagulated via coumarin therapy, or exposed to heparin or calcium chelators.

Then, in order to avoid local trapping and hamper further spread, *S. aureus* needs to control local coagulation. To this end, coagulase is only transiently produced in the early exponential phase of growth. Moreover, *S. aureus* can escape clots by secreting staphylokinase (Sak), a protease that activates host plasminogen into active plasmin, which in turn disintegrates fibrin clots[111] and promotes extension of local infections.[112] Sak also cleaves complement opsonin C3b and preformed antibodies, contributing to the antiphagocytic properties of *S. aureus* (see "Immune Evasion" later). Sak is produced in both the early exponential and the late stationary growth phases. Its gene *(sak)* is located, together with SCIN (for staphylococcal complement inhibiting protein) and CHIPS (for chemotaxis inhibitory protein of *Staphylococcus*), on a so-called immune escape cluster (IEC) as part of a ϕSa3 β-hemolysin–converting prophage, which is present in >90% of human *S. aureus* isolates, but usually not in animal isolates.[113,114] Thus, the ϕSa3 prophage and its IEC cargo are believed to participate to the *S. aureus* specificity for human hosts.

Contribution of Coagulation

It was originally thought that *S. aureus* surface MSCRAMMs would mediate direct binding to ligands present in target organs, such as binding to collagen via collagen-binding protein (Cna) in osteoarthritis.[115,116] However, in the case of hematogenous dissemination, reaching the target organ requires prior *S. aureus* extravasation from the microcirculation. It was shown that ClfA and FnBPA are critical to colonize and invade damaged or inflamed endothelia, and this presumably occurred by direct attachment to the injured tissues.[81] However, in the bloodstream, ClfA and FnBPA become rapidly saturated with soluble fibrinogen and fibronectin, which interfere with direct binding to injured vessels.[109] Thus there is a missing link, which *S. aureus* circumvent by hijacking the coagulation system.[117]

While circulating *S. aureus* organisms become saturated with soluble fibrinogen and fibronectin, ClfA and FnBPA induce a fibrinogen conformational change that triggers its docking to the platelet GPIIb/IIIa receptor and activates platelets.[118] In addition, preexisting anti-ClfA or anti-FnBPA antibodies, if present, activate platelets by docking to the immunoglobulin G (IgG) platelet receptor FcγRIIa. These microaggregates are then conveyed through the blood to inflamed endothelia or to nascent platelet-fibrin clots present on injured tissues, to which activated platelets attach.

The convergence of these *S. aureus*–platelet microaggregates to inflamed endothelia is further strengthened by the second staphylocoagulase vWbp.[110] vWbp has the dual capacity to activate blood coagulation and to bind endothelial-attached von Willebrand factor (vWF). vWF is secreted as monomer by inflamed endothelia or activated platelets. vWF monomers attach to injured tissues and polymerize into discrete strings floating in the vascular flow, which bind activated platelets and microaggregates in a sheer-dependent manner.[119] *S. aureus*–secreted vWbp binds on one hand to the floating WWF strings, and on the other hand to *S. aureus*–attached ClfA,[120] thus also favoring the halt of circulating *S. aureus* onto inflamed endothelia.

This platelet-staphylothrombin scenario was validated in experimental models of endovascular colonization and endocarditis, in which prophylaxis with antiplatelet (acetylsalicylic acid and ticlopidine) or antithrombin (dabigatran) agents successfully prevented *S. aureus* endovascular infections.[121,122] In contrast, coumarin anticoagulation,

which relies on a different mechanism, did not prevent experimental endovascular infection.

Of note, platelet activation may be a double-edged sword in that platelet degranulation produces platelet-microbicidal peptides (PMP) that destabilize bacterial membranes and can kill bacteria.[123] However, *S. aureus* strains that produce successful endovascular infection are known to resist PMP-induced killing via plasma membrane modification.[124,125]

Eventually, inflamed endothelial cells also express α5β1 integrins, which bind soluble fibronectin and normally act as a landing runway for neutrophils. However, α5β1-bound fibronectin also promotes *S. aureus* attachment via FnBPA, which triggers active bacterial internalization by endothelial cells.[81] Local tissue destruction ensues, and more specific molecules can enter into action, including specific MSCRAMMs (e.g., Cna),[115,116] biofilm facilitating factors,[126] hemolysins, and immune evasion molecules.

Immune Evasion

Along with invasion and tissue colonization, *S. aureus* has to confront several layers of host defenses against which it applies an extremely sophisticated immune evasion armamentarium, which is briefly described in the following sections (Table 194.4) (for review, see Foster,[9] Kim and colleagues,[127] and Guerra and colleagues[128]).

Escaping Phagocytosis

The first line of anti–*S. aureus* host defense is phagocyte engulfment, primarily by neutrophils, either by direct recognition of pathogen-associated molecular patterns (PAMPs),[129] or via complement-mediated opsonization. Direct PAMP recognition is hampered by the production of polysaccharidic capsules (mostly type 5 or 8 in human *S. aureus* isolates), which are not recognized by professional phagocytes and physically block their access to underlying PAMPs, such as teichoic acids, lipoteichoic acids, peptidoglycan, and even C3b complement opsonins attached to these PAMP structures.

In addition, *S. aureus* uses several secreted and SortA-LPXTG anchored surface factors to counters phagocytosis. Secreted factors include the chemotaxis inhibitory protein CHIPS[111] and the extracellular adherence protein Eap (or Map).[130] CHIPS belongs to the ϕSa3 prophage IEC and blocks the neutrophil receptor for formyl-peptides, a universal signature of bacterial protein synthesis, and the C5a receptor for chemotaxis.[111] Eap binds intercellular adhesion molecule 1 (ICAM-1) and fibrinogen and vitronectin, and blocks leukocyte adhesion and neutrophil recruitment mediated by β2-integrin and urokinase receptors in vitro and in vivo.[130]

SortA-LPXTG anchored molecules include protein A (Spa), ClfA, and adenosine synthase A (AdsA, also called SasA) (see Tables 194.3 and 194.4). Spa blocks antibody-mediated phagocytosis by binding IgGs by their Fc fragments and exposing the Fab fragments instead, which are not recognized by complement.[127] ClfA interferes with phagocytosis in a fibrinogen-dependent manner (probably by bacterial shielding) and an as yet unclear fibrinogen-independent manner.[131] AdsA converts adenosine monophosphate into adenosine, a dual immuno-modulator compound that has proinflammatory antiinflammatory properties.[132] *S. aureus*–generated adenosine was shown to impede neutrophil-mediated bacterial clearance and to promote abscess formation in a mouse model of sepsis and kidney abscess.[133]

Luring Complement

If not directly triggered by PAMPs, phagocytosis may be promoted by complement-mediated opsonization. The lectin and the alternative complement pathways are mainly involved against *S. aureus*. The classical pathway, which requires prior antibodies, is largely hampered protein A (Spa) and Sak, as mentioned earlier,[9] and by secreted staphylococcal binder of immunoglobulin (Sbi).[134] Sbi is both secreted and loosely attached to the bacterial envelope. Its envelope-attached form binds immunoglobulin Fc fragments similarly to Spa, and its secreted form binds complement factor H and C3d, which accelerate the decay of preopsonin C3. Soluble Sbi-C3d-factor H complexes also bind the complement receptor CR2 of B lymphocytes, promoting their apoptosis and impeding antibody production.[127] In addition, direct complement-induced bacterial killing via the C8-C9 polymerization membrane attack complex (MAC) is not effective against gram-positive bacteria, because their plasma membrane is physically protected from MAC by the thick peptidoglycan cell wall (for review see Zipfel[135]).

The lectin and alternative complement pathways are triggered by PAMPs, which activate the lectin or alternative pathway-dependent convertases C4b2a and C3bBb. The convertases cleave C3 into C3a, which amplifies the chemoattractant loop, and C3b, which binds to staphylococcal teichoic acids and attracts phagocytes.[135] *S. aureus* counteracts complement-mediated opsonization by means of several mechanisms. First, as mentioned earlier, it can produce polysaccharidic capsules, hindering phagocyte access to teichoic acid–attached C3b. Second, it secretes Sak, which cleaves C3 and C3b. Third, it produces staphylococcal complement inhibitory protein SCIN, which binds to and inhibits the C4b2a and C3bBb convertases, thus blocking the production of C3a and C3b.[111] Like Sak, SCIN and CHIPS are located on the ϕSa3 prophage EIC.[113] They are expressed in the exponential phase of growth, whereas Sak is also expressed later in the late stationary growth phase.[111]

A fourth mechanism involves secreted extracellular fibrinogen binding protein (Efb), a dual adhesin capable of binding bacterial-attached C3b proximally, and soluble fibrinogen distally. As a result, Efb contributes an additional external fibrinogen shield, preventing the contact of neutrophils with C3b.[136]

Finally, a most astounding host-hijacking mechanism is conferred by SortA-LPXTG anchored SdrE. In addition to binding fibrinogen in vitro, SdrE binds to and attracts complement factor H on the *S. aureus* surface.[137] Factor H is a complement regulatory protein that normally binds to host cells and accelerates the decay of C3b in order to protect them from nonspecific assaults from self-host defenses. By attracting factor H on the *S. aureus* surface, SdrE usurps the complement host control system to its advantage.

Resisting Oxidative Burst

Activated neutrophils trigger oxidative burst and bacterial killing via NADPH oxidase and myeloperoxidase (MPO), or via nitric inducible oxide synthase (iNOS).[138] The cascade uses superoxide (O_2^-) to produce highly oxidative molecules such as H_2O_2 or hypochlorous acid (HOCl). Oxidation results in protein, lipid, and nucleic acid damage that can kill pathogens either extracellularly or inside phagolysosomes. Extracellular oxidative burst is exemplified by the neutrophil extracellular traps (NETs), which are constituted of neutrophil granules and chromatin proteins and contain up to 80% of neutrophil-released MPO.[139] *S. aureus* can disable these mechanisms by reducing enzymes such as catalase, which converts H_2O_2 to water and O_2, or direct rescue of oxidized molecules by means of several reducing agents listed in Table 194.4.

Resisting Antimicrobial Peptides

Insects and animals produce an array of antimicrobial peptides (AMPs) consisting most often of 20– to 100–amino acid pore-forming β-sheet structures.[140] Human produces various AMPs in skin and mucosal tissues, and large quantities that are stored in granules of neutrophils and platelets (see "Contribution of Coagulation" earlier). A hallmark of these AMPs is that they are positively charged and are attracted by the negatively charged wall teichoic acids (Fig. 194.7) and membrane phospholipids of the gram-positive bacterial envelope. *S. aureus* modulates its susceptibility to AMPs by modulating its surface charge, either by means of the D-alanine lipoteichoic acid ligase *(dlt)* operon, which decorates teichoic acids with alanine residues, or by means of a lysyltransferase that transfers lysine residues to membrane phospholipids.[141,142] Both mechanisms result in a more positively charged bacterial envelope and thus in AMP repulsion. In particular, successful endocarditis *S. aureus* strains were shown to be consistently resistant to platelet-secreted PMPs,[125] a property that may discourage the development of AMPs for therapeutic purposes.

Killing Leukocytes

S. aureus kills eukaryotic cells via secreted hemolysins, leukocidins, and phenol-soluble modulins (PSMs).[11,143,144] There are four types of hemolysins, referred to as α-hemolysin (Hla), β-hemolysin (Hlb), δ-hemolysin (Hld), and γ-hemolysin (Hlg).

TABLE 194.4 Main Immune Evasion Determinants

INTERFERENCE WITH	DETERMINANT	LOCATION	FREQUENCY IN *STAPHYLOCOCCUS AUREUS* ISOLATES	ACTION
Neutrophil chemotaxis, migration and phagocytosis	Polysaccharidic capsule (mainly types 5 and 8)	Core genome	20%–60%	Not recognized as PAMPs Steric blockage of neutrophil access to deeper cells wall structures including wall attached C3 and C3b
	CHIPS (chemotaxis inhibitory protein of *Staphylococcus*)	ϕSa3 IEC	>60%	Blocks neutrophil C5a receptor Blocks neutrophil formyl-peptide receptor
	Eap (or MAP) (extracellular adherence protein)	Core genome	>95%	Interferes with neutrophil migration and extravasation by blocking the docking of neutrophil LFA-1 to endothelial ICAM-1 (See anticomplement activity below)
	AdsA (or SasH) (adenosine synthase A)	Core genome	70%–80%	Converts adenosine monophosphate to adenosine Interferes with inflammation and phagocytosis
Complement	SdrE (serine-aspartate repeat protein)	Core genome	40%–60%	Antiopsonic Binds factor H on the *S. aureus* surface
	SCIN	ϕSa3 IEC	>60%	Antiopsonic Inhibits lectin and alternative complement pathways Binds to and inhibits the C3 convertases C4b2 and C3bBb, thus blocking the generation of the C3b opsonin
	Sak (staphylokinase)	ϕSa3 IEC	>60%	Antiopsonic Converts plasminogen into plasmin which cleaves fibrin (solubilizing clots), IgGs, and bacterial attached C3b (See anti-AMP activity below)
	Eap (or MAP) (extracellular adherence protein)	Core genome	>95%	Antiopsonic Inhibits classical and lectin complement pathways Binds to and inhibits C3 convertase C4b2 and further C3b-mediated opsonization
	Efb (extracellular fibrinogen-binding protein)	IEC2	60%–70%	Antiopsonic Binds C3 components and fibrinogen Binds to staphylococcal wall-attached C3b and attracts plasma fibrinogen over it, thus shielding it from recognition by neutrophils
	Ecb (Extracellular complement binding protein)	IEC2	>95%	Antiopsonic Blocks binding of the neutrophil complement receptor CR1 to bacterial-attached C3b, thus preventing phagocytosis
Oxidative burst	SodA and SodM (super oxide dismutases)	Core genome		Convert superoxide radicals to H_2O_2 and O_2 (using manganese as a co-factor)
	KatA (catalase)	Core genome		Converts H_2O_2 into H_2O and O_2
	AhpC and AhpF (alkyl hydroperoxide reductases)	Core genome		Convert H_2O_2 to H_2O and O_2 Convert alkyl hydroperoxides to alcohol and water
	bNOS (bacterial nitric oxide synthase)	Core genome		Scavenges HOCl (hypochlorous acid).
	Bacillithiol Coenzyme A Staphyloxanthin	Core genome		Protection by *S*-thiolation of oxidants Antioxidant mechanism unclear
Antimicrobial peptides (AMPs)	Dlt (D-alanine (lipo)teichoic acid ligase)	Core genome	>95%	Neutralizes negatively charged wall surface by alanine substitution of ribitol teichoic and lipoteichoic acids Decreases surface affinity for positively charged AMPs
	MprF (muropetide resistance factor)	Core genome	>95%	Idem by adding L-lysine residues to phosphatidylglycerol at the extracellular side of the plasma membrane
	Sak (staphylokinase)	ϕSa3 IEC		Proteolytic degradation of fibrin (via plasmin activation), C3 components, IgGs, and AMPs
	Aur (aureolysin)	Core genome		Proteolytic cleavage of cathelicidin AMPs
Leukocyte lysis	Hla (α-hemolysin)	Core genome		Forms heptamer barrels in the plasma membrane of target cells
	Hlg (γ-hemolysin)	Core genome	>95%	Bicomponent leukocidin Lyses both erythrocytes and leukocytes
	Luk E/D (leukocidin E/D)	Genomic island beta	30%–40%	Synergohymenotropic bicomponent leukodicin
	Luk F/M (leukocidin F/M)	ϕSa1		Synergohymenotropic bicomponent leukocidin
	Panton-Valentine leukocidin	ϕSa2	2%–4%	Synergohymenotropic bicomponent leukocidin
	Hld (delta-hemolysin)	Core genome	>95%	Idem phenol-soluble modulins (PSMs) below
	PSM alpha 1–4	Core genome	100%	Phagocyte lysis by membrane destabilization
	PSM beta-1 and 2 (phenol-soluble modulins)			Mechanism analogous to the delta-hemolysin mechanism of membrane damage

AMPs, Antimicrobial peptides; *ICAM-1,* intercellular adhesion molecule 1 (ligand of LFA-1); *IEC,* immune escape cluster; IgG, immunoglobulin G; *LFA-1,* lymphocyte function-associated antigen h1; *MAP,* major histocompatibility complex class II analogous protein; *PAMPs,* pathogen associated molecular patterns; *ϕSa1* to *ϕSa3,* *Stapylococcus* prophages 1 to 3; *SasH, Staphylococcus* surface protein H; *SCIN,* staphylococcal complement inhibitory protein.

Modified from McCarthy AJ and Lindsay JA. S. aureus *innate immune evasion is lineage-specific: a bioinfomatics study. Infect Genet Evol. 2013;19:7–14; Lindsay JA.* S. aureus *evolution: lineages and mobile genetic elements (MGEs). In: Lindsay J, ed.* Staphylococcus aureus *Molecular Genetics. Norfolk, UK: Casiter Academic Press; 2008:45–69; and Peakock SJ, et al. Virulent combinations of adhesin and toxin genes in natural populations of* Staphylococcus aureus. *Infect Immun. 2002;70:4987–4996.*

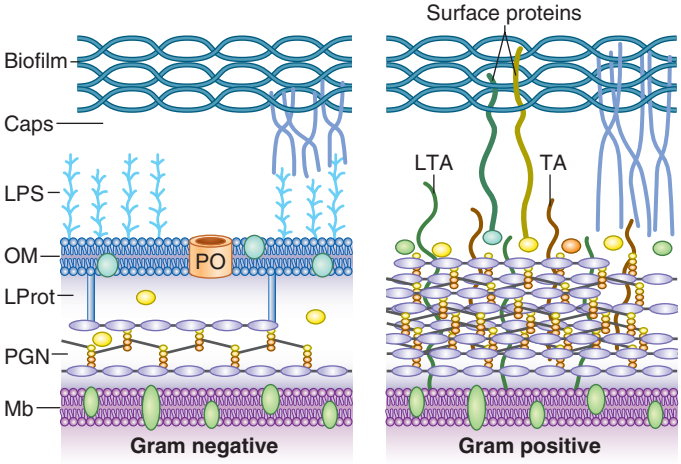

FIG. 194.7 Schematic representation of gram-negative *(left)* and gram-positive *(right)* bacterial envelopes. Gram-negative bacteria have very thin peptidoglycan *(PGN)* and an outer membrane *(OM),* made of lipopolysaccharide *(LPS),* which are not present in gram-positive bacteria. Peptidoglycan is very conserved and constituted of glycan chains made of *N*-acetylglucosamine and *N*-acetylmuramic acid disaccharide subunits, in which the *N*-acetylmuramate moiety is linked to highly conserved pentapeptide or tetrapeptide stems (L-alanine–D-isoglutamine–L-lysine–D-alanine–D-alanine). The chains of disaccharide peptide are cross-linked via peptide bridges between the penultimate D-alanine and the diamino acid L-lysine located in position 3 of a neighboring stem peptide. In *S. aureus,* the interpeptide bridge typically contains a polyglycine linking piece that comprises one to five glycine residues. The addition of glycines to the wall precursors is driven by the *femABC* and *fmhB* genes (see Fig. 194.13). Teichoic acids *(TA)* represent up to 50% of the dry weight of purified staphylococcal walls and are made of polyribitol-phosphate polymers cross-linked to *N*-acetylmuramic acid residues of the peptidoglycan and decorated with D-alanine and *N*-acetylglucosamine residues. Lipoteichoic acids *(LTA)* are the plasma membrane–bound counterparts of teichoic acids. They are made of polyglycerol phosphates chains and are linked to a diacylglycerol moiety that serves as a plasma membrane anchor. TA and LTA are not present in gram-negative bacteria. *Caps,* Capsule; *Lprot,* lipoprotein; *Mb,* plasma membrane; *PO,* porin. *(Modified from Gotz F, Bannerman T, Schleifer K-H. The genera* Staphylococcus *and* Macrococcus. *In: Balows A, Truper AG, Dworkin M, et al, eds.* The Prokaryotes. *Vol. 4. New York: Springer Science+Business Media; 2006:41–75.)*

FIG. 194.8 Fulminant hemorrhagic pneumonia in 20-year-old patient infected with Panton-Valentine leukocidin–producing *Staphylococcus aureus*.

Hla and Hld are secreted in nontoxic soluble forms and multimerize on eukaryotic membranes to form lytic pores. α-Hemolysin (or α-toxin) is involved in a great variety disease.[145,146] It multimerizes as heptamers on phosphocholine-containing membranes, a process which depends on the presence of host cell transmembrane protein ADAM10, which reunifies both metalloprotease and disintigrin (integrin-binding) properties.[147] Moreover, α-hemolysin interferes with adherens junction proteins to induce cell killing, most notably plekstrin-homology domain-containing protein 7 (PLEKHA7). Indeed, PLEKHA7-deficient cells can readily recover from Hla cytotoxicity.[148] Hence, polymorphism in this determinant could influence individual susceptibility to infection.

Hld acts in a similar way and belongs to the same family as PSMs, which were described more recently.[144] There are four PSMα types (PSMα_{1-4}), consisting of approximately 20 amino acids, and two PSMβ types (PSMβ_{1-2}), consisting of approximately 40 amino acids, the genes for which are located on the staphylococcal chromosome. Hld is located upstream of *agr* RNAIII regulatory RNA (see "Regulation" section). The structure of Hld and PSMs consists of amphipathic α-helices, which confer several properties aside from membrane cell damage, including biofilm turnover and inflammatory responses.[149]

Hlb is distinctive because it is a sphingomyelinase that damages membranes by means of enzymatic alteration of their lipid content.

Hlg is also peculiar in that it is composed of two types of proteins called S and F, for slow and fast elution at chromatography. It promptly lyses white blood cells in addition to other cells and is sometimes referred to as leukocidin. It is encoded by two distinct operons, one that encodes a unique HlgA (S protein) and another that encodes for one S protein (HlgC) and one F protein (HlgB). S and F proteins must assemble to form membrane-perforating complexes. Therefore, this class of hemolysins is also referred to as synergohymenotropic toxins. Active α-Hemolysin exists in two bioactive forms: HlgA-HlgB and HlgA-HlgC.

Panton-valentine leukocidin. PVL is a peculiar homologue of Hlg, which was originally reported in 1932 by Panton and Valentine.[150,151] PVL is encoded by two genes, *lukS* and *lukF,* which can assemble either between themselves or with the components of Hlg, thus producing chimera complexes. Like the other hemolysins, PVL is regulated by *agr* (see Table 194.1). Unlike the other hemolysins, PVL is encoded by mobile phages, including ϕSLT, ϕSa2958, ϕSa2MW, ϕPVL, ϕ108PVL, ϕ7247PVL, ϕSa119, ϕTCH60, and ϕSa2USA, which can transfer PVL to other strains.[151,152] Also unlike the other hemolysins, the prevalence rate of PVL is usually low (≤2%) in MSSA and health care–associated MRSA (HCA-MRSA),[150] whereas it is present in almost 100% of isolates of the community-acquired MRSA (CA-MRSA) USA300 cluster, which is peculiarly prevalent in North America.[151,153]

PVL-producing *S. aureus* is associated with skin and soft tissue infection (SSTI) and severe hemorrhagic pneumonia in children and young adults.[154] In contrast, it is rarely responsible for other infections, such as osteomyelitis, septicemia, and endocarditis. The reason for clustering in young patients is unclear. The clustering could be linked to an age-related permissive milieu or permissive immunologic window. Nevertheless, the connection is important; a young adult with recurrent boils and pneumonia should receive particular attention because the mortality rate of hemorrhagic lung disease is high (Fig. 194.8).

Escaping Cell-Mediated Immunity

Among the first lines of skin and mucosal innate defenses are γ/δ T cells and antigen-presenting Langerhans cells. γ/δ T cells are not major histocompatibility complex (MHC) restricted and respond to epithelial stress and injury. They promote healing via the production of growth factors and attract neutrophils and T cells via the production of IL-17A,[155] which also upregulates the production of AMPs.[156] Stimulation of

neutrophils by IL-17A decreased the severity of experimental *S. aureus* SSTIs and facilitated *S. aureus* nasal eradication.[156,157] Thus, γ/δ T cells and the production of IL-17A comprise an important nonspecific first-line defense against invading microbes. Besides, Langerhans cells should phagocytose invading organisms and present surface antigens to boost humoral immunity, thus completing the continuum from innate to acquired host immunity.

However, *S. aureus* is well equipped to counter recognition by phagocytes and migration of neutrophil and lymphocytes, and impede cytokine-mediated cell recruitment and antibody production, including Eap (or Map), which interferes with lymphocyte migration,[130] Spa, Sbi,[134] and Sak (see "Escaping Phagocytosis" earlier). In addition, the most impressive interference of *S. aureus* with cell immunity is the ubiquitous production of SAgs, which trigger massive and nonspecific activation of the T-lymphocyte compartment, resulting in TSS.[2] One consequence of this T-cell distraction is immune paralysis and anergy. SAgs also aggravate atopic dermatitis and psoriasis by promoting local inflammation, serum suffusion, and access to nutrients.[158] SAgs, of which TSST-1 is a paradigm, are discussed in the "Superantigens" section later.

Producing Biofilm

Biofilm is an ultimate way to settle and escape host defenses. It consists in an extracellular polysaccharidic and proteinaceous meshwork that gathers bacterial communities within a mechanically cohesive scaffold. Biofilm-trapped bacteria cannot be physically phagocytized, a phenomenon referred to as frustrated phagocytosis,[159] and are dormant.[88] As result, they are phenotypically tolerant to antibiotic-induced killing.

Biofilm formation is a major therapeutic problem.[160] It was widely described in CoNS but is also formed by *S. aureus,* especially in the settings of colonization of catheters and biomaterials. Biofilm-producing staphylococci were associated with persistence and virulence in various experimental models, including *Caenorhabditis elegans* and mice with foreign-body infection.[161]

Biofilm formation evolves in three steps, starting with nonspecific adherence of individual cells to the materials, followed by growth and biofilm formation, and ending with detachment of surface bacteria. In CoNS, it is associated with the production of polysaccharide intercellular adhesion (PIA), which consists of β-1,6-glucosamine chains that are *N*-substituted with succinate residues.[162] PIA is synthesized by an operon called *ica* composed of a regulator *(icaR)* and biosynthetic *(icaADBC)* genes.[163]

An *ica* homologue has also been described in *S. aureus.* Its role in colonizing amorphous surfaces might be identical to that shown in CoNS. However, its role in disease initiation is debated.[164] In *S. aureus,* biofilm production relates to a large network of genes including surface-attached and secreted proteins in addition to complex regulatory circuitries.[126] For instance, although biofilm deep-seated bacteria must express adherence molecules, surface bacteria must be prone to detach in order to colonize additional organs. Detachment depends on, among other factors, *agr* expression, which represses expression of adhesins and promotes that of secreted factors including PSMs.[87] In turn, PSMs are involved in remodeling biofilm surfaces and creating channels to feed inner parts of the structure.[165] Thus, *ica* could be a relatively ancestral colonization mechanism that is still present in *S. aureus* but is surpassed by more effective means.

Taken together, the myriad immune evasion strategies collected by *S. aureus* highlight its remarkable adaptation to the animal world and make it a major challenge for host defense-mediated elimination. This explains the as yet unsuccessful attempts to develop an antibacterial vaccine against it, leaving only hope for antitoxin neutralizing vaccines, which will not eradicate the bacterium but might help reduce tissue destruction and disease symptoms.

Exfoliative Toxins and Staphylococcal Scalded Skin Syndrome

SSSS is a superficial skin disorder that varies from local blistering to impressive generalized scalding (Fig. 194.9). It was originally described by the German physician Baron Gotfried Ritter von Rittershain, who published a series of 297 cases in young children in 1878.[166] Hence, the syndrome is sometimes referred to as Ritter disease. SSSS clusters in neonates and infants younger than 1 year and rarely in adults. It is typically the result of mucosal or skin colonization (e.g., umbilical cord) with a toxigenic *S. aureus* strain that produces either ETA or ETB, encoded by the *eta* and *etb* genes, respectively. The toxin genes are located either on a phage *(eta)*[8] or on a plasmid *(etb).* Two additional isoforms of SSSS toxins (exfoliative toxins C [ETC] and D [ETD]) were isolated through pathologic observations in animals and with genome screen, but seem not to be involved in humans.[167,168]

FIG. 194.9 Staphylococcal scalded skin syndrome. Blisters are expression of toxin-related (exfoliative toxin A or B) distant disease and usually do not contain microorganisms.

A US study estimated the annual incidence of SSSS to be 8 cases per million US children, increasing to 45 cases per million in children younger than 2 years.[169] The crude inpatient mortality was low (0.33%) and similar to that in children without SSSS sharing comparable clinical conditions.[169] Similar figures were reported in France.[4] SSSS is often related to *S. aureus* infections or carriage in close contacts, and may evolve as small epidemics that result from clonally related strains, usually in nurseries. Nasal carriage of the organism may be found among the medical staff, and all caretakers should be screened for this possibility. The proportion of *S. aureus* carrying *eta* or *etb* in overall staphylococcal nasal carriers or clinical isolates is low (≤2% of isolates),[170] which explains the rarity of the disease and its clustering in favorable milieus.

The toxins act by a direct effect on the stratum granulosum of the epidermis. Mucosa are never involved. This consideration is important for differential diagnosis with more severe Lyell syndrome, which usually involves mucosa.[3] Lyell syndrome, or toxic epidermal necrolysis, results from cleavage below the dermoepidermal junction. It is associated with a reaction to more than 100 drugs and sometimes vaccination and has a high fatality rate.

Molecular Pathogenesis of Staphylococcal Scalded Skin Syndrome

The toxin is released by staphylococci locally, passes through the body, and localizes at the level of the stratum granulosum. The toxin is a glutamate-specific serine protease whose molecular target is desmoglein-1 (Dsg1). Dsg1 is a transmembrane desmosomal glycoprotein that is important to maintenance of interkeratinocyte adhesion.[171] The human skin harbors four Dsg isoforms (Dsg1 to Dsg4) that are localized in various layers of the epidermis, but only Dsg1 is present at the level of the stratum granulosum and is the target of SSSS toxins, which remove its amino-terminal extracellular domain.[172]

An incompletely solved question is why the disease primarily affects children and adults with peculiar skin diseases. One hypothesis is that the toxin targets Dsg1 in the vicinity of the cell membrane ganglioside (GM4), which is present only in the skin of young children or in adults with peculiar skin diseases. This could explain the clustering of SSSS in these particular populations. GM4-like gangliosides are present in the skin of suckling mice and can inhibit the effect of the toxin when coincubated before injection to susceptible animals.[3] The toxin has a serine protease activity, but only after it has reached the skin, which

suggests that a locally induced conformational change is needed for activity.

Clinical Aspects

The two forms of SSSS are a generalized form and a localized form. In the generalized form, the toxin spreads throughout the body and localizes at the level of the skin, where it produces generalized scalding (see Fig. 194.9). The skin easily detaches by mere rubbing (Nikolsky sign). The blister liquid is clear. Because scalding is the expression of a distantly secreted toxin, the responsible staphylococci are usually not found in the lesions. The disease is self-limited and wanes within 4 to 5 days, which probably parallels the appearance of specific antitoxin immunoglobulins. Indeed, in addition to age-related expression of GM4 or other specific factors in the skin, the presence of antitoxin antibodies in older children and adults also explains the restriction of SSSS to the younger age groups.

The localized form of SSSS is sometimes referred to as bullous impetigo (Fig. 194.10). It results from the local spread of the toxin around a colonized wound in individuals who already bear some immunity against the toxin, as is the case in neonates still benefiting from passive maternal immunity (often around the umbilicus), or in older individuals who are already immunized. The presence of antibodies hinders distant dissemination of the toxin but not local spread around the colonized area. Unlike the generalized form, scalding is localized and the blister liquid often contains bacteria and sometimes white blood cells.

Patients may have general symptoms that include fever and lethargy, especially in the generalized form. Treatment includes general measures such as antiseptic wound dressing and fluid support, specific antibiotic therapy to eradicate the causative agent, and screening and decontamination of caretakers, especially in nurseries. If appropriately handled, the prognosis of SSSS in children is usually good and, as mentioned, the mortality rate far less than 5%.[169] In contrast, the mortality rate can be very high in adults (>50%) and is usually associated with an underlying condition.

As mentioned, the differential diagnosis with Lyell syndrome (toxic epidermal necrolysis) is critical because the etiology, treatment, and prognosis of the diseases are different. In doubtful cases, skin biopsy is useful to provide the definitive answer.

Superantigens

TSST-1 and staphylococcal enterotoxins (SEs) are the paradigm of a large family of pyrogenic exotoxins called *superantigens*.[2,5,173] SAgs are proteins that do not activate the immune system by means of normal contact between antigen-presenting cells and T lymphocytes. Normally, antigens are taken up by antigen-presenting cells, hydrolyzed, and presented as restricted peptides to cognate T lymphocytes. The peptides are expressed within a groove on the MHC class II receptor on the surface of the antigen-presenting cell. Cognate T cells recognize the peptide–MHC class II complex by specific contacts with the five variable domains of the α and β chain of the T-cell receptor (Vβ, Dβ, Jβ, Vα, Jα).

SAgs can bypass this highly specific interaction. They attach to an external portion of the Vβ domain from large quantities of lymphocytes and directly wedge them to the MHC class II receptors of antigen-presenting cells. This nonspecific contact activates up to 20% of the total pool of T cells, instead of approximately 1 per 10,000 during normal antigen presentation. The consequence is a massive burst in cytokine release, which drives an overwhelming inflammatory response that results in endotoxin-like shock, including endothelial leakage, hemodynamic shock, multiorgan failure, and possibly death.

S. aureus can produce a large number of SAgs. Aside from TSST-1, it can produce at least 15 different enterotoxins (SEs A, B, C_n, D, E, G, H, I, J, K, L, M, N, O), which by definition are emetic when administered to rodents. However, the nomenclature has become more complicated with the discovery of enterotoxin homologues that did not have emetic properties, and which are now called SEl, for "staphylococcal enterotoxin-like," followed by specific letterings.[173] Moreover, additional screens have revealed a family of at least 14 proteins based on homologies in the conserved C- and N-terminal domains of SAgs.[174] These proteins do not bind MHC class II molecules but can variously interact with immunoglobulins and complement. They are referred to as SSL for "staphylococcal superantigen-like" and tend to cluster together on staphylococcal pathogenicity islands (discussed in the "Genomics and Mobile Genetic Elements" section later).

Although quite some variation exists in the primary structure of many SAgs, they all share a common architecture, as shown with crystallography. They consist of A and B globular domains, which are made of β-sheet barrels and α-helices and rejoined by a discrete linking piece. In TSST-1, the region binding to the Vβ chain of the T-cell receptor has been mapped at the A-B hinge region.

A genealogy of SAgs was built on the base of their sequence homologies. The SAgs studied were segregated into five groups.[5] Group I was represented only by TSST-1. Group III contained only staphylococcal SAgs (SEs H, I, K, L, and P), and group IV contained only streptococcal SAgs (SPs E, C, G, and SME Z). On the other hand, groups II and V contained both staphylococcal and streptococcal SAgs. Group II contained staphylococcal SEs B, C, and G and streptococcal SSA and SPE A, and group V contained staphylococcal SEs I, K, L, and P and streptococcal SPs E and H. This underlines the likelihood of horizontal gene transfer between these two genera, a fact that is becoming increasingly apparent with genome comparisons.[2]

Toxic Shock Syndrome

TSS has been sporadically reported as staphylococcal scarlet fever since 1927.[175] Interest in TSS dramatically increased in the early 1980s, when a number of staphylococcal TSS cases occurred in young women who used high-absorbency tampons during menses.[176] The disease was associated with a toxin called TSST-1 that was secreted locally by toxigenic strains. TSST-1 and other SAgs can cross the mucosal membrane by several means. At the level of mechanical barriers, tight junctions are not uniformly present on mucosal surfaces, and staphylococcal Hla may help further disrupt their surface. At the level of SAgs, a conserved dodecapeptide (YNKKKATVQELD) was shown to mediate transcytosis of the toxin to deeper mucosal layers, promoting contact with immune cells and triggering inflammation—a phenomenon referred to as outside-in signaling mechanism.[5,177] There are two clinical forms of TSS: menstrual TSS and nonmenstrual TSS.

Menstrual Toxic Shock Syndrome

Menstrual TSS starts within 2 days of the beginning or the end of menses and is primarily associated with the use of high-absorbency tampons. Clinical signs include high fever, capillary leak syndrome with hypotension and hypoalbuminemia, generalized nonpitting edema, and a morbilliform rash, followed by desquamation after a few days. The toxin is produced locally, and blood culture results are typically negative. The organisms responsible were represented by a single clone in most reported cases.

The disease proceeds by SAg-induced hyperactivation of the immune system (see previous discussion). TSST-1 production is regulated by

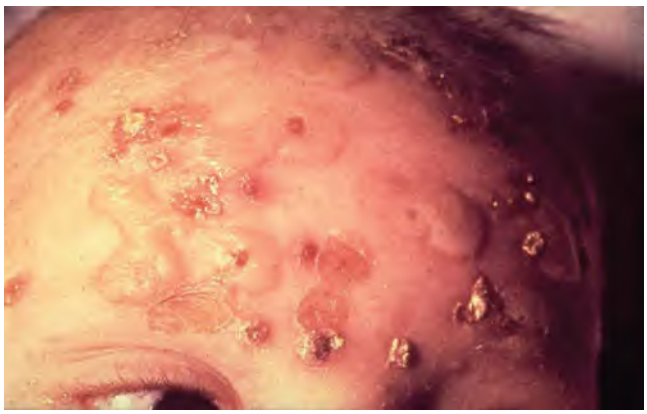

FIG. 194.10 Localized staphylococcal scalded skin syndrome, also called bullous impetigo. Disease is caused by local production of exfoliative toxins, and bacteria may be found in blister liquid.

agr (see Table 194.1). However, its expression requires specific conditions that include (1) an elevated protein level; (2) a relatively neutral pH (6.5–8); (3) an elevated pCO_2; and (4) an elevated pO_2.[68] All four conditions are met when menstruation is combined with the use of high-absorbency tampons. The high protein concentration and neutral pH are provided by blood proteins and their buffering capacity. The high PCO_2 is ensured by the higher than atmospheric CO_2 content of venous blood. Eventually, the high concentration in O_2 is introduced into the vaginal anaerobic flora by the high-absorbency tampon. Thus, the O_2 brought in by the tampon might be the trigger that modifies an otherwise equilibrated ecosystem and stimulates the production of TSST-1 by colonizing staphylococci.

TSST-1–producing *S. aureus* may be found in up to 20% of isolates from both carrier and clinical specimens, and higher in MSSA than in MRSA.[27,170] The fact that TSST-1 expression has special requirements may partially explain the comparatively low prevalence rate of the disease (approximately 1–3 cases per 100,000 patient-years).[2]

Nonmenstrual Toxic Shock Syndrome

Nonmenstrual TSS has attracted less attention than menstrual TSS, yet it can occur in any patient. In addition to TSST-1, nonmenstrual TSS can be the result of enterotoxins SEB and SEC, which are *agr* regulated (see Table 194.1). Responsible organisms may colonize virtually any site of the body, including surgical wounds (surgical TSS), lung (influenza-associated TSS), mucosa or skin (recalcitrant desquamative syndrome in patients with acquired immunodeficiency syndrome [AIDS]), contraceptive diaphragms, and dialysis catheters in patients undergoing chronic peritoneal dialysis. The development of general symptoms with high fever and cutaneous rash should suggest the possibility of nonmenstrual TSS in such patients.

A special feature of wound colonization is that the affected tissues often do not appear inflamed. This is believed to result from the toxin itself, which is able to prevent the influx of professional macrophages.

Predisposing Factors

In addition to the use of high-absorbency tampons or colonization with a toxigenic strain, most patients who are TSS susceptible also lack specific antibodies that block the responsible SAg. In one study, antibody titers considered protective against TSST-1 (≥1:100) were detected in 30% of 2-year-old children and in more than 90% of women and men 25 years of age. However, low or negative titers of anti–TSST-1 antibodies (<5) were found in acute-phase sera from 90.5% of patients with menstrual TSS, and less than 50% had positive titers of anti–TSST-1 antibody that developed during convalescence.[2,178] Hence, some patients remain susceptible to recurrent TSS.

An interesting feature of SAgs is that they primarily trigger a CD_4^+ T-cell response, which privileges a helper T-cell type 1 (Th1) cytokine release response without a significant type 2 (Th2) response. A consequence of the dominant Th1 response is a decreased antibody expression, which could explain the relative lack of antibody response in patients with TSS. An additional explanation for the anergy could be SAg-induced apoptosis of responsive T cells, which could account for the prolonged anergy toward the deleterious toxin.

Diagnosis

The diagnosis of TSS is based on a constellation of clinical and laboratory signs as proposed by the Centers for Disease Control and Prevention.[179] Table 194.5 also proposes additional laboratory features, such as isolation of a toxin-producing organism to broaden the diagnostic tools.[180] The criteria of streptococcal TSS, from toxigenic *S. pyogenes* isolates, are presented for comparison. Although both syndromes are the results of similar kinds of SAgs, they differ in two important aspects. First, in contrast to staphylococcal TSS, streptococcal TSS is almost always associated with the presence of streptococci in deep-seated infections, such as erysipelas or necrotizing fasciitis, which has been referred to as flesh-eating disease. Second, mortality rates are very different in staphylococcal and streptococcal TSS. Mortality rates of menstrual and nonmenstrual (in children) staphylococcal TSS were reportedly <1%.[181,182] In contrast, mortality of streptococcal TSS in children was 28%[182] and up to 45% in adults,[183] especially in cases of necrotizing fasciitis, which necessitates aggressive treatment with generous surgical débridement of infected tissues, and sometimes amputation.

TABLE 194.5 Diagnostic Criteria for Staphylococcal and Streptococcal Toxic Shock Syndrome

STAPHYLOCOCCAL TOXIC SHOCK SYNDROME[a]	STREPTOCOCCAL TOXIC SHOCK SYNDROME
Fever	Isolation of group A streptococci from:
Hypotension	Sterile site for definite case
Diffuse macular rash with subsequent desquamation	Nonsterile site for probable case
Three of following organ systems involved:	Hypotension
Liver	Two of the following symptoms:
Blood	Renal dysfunction
Renal	Liver involvement
Mucous membranes	Erythematous macular rash
Gastrointestinal	Coagulopathy
Muscular	Soft tissue necrosis
Central nervous system	Adult respiratory distress syndrome
Negative serologic studies for measles, leptospirosis, and Rocky Mountain spotted fever and negative blood or cerebrospinal fluid cultures for organisms other than *Staphylococcus aureus*	

[a]Proposed revision of diagnostic criteria for staphylococcal toxic shock syndrome (TSS) includes (1) isolation of *S. aureus* from mucosal or normally sterile site; (2) production of TSS-associated superantigen by isolate; (3) lack of antibody to implicated toxin at time of acute illness; and (4) development of antibody to toxin during convalescence.

Modified from McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol. *2001;55:77–104.*

Therapy and Prevention

Treatment of staphylococcal TSS consists of elimination of the causative agent with antibiotic treatment and appropriate drainage of affected tissues if necessary. Antibiotic regimens should include active drugs such as β-lactams or vancomycin (in case of MRSA) plus protein inhibitors such as clindamycin or linezolid, which block the production of toxins.[184,185] Supportive care that includes intravenous fluid and vasopressors might be necessary. The immunologic gap that allows the toxin to be active in susceptible patients suggests that passive immunotherapy such as intravenous immune globulin (IVIG) could be effective. However, the success of IVIG therapy has been disputed in several recent analyses.[186,187] Because the mortality of menstrual staphylococcal TSS is low, immunotherapy should be considered only for life-threatening cases of streptococcal TSS.

Prevention is aimed at avoiding the use of hyperabsorbent tampons and preventing staphylococcal colonization of wounds and mucosa. In the case of nasal carriage, this is achieved with topical application of antibacterial agents such as mupirocin. In the case of extranasal colonization, additional complete body washing with antiseptics such as chlorhexidine is recommended for at least 1 week (see Table 194.8 later). Control cultures should be taken thereafter.

Active immunization with a TSST-1 vaccine could be a potential alternative. A phase I trial with recombinant TSST-1 demonstrated good tolerance and immunogenicity.[188] Further evaluation is awaited.

Enterotoxins and Food Poisoning

S. aureus harbors up to 15 enterotoxins, which are defined as SAgs able to produce gastrointestinal symptoms that include vomiting and diarrhea in primate models.[2,5] Although many of these toxins have potential SAg activity, not all of them have a clear role in human disease. As mentioned, SEB and SEC are associated with nonmenstrual TSS. Likewise, SEA is by far the most frequent culprit of food poisoning, whereas SED, SEB, and SEC are less frequently involved.[189]

Foodborne disease is a major public health problem that may account for 6 to 8 million cases per year in the United States. *S. aureus* food poisoning follows ingestion of toxins that have been released into

contaminated food stocks or beverages. The toxins are heat stable and thus are not denatured by cooking. The disease typically starts 2 to 6 hours after ingestion with general malaise, nausea, vomiting, abdominal pain, and diarrhea. No fever occurs, but the symptoms may be distressing enough to justify hospital consultation in approximately 10% of patients. The symptoms spontaneously resolve within 6 to 12 hours, and the prognosis is excellent, except in the case of severe dehydration in young children and elderly patients.

Although the mode of action of SAgs at the level of T lymphocytes is known, their mechanism at the surface of the intestinal mucosa is less clear. They might share transcytosis mechanisms with TSST-1.[5,177]

Other Implications of Superantigens

Although SAgs can result in dramatic subversion of the host immune system, they are not ultimate bacterial weapons because they affect only a restricted subgroup of patients who do not mount an appropriate blocking antibody response. Many of these staphylococcal genes are physically contiguous, which suggests that they may have arisen by duplication, maybe for the purpose of diversity.[8,190] The versatility of SAgs is further supported by the discovery that one of them (i.e., SHE) develops its SAg activity by binding to the Vα rather than the Vβ domains of the T-cell receptor, thus expanding different sets of T-cell lineages than classic SAgs.[191]

The clinical relevance of this multiplicity of toxins is not entirely understood. Toxin genes are dispensable elements that are not needed for growth in rich media and in the absence of competition. Some SAgs (e.g., TSST-1 and SEA, SEB, and SEC) obviously provide a way for the bacterium to escape host immunity. For instance, SAgs have been involved in the etiology of psoriasis and atopic dermatitis,[192] where SAg-induced skin modification could promote bacterial survival. On the other hand, the survival advantage of provoking allergic diseases including rhinitis and asthma[193,194] is less intuitive, except maybe to promote airborne dispersal (see Fig. 194.5). Altogether, the multiplicity of SAgs could enable *S. aureus* to interfere with the immune response of various animal species, thus broadening its host spectrum.

Genomics and Mobile Genetic Elements

At the time of writing, several thousands of *S. aureus* genome assemblies and annotation reports are available in public databases (www.ncbi.nlm.nih.gov/genome/genomes/154). *S. aureus* genomes are circular and contain approximately 2.8 million base pairs that represent up to 2700 coding sequences, plus structural and regulatory RNAs. They are divided into (1) a core genome, which contains mostly housekeeping genes, is quite conserved along various staphylococcal species, and accounts for about 80% of the whole DNA, and (2) an accessory genome, which carries mobile DNA (MGEs), contains most *S. aureus* pathogenic and drug-resistance features, and may vary among different species and strains.[7,8,10,16–18] In addition, certain elements of the core genome can vary according to lineages—for example, by the presence or absence of core genes that are specific of given clades. Therefore the core genome is sometimes subdivided into the core-stable and core-variable genome.[7,10]

Comparative Genomics and Evolution

Genome evolution is driven by random point mutations that lead to single nucleotide polymorphism (SNP), larger variations in core genes (e.g., deletions or duplication of repeat regions) that may differ between lineages, and MGEs that include insertion sequences, transposons, viruses, and pathogenicity and genomic islands.[7,8,10,16–18]

Beyond academic interest, understanding the evolution of *S. aureus* may help understand the fundamentals of successful clones and eventually help design strategies to block their spread. Based on 7- and 14-gene MLST analyses,[195,196] an evolutionary scenario was proposed in which a common ancestor of *Staphylococcus* spp. first segregated into non–*S. aureus* and *S. aureus* species (Fig. 194.11). The *S. aureus* branch acquired the genomic islands νSaα and νSaβ (see later), which are absent from other staphylococci and encode for type I restriction modification systems, and further evolved into two subbranches that gave rise to different STs and CCs. Although still discussed,[196,197] it seems that the global regulator *agr* groups I to IV differentiated early in these two

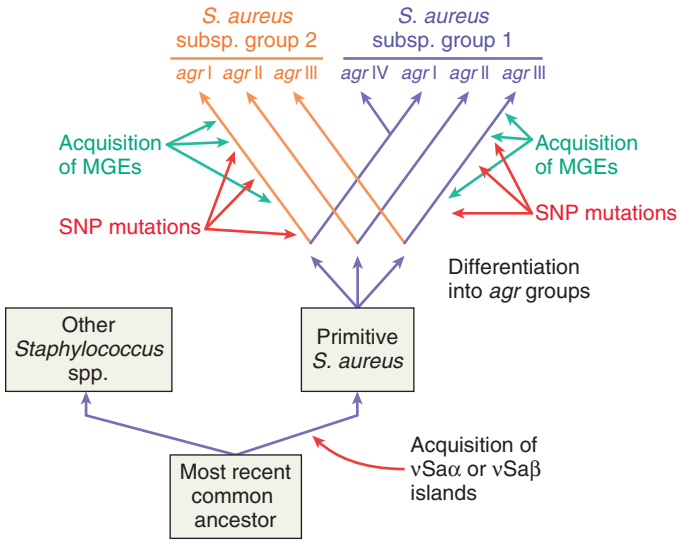

FIG. 194.11 Molecular evolution of *Staphylococcus* spp. into species and subspecies. *Staphylococcus* spp. evolved from a common ancestor into non–*S. aureus* and *S. aureus* species. The *S. aureus* branch distinguishes itself by the early acquisition of the νSaα or νSaβ genomic islands, which carry the genes for type I restriction modification systems. *agr*-interference groups differentiated shortly after, primarily by mutations. However, the *agr* two-component regulatory system is likely to be very old, because it is also present in non–*S. aureus* species. Sequence types (STs) and clonal clusters (CCs) evolved later, mostly via mutations, and determined two *S. aureus* subspecies (subsp. 1 and subsp. 2). Accordingly, the two subspecies contain different CCs, such as notoriously successful CC30 in subsp. 1 and CC5 in subsp. 2. On the other hand, they may contain similar *agr* interference groups, because these interference groups differentiated earlier, with the exception of group IV. *(Modified from Robinson DA, Monk AB, Cooper JE, et al. Evolutionary genetics of the accessory gene regulator* (agr) *locus in* Staphylococcus aureus. J Bacteriol. *2005;187:8312–8321; and Feng Y, Chen CJ, Su LH, et al. Evolution and pathogenesis of* Staphylococcus aureus*: lessons learned from genotyping and comparative genomics.* FEMS Microbiol Rev. *2008;32:23–37.)*

branches, which explains why the four *agr* groups may be found in different downstream CCs (see Fig. 194.11). Eventually, MGEs were acquired later and are dispersed in almost any of the STs or CCs. Yet, a few exceptions to this rule exist. Indeed, the TSST-1 gene and the PVL gene locus are classically associated with *agr* group III, the exfoliatin genes with *agr* group IV, and the vancomycin intermediate-resistance phenotypes with *agr* groups I or II.[7] Whether this is due to peculiarly favorable *agr*-related genetic backgrounds or to favorable contemporary conditions for the extension of specific clones is unclear. Moreover, a functional expression of *agr* is dispensable in certain circumstances, as for instance in biofilms.[87,88,165] Indeed, the association of clinical outcome with *agr* dysfunction and SCC*mec* type was observed in a study from South Korea, in which MRSA bacteremia-associated mortality was highest with types II/III, in which *agr* dysfunction is significantly increased.[198]

Using whole-genome sequencing, McAdam and colleagues[199] traced back the evolution of notoriously virulent CC30 to over 100 years ago. In the late 1800s, CC30 first segregated into phage type 80/81 and Southwest Pacific clades, which encoded PVL and produced severe community- and hospital-acquired infections due to penicillin-resistant (but methicillin-susceptible) strains in the mid 1900s. In the mid 1950s, a third hospital-related clade emerged (EMRSA-16), which was devoid of PVL but had acquired a new methicillin-resistance MGE named staphylococcal cassette chromosome *mec* (SCC*mec;* see later discussion). All three clades gave downstream variants related to further SNPs or MGE acquisitions. Moreover, the CC30 evolution demonstrates acquisition, loss, and reacquisition of PVL and other MGEs, underlying the virulence plasticity of this particular organism. It will be important to understand whether the success of CC30 is due to a peculiar ability to capture useful

MGEs or rather to contemporary environmental conditions that favored clonal expansion (e.g., in the hospital), or perhaps both.

Staphylococcus aureus Cross-Species Jump Between Humans and Animals

S. aureus is also an animal pathogen that raises particular concerns in livestock and cattle. Human and animal *S. aureus* strains tend to segregate in different lineages.[200] However, cross-species jumps exist and need to be considered. Companion animals and veterinary providers have been shown to share strains.[201] Two outbreaks have been described in Israel involving horses and veterinary personnel.[202,203]

Another example is the swine-related MRSA CC398, which appeared to colonize swine husbandries since the early 2000s.[204] Price and colleagues[205] proposed that CC398 was first transferred from human to swine. Indeed, CC398 is also present in humans and had been devoid of the SCC*mec* cassette (and thus was methicillin susceptible).[206] The swine CC398 has lost the ϕSa3 β-hemolysin–disrupting prophage, which is present in the human CC398. As mentioned, in human strains prophage ϕSa3 disrupts the β-hemolysin gene and simultaneously imports virulence factors, including the IEC carrying Sak, CHIPS, and SCIN (discussed under "Immune Evasion" earlier).[207] In swine CC398, the loss of prophage ϕSa3 restores the β-hemolysin gene, which may be important for skin or mucosal colonization in animals.[114,208] The swine CC398 also acquired a new SCC*mec* cassette that may carry useful determinants for survival in swine husbandries, including antibiotic use. Thus, CC398 was first a human MSSA. It was transferred to swine with the parallel loss of ϕSa3 and acquisition of a new SCC*mec*. The loss of prophage ϕSa3 seems critical in this evolution. This raises the question as to whether reacquisition of prophage ϕSa3 by swine CC398 could promote its reestablishment in humans, carrying along a new SCC*mec* cassette. Indeed, cases of invasive human infection with swine-CC398 MRSA are increasingly reported.[209] Likewise, a linear increase of 66% of human CC398 MRSA cases occurred in Denmark from 2004 to 2011; one-third of these patients reported no livestock exposure.[210] Most important, an epidemiologic evaluation in the Republic of Ireland has revealed an elaborate pattern of cases mixing human and animal disease, wherein a few human CC398 MRSAs did not carry the ϕSa3 prophage IEC cluster, thus resembling typical animal strains, whereas some animal CC398 MRSAs did carry the ϕSa3 prophage IEC cluster, thus resembling human strains.[211] In this line, a large study in Iowa found higher rates of *S. aureus* carriage in swine workers, with more than a third carrying livestock-associated strains that did cause infection in humans.[212] This strongly supports the likelihood constant of interspecies passage.

Similar scenarios were described with the ST5 *S. aureus* strain that invades poultry[213] and with the notoriously virulent human CC8 *S. aureus* strain that infects cows.[114,214] As in swine CC398, the bovine version of CC8 first lost the ϕSa3 prophage and then acquired a new composite SCC element, which is as yet devoid of the methicillin resistance *mecA* gene but carries a new LPXTG surface protein that might be responsible for colonization of the bovine mammary gland. Thus, gene trafficking and genome evolution should be apprehended globally.

Pathogenicity and Genomic Islands

S. aureus pathogenicity (SaPI) and genomic islands (νSa) are continuous structures that vary in size from approximately 15 kb to 70 kb and can harbor many virulence or resistance genes. They mostly contain heterologous DNA that indicates exogenous acquisition. A common feature of these elements is that they are bracketed by direct or inverted repeats and carry recombinase genes. The repeats serve as an attachment site *(att)* for integration into homologous regions of the bacterial chromosome. The recombinase, which is often an integrase, catalyzes integration into the chromosome.[215] At least seven SaPIs have been described and are reunified in four groups based on the homology of their integrase genes.[10] SaPI1 and SaPI2 harbor the gene for TSST-1 and are responsible for most cases of TSS. SaPI3 and SaPI4 contain numerous enterotoxin genes. SaPIbov encodes for a bovine version of TSST, and SaPIbap encodes a bovine adherence protein that might play a role in bovine mastitis.

The *S. aureus* chromosome also carries two larger elements called genomic islands (νSaα and νSaβ), which were acquired early during staphylococcal speciation (see Fig. 194.11).[8,17,58,190] νSaα and νSaβ carry not only restriction modification systems, but also a variety of SEs and other virulence genes and thus have been variously referred to as an enterotoxin gene cluster *(egc)* or virulence gene nursery.[6,173]

Pathogenicity and genomic islands are terminology variations of mobile elements deriving from ancestral prophages, and their mobilization is still dependent on so-called helper prophages.[13] A seminal study showed that *S. aureus* SaPI1 could be mobilized from the bacterial chromosome by ϕ80α and ϕ11 and transferred into naïve recipients thereafter.[216] SaPIs often carry remnants of integrase/excisase *(int/xis)* genes, and λ phage *cI*-like repressor genes that repress the expression of *int*/*xis* and forbid spontaneous excision of their cognate SaPIs. When induced, helper prophages produce a nonessential protein that blocks the SaPI *cI*-like repressor, thus freeing the expression of *int*/*xis* genes and promoting SaPI excision. Excised SaPI can replicate and then undergo illegitimate packaging into nascent phage capsids of the helper prophage via *pac* sites. Following phage-induced bacterial lysis, capsids that are illegitimately hijacked by SaPIs can bind to new *S. aureus* recipients and inject their SaPI-DNA cargo, a process called generalized transduction. The delivered SaPI DNA then undergoes Campbell-like site-specific integration into the bacterial chromosome using its *att* site and its own integrase.[13,217] Such phage mobilization was observed with other pathogenicity islands (e.g., SaPI2) and is likely to be a general mechanism of gene transfer for these large elements. Excision and transfer of such elements are triggered by stresses such as exposure to ultraviolet light and certain antibiotics in vitro.[216–218] They are also likely to be promoted by antibiotics in the clinical setting as well.

Resistance Island Staphylococcal Cassette Chromosome *mec*

MRSA contains one resistance island called *staphylococcal cassette chromosome* (SCC) *mec,* wherein *mec* is the genetic element that confers resistance to methicillin.[219] SCC*mec* is an exogenous piece of DNA that may vary between 15 and 60 kb. It inserts at the 5′ end of the *orfX* gene at a conserved 15–base-pair chromosomal attachment site *(attB)* that recombines with an homologous site on the cassette *(attS),* generating two flanking direct repeats (*attL* and *attR*) after insertion. The SCC*mec* critical genes are the recombinases *ccrA*/*ccrB* and *ccrC,* which can mediate mobilization of the whole element, and the *mecA* gene, which mediates β-lactam resistance. The rest of SCC*mec* contains various additional determinants and is referred to as "J" for junkyard.[220]

mecA encodes for penicillin-binding protein 2A (PBP2A), which has a low affinity for methicillin and most other β-lactam drugs and confers intrinsic resistance of MRSA to almost all β-lactams (see "Mechanism of Methicillin Resistance").[221] The *mecA* gene is preceded or not by the *mecRI* and *mecI* regulator determinants, which are homologues of the *blaRI* and *blaI* regulators of penicillinase *(bla)* genes. *mecRI* (and *blaRI*) encodes for a membrane receptor, and *mecI* (and *blaI*) encodes for a gene repressor. In the presence of penicillin, the extracellular portion of the membrane *mecRI (blaRI)* receptor triggers an autocatalytic cleavage of its intracytoplasmic portion. The liberated intracytoplasmic peptide acts as a metalloprotease, which further cleaves the *mecI (blaI)* repressor, thus derepressing gene expression.[222] The *mecA* gene is bracketed by one or two copies of IS431, which is believed to serve as a gene collector and might promote the local insertion of additional determinants, such as antibiotic resistance genes.[220]

Several types of SCC*mec* have evolved from more generalist SCC cassettes, which are structures able to integrate a variety of genes and are functional equivalents to integrons in gram-negative bacteria. Up to 12 SCC*mec* types have been described according to a consensus classification system.[223,224] The classification is based on the *mecA* complex, which encompasses the *mecA* gene and its surrounding regulatory genes, and the *ccr* complex, which includes the *ccrA*/*ccrB* and *ccrC* genes.[223] The *ccrA*/*ccrB* and *ccrC* genes do not come together on the same SCC*mec*. *ccrA*/*ccrB* are cotranscribed and come in four different alleles, whereas *ccrC* has only one allele. Up to now there are nine types of SCC*mec* that carry various combinations of *ccrA* and *ccrB* and three types that carry one of two different *ccrC* alleles, including one in type V and VIII and a newer one in type XII.[224]

SCC*mec* types mirror major original MRSA clones. Types I, II, and III were shown to belong to HCA-MRSA. They harbor multiple resistance determinants, they have relatively large sizes (35–60 kb), and they are therefore difficult to mobilize. Types IV, V, and VI were associated with CA-MRSA.[225,226] They are much smaller (about 15 kb) than their hospital congeners and do not carry multiple antibiotic-resistance genes. However, they appear to be associated with other elements in the same bacterium, including prophage-related PVL and multiple SE genes.[8] One particularly successful clone of CA-MRSA (clone USA300 of CC8 lineage) has also acquired a so-called arginine catabolic mobile element (ACME) inserted downstream of the SCC*mec* cassette.[227] ACME was acquired from *S. epidermidis* and confers survival advantages in acidic and maybe other environments. It improved survival and fitness of USA300 in a rabbit model of bacteremia.[228] Together these elements may render the organism particularly fit and virulent.

Whereas HCA-MRSA types are clonal and carry large and difficult-to-mobilize SCC*mec* cassettes, CA-MRSA types carry small SCC*mec* cassettes, which are more prone to mobilization[220] and less clonal than HCA-MRSA, at least in Europe, where the USA300 clone is rather sporadic.[229,230] CA-MRSA did not arise from HCA-MRSA that permeated the community, but emerged independently by acquiring SCC*mec* most likely from CoNS donors.

Recently, a new SCC*mec* carrying a *mecA* variant gene ($mecA_{LGA251}$) with only 70% homology to *mecA* was missed by molecular probing and incidentally identified through methicillin-resistance phenotyping.[48] This variant, renamed *mecC*,[47] was mainly found in livestock-associated MRSA (e.g., CC130, CC1943, and ST425). Although few *mecC*-positive MRSA strains could be traced back to the mid-1970s,[231] they have clearly been increasing since 2003, representing almost 3% of all MRSA isolates recovered in Denmark in 2011.[231] This adds to the need to closely follow staphylococcal cross-species jumps between humans and animals.

Beyond epidemiologic implications, the use of molecular diagnostics insensitive to non-*mecA* methicillin resistance should be considered, and a newer generation of the Cepheid Xpert (new Xpert MRSA Gen 3; Cepheid, Sunnyvale, CA) PCR assay includes *mecC* as a target.[212] The therapeutic implications of *mecC* have not been reported, but this variant PBP has a higher affinity for penicillins than for cephalosporins, and in vitro, *mecC* isolates are susceptible to the addition of clavulanate to penicillin.[232] This is reminiscent of the greater affinity of old penicillin G and amoxicillin for PBP2A, and the fact that amoxicillin-clavulanate combinations successfully cured experimental infections due to MRSA, with clavulanate being required to block secreted penicillinase.[233] Moreover, expression of methicillin-resistance by *mecC*-positive MRSA appears to be temperature dependent. MICs of flucloxacillin (an analogue of methicillin) were much lower when tested at 37°C than at 30°C. As a result, flucloxacillin successfully cured experimental endocarditis due to *mecC*-positive MRSA, whereas it is known to fail against *mecA*-positive MRSA.[234]

ANTIBIOTIC RESISTANCE

S. aureus has developed resistance to virtually all antibiotic classes available for clinical use. These encompass cell wall inhibitors such as β-lactams and glycopeptides; ribosomal inhibitors that include macrolide-lincosamide-streptogramin B (MLS_B), pleuromutilins, aminoglycosides, tetracyclines, fusidic acid, and the new oxazolidinones; the RNA polymerase inhibitor rifampin; the DNA gyrase–blocking quinolones; the antimetabolite TMP-SMX and iclaprim; and the newer lipopeptides and lipoglycopeptides.[235–238] FDA-approved versions of such drugs and the main resistance mechanisms of *S. aureus* are summarized in Table 194.6. Some are discussed subsequently.

β-Lactams

β-Lactams inhibit bacterial growth by interfering with cell wall assembly. They bind to the active site of a series of membrane-bound enzymes responsible for inserting the peptidoglycan precursors into the nascent wall (Fig. 194.12).[239] Certain enzymes are bifunctional and carry both transglycosidase and transpeptidase activity. Transpeptidation takes place at the D-ala-D-ala terminal of the precursor. It hydrolyzes the covalent bond between the penultimate and the terminal D-ala and then transfers the penultimate D-ala to a free NH_2 terminal (the ε—NH_2 of lysine) of neighboring peptidoglycan stem peptides. The terminal D-ala is released, and a new stem peptide cross-link is created (see Fig. 194.12A).

Penicillin and other β-lactams are steric analogues of the cell wall D-ala-D-ala terminal of the precursors. They are mechanism-based inhibitors that compete with D-ala-D-ala binding to the active site of the membrane-bound transpeptidase, which they block irreversibly—hence the term *penicillin-binding protein* (PBP) coined for these enzymes.

Resistance to Penicillin

The most common resistance mechanism of *S. aureus* to β-lactams is penicillinase, which is encoded by the *bla* gene usually carried on a plasmid. The gene is inducible and preceded by the *blaRI* and *blaI* regulatory determinants (see "Resistance Island Staphylococcal Cassette Chromosome *mec*"). Penicillinase is a secreted enzyme that hydrolyzes penicillin and other penicillinase-susceptible compounds into inactive penicilloic acid. Penicillinase-producing *S. aureus* emerged rapidly after penicillin was introduced as a therapeutic agent in the mid 1940s. It is now prevalent both in the hospital and in the community, where it represents close to 80% of the isolates.[240,241]

The minimal inhibitory concentration (MIC) of penicillin G for fully susceptible *S. aureus* is approximately 0.01 mg/L. In contrast, the MIC of penicillinase-stable drugs such as nafcillin, oxacillin, flucloxacillin, or cephalosporins is 10-fold greater. Thus, penicillin G remains the best choice against penicillin-susceptible staphylococci. However, major concerns remain in the United States about recommended detection of penicillin susceptibility and resistance, because currently recommended susceptibility assays may miss more than 35% of β-lactamase–producing *S. aureus*.[242,243] Moreover, the use of a single β-lactam, such as oxacillin or cefoxitin, to predict susceptibility of *S. aureus* to other β-lactams may have limitations. For example, an analysis of *S. aureus* MICs found that oxacillin was a better predictor than cefoxitin for susceptibility to ceftriaxone, ceftaroline, and cefazolin.[244]

Methicillin-Resistant *Staphylococcus aureus*

The first penicillinase-stable β-lactams such as cephalosporins and semisynthetic methicillin and nafcillin became available in the late 1950s. Ironically, MRSA was first described at about the same time.[245] The prevalence of MRSA progressively increased thereafter. One survey of the National Nosocomial Infections Surveillance System (NNIS) found that the hospital prevalence of MRSA increased from 2.1% in 1975 to 35% in 1991.[246] It went up to 60% in certain centers in the United States,[52] and more than 70% in a report from Shanghai,[247] but great geographic variations exist worldwide. Several reports have indicated a declining trend in the prevalence of MRSA over the last decade, both in Europe and in North America.[53,248–250] Although this decline most probably results from hospital hygiene measures, the exact reason may be more intricate, such as a better fitness of MSSA replacing MRSA in the absence of antibiotic pressure.[251] This was elegantly demonstrated in competition experiments showing that antibiotic concentrations that were several orders of magnitude below the MIC of susceptible strains favored the growth of MRSA over MSSA, whereas MSSA overgrew MRSA in the total absence of antibiotics.[252] Such low antibiotic concentrations are found in sewages of urban environments[253] and are likely to be decreasing with antibiotic consumption control, thus favoring MSSA over MRSA resurgence.

Health Care–Associated Versus Community-Acquired Methicillin-Resistant *Staphylococcus aureus*

Although originally confined to the hospital environment, MRSA has emerged as a community-associated infection over the past 2 decades.[25,153,254] CA-MRSA is different from HCA-MRSA from both epidemiologic and molecular points of view. Case-definition studies showed that HCA-MRSA and CA-MRSA represented different organisms that produced different clinical syndromes.[255,256] HCA-MRSA was associated with risk factors that included recent hospitalization or surgery, residence in a nursing home, or presence of an indwelling catheter or device. It produced mostly hospital-related pneumonia and

TABLE 194.6 *Staphylococcus aureus* Resistance Mechanisms to Major Classes of Antibiotics

ANTIMICROBIAL AGENTS	RESISTANCE MECHANISMS: TARGET MODIFICATION	RESISTANCE MECHANISMS: DRUG INACTIVATION	RESISTANCE MECHANISMS: DECREASED ACCUMULATION	RESISTANCE GENE: NATURE	RESISTANCE GENE: ORIGIN	RESISTANCE GENE: LOCATION[a]
β-Lactams						
Penicillinase-S	Yes	Yes	No	Penicillinase	Acquired	Plasmid
				PBP2A[b]	Acquired	SCC*mec* (chromosome)
				(PBP2C in some livestock animals)		
Penicillinase-R	Yes	No	No	PBP2A[b]	Acquired	SCC*mec* (chromosome)
				(PBP2C in some livestock animals)		
Glycopeptides						
Intermediate resistance	Yes	No	No	Mutations in wall-building genes	Intrinsic	Chromosome
Full resistance	Yes	No	No	*vanA* and *vanH*	Acquired	SCC*mec* (chromosome)
Lipoglycopeptides						
Daptomycin	Yes	No	No	Mutations in genes involved in wall-building and membrane charges *(mprF)*	Intrinsic	Chromosome
Macrolide-lincosamide-streptogramin B						
Macrolides	Yes	No	Yes	*erm*	Acquired	Plasmid or chromosome
				msrA	Acquired	Plasmid
Lincosamide[c]	Yes	Yes	No	*erm*	Acquired	Plasmid or chromosome
				linA	Acquired	Plasmid or chromosome
Streptogramin B[c]	Yes	Yes	Yes	*erm*	Acquired	Plasmid or chromosome
				vgb (rare)	Acquired	Plasmid or chromosome
				msrA (rare)	Acquired	Plasmid or chromosome
Streptogramin A	No	Yes	Yes	*vat, vatA* (rare)	Acquired	Plasmid or chromosome
Quinupristin-dalfopristin	Yes	Yes	Yes	*vga, vgaB* (rare); combinations of above (rare)	Acquired	Plasmid or chromosome
Linezolid	Yes	No	No	Mutation in 23S rRNA gene	Intrinsic	Chromosome
				cfr	Acquired	Plasmid
Tetracyclines	Yes	No	Yes	*tet*(M), tet(O)	Acquired	Plasmid or chromosome
				tet(K), *tet*(L)	Acquired	Plasmid or chromosome
Gentamicin	No	Yes	Yes	*aac*(6′)-*aph*(2′)	Acquired	Plasmid or chromosome
				Respiratory chain mutants		Chromosome
Chloramphenicol	No	Yes	No	*cat*	Acquired	Plasmid or chromosome
Fusidic acid	Yes	No	Yes	*fusA* mutation	Intrinsic	Chromosome
				pUB101	Acquired	Plasmid
Rifampin	Yes	No	No	*rpoB* mutation	Intrinsic	Chromosome
Fluoroquinolones	Yes	No	Yes	*grlA* and *gyrA*	Intrinsic	Chromosome
				norA	Intrinsic	Chromosome
Trimethoprim	Yes	No	No	*dfrA* mutation	Intrinsic	Chromosome
				dfrA	Acquired	Plasmid or chromosome (acts by mutation or overproduction)
Sulfamethoxazole	Yes	No	No	*dpsA*	Intrinsic	Chromosome
					Acquired	Plasmid (probable) (acts by mutation or overproduction)

[a]*SCC*mec, Staphylococcal chromosomal cassette *mec* (see text for details).
[b]*PBP2A,* Penicillin-binding protein 2A; *PBP2C,* penicillin-binding protein 2C.
[c]*erm* gene must be induced or constitutively expressed to confer resistance to lincosamides and streptogramins B. Only macrolides are good inducers. Lincosamides and streptogramins do not induce resistance but are inactive against constitutively MLS_B-resistant strains.
aac(6′)-*aph*(2′), Bifunctional aminoglycoside acetyl-transferase and phosphor-transferase determinant, present on transposons Tn*4001*; *cat,* chloramphenicol acetyl-transferase; *cfr (chloramphenicol-florfenicol resistance),* 23S rRNA methyltransferase; *dfrA,* dihydrofolate reductase gene; *dpsA,* dihydropteroate synthase; *erm,* erythromycin-resistance methylase, mainly *ermA* (chromosome, transposons Tn*554*) and *ermC* (plasmid); *fusA,* gene encoding elongation factor G (EF-G); *grlA* and *gyrA,* genes encoding for the DNA topoisomerase and gyrase, respectively; *linA,* lincosamide nucleotidyl transferase; *mprF* (muropeptide resistance factor), lysylphosphatidylglycerol synthase; *msrA,* macrolide-streptogramin resistance, ABC-transporter; *norA,* gene encoding for staphylococcal efflux pump; *pUB101,* plasmid encoding penicillin-resistance (penicillinase), cadmium-resistance, and a protein (Far1) conferring impermeability to fusidic acid; *rpoB,* gene encoding for β subunit of RNA polymerase; *tet(M) and tet(O),* responsible for ribosomal-modification and protection; *tet(K) and tet(L),* responsible for active efflux of tetracyclines; *vanA* and *vanH,* vancomycin resistance A and H genes (see text for details); *vat* and *vatA,* acetyl transferase genes; *vga* and *vgaB,* streptogramin A efflux gene, ABC-transporter; *vgb,* virginiamycin hydrolysis.

bacteremia. In contrast, CA-MRSA was not associated with any risk factors and produced primarily SSTIs (often furunculosis), and sometimes rapidly fatal necrotizing pneumonia. It was also described as being responsible for necrotizing fasciitis and bone and joint infections.[257,258] In addition, whereas HCA-MRSA was multiresistant and highly clonal, CA-MRSA was pauciresistant[259,260] and seemingly more polyclonal, at least in Europe and Australia.[229,260] Indeed, CA-MRSA has been highly clonal in North America, with a first clone (USA400 and ST1) prevailing until approximately 2000. Since then, it has been replaced with the very successful and highly prevalent clone USA300 (ST8),[153] accounting for 61% of all MRSA isolates in the United States in one recent surveillance survey.[261] Most interesting, Strauss and colleagues[262] traced USA300 back

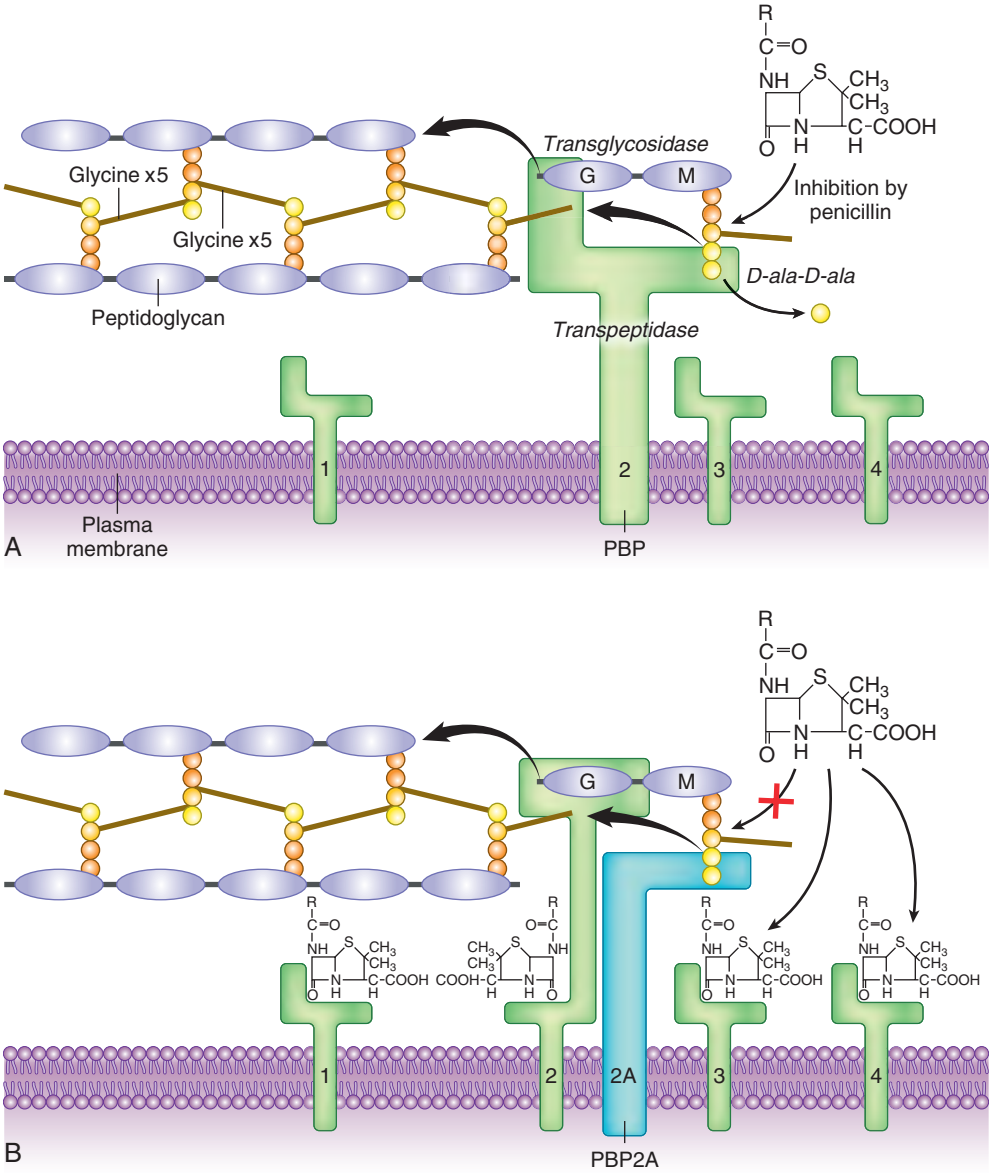

FIG. 194.12 Peptidoglycan assembly in wild-type *Staphylococcus aureus* and in methicillin-resistant *S. aureus* (MRSA). (A) Cell wall precursors consist of units of disaccharides-peptides where disaccharides (*N*-acetylglucosamine–*N*-acetylmuramic acid) are linked to pentapeptides (L-ala–D-glu–L-lys–D-ala–D-ala) at the *N*-acetylmuramate residue. After membrane translocation, precursors are handled by membrane penicillin-binding proteins *(PBPs).* High-molecular-weight PBPs are bifunctional enzymes that perform both a transglycosidase step, linking incoming *N*-acetylglucosamine *(G)* to muramic acid *(M)* in nascent wall, and a transpeptidase step, linking penultimate D-ala to glycine acceptor in nascent wall. In *S. aureus,* lysine in position 3 of stem peptide is almost always decorated with pentaglycine side chain *(orange bars).* Penicillin is a mechanism-based inhibitor of the transpeptidase domain of PBPs. (B) MRSA carries an additional PBP called PBP2A, which has very low affinity for most available β-lactam drugs. Therefore, when β-lactams are present, they block normal PBPs but not PBP2A. PBP2A has only a transpeptidase domain and must "hijack" the transglycosidase domain of normal PBP2 to be active. *(From de Lencastre H, Wu SW, Pinho MG, et al. Antibiotic resistance as a stress response: complete sequencing of a larger number of chromosomal loci in* Staphylococcus aureus *strain COL that impact on the expression of resistance to methicillin.* Microb Drug Resist. *1999;5:163–175.)*

to Europe—not to Australia, as formerly thought—where it became prevalent as PVL-negative MSSA in the mid-1800s. It then spread through Europe and invaded the United States in the early 1900s, where it acquired PVL-containing prophage ϕSA2 in the late 1930s, followed by the ACME locus and then several introductions of SCC*mec* after the 1960s. Since then, it has spread transcontinentally and intercontinentally to South America and Africa but as yet has not become prevalent in Europe.

As mentioned, CA-MRSA is associated with SCC*mec* type IV (and type V and VI in a few cases) and almost always carries PVL.[153,259,263] PVL is epidemiologically associated with SSTI and necrotizing pneumonia, but its specific role in disease has been controversial because, among other considerations, experimental results were opposite in rabbit versus mouse experimental models.[228,264,265] This issue was solved by two more recent studies. The first one demonstrated that PVL toxicity was host specific.[266] PVL appeared to bind to and lyse neutrophils via the C5a receptor and was effective against human and rabbit versions of C5a, but not against mouse and dog C5a, thus explaining the rabbit and mouse differences. The second demonstrated that PVL was toxic in humanized SCID mice, but not in wild-type mice.[267] These are important findings because they provide a rationale for treating PVL-producing staphylococcal infection with toxin-blocking antimicrobial combinations containing, for instance, clindamycin or linezolid,[268] or maybe passive immunotherapy.[269]

Thus, HCA-MRSA and CA-MRSA are not alike. Practically, MRSA in patients at risk is likely to be of the multiresistant hospital type,

whereas MRSA in patients without risk factors is likely to more susceptible to antibiotics but more invasive.

Mechanism of Methicillin Resistance

The main mechanism of methicillin resistance is not mediated by penicillinase but by the newly acquired PBP2A, encoded by *mecA*.[221] The few staphylococci that express borderline methicillin resistance from the overexpression of penicillinase are usually not considered as clinically relevant, although awareness of their possible implication in poor therapeutic outcome is resurging.[270] Because of its low β-lactam affinity, PBP2A can take over the cell wall assembly when normal staphylococcal PBPs are blocked by β-lactams (see Fig. 194.12B).[271] However, although this confers high intrinsic resistance to virtually all β-lactams, PBP2A has a special requirement for particular cell wall precursors. These must contain a pentaglycine decorating side chain attached to the position 3 L-lysine of their stem peptide and other specificities, such as an amidated D-glutamine in position 2 of the peptide (Fig. 194.13).

Providing this adequate substrate to PBP2A requires the functionality of several additional genes implicated in the normal wall building machinery, including 14 or more accessory determinants.[272] Some of them (*femABC* and *fmhB*) are responsible for adding the glycine side-chain residues critical for the PBP2A function.[273] Any alteration in these elements decreases the expression of methicillin resistance in spite of the fact that PBP2A is present.

Another fragility of PBP2A is that it carries only a transpeptidase domain and misses a transglycosidase activity (see Fig. 194.12B). Thus, for successful assembly of the peptidoglycan, PBP2A needs the assistance of the transglycosidase domain of normal staphylococcal PBP, namely PBP2.[274] This is a salient example of protein cooperation in antibiotic resistance but also represents the Achilles' heel of the system. Because most β-lactams can readily block the normal staphylococcal PBPs, further drug development needs only to target additional PBP2A to be effective. Both experimental work and recent crystallographic evidence indicate that such an approach is feasible.[275] Successful treatment of experimental endocarditis from MRSA was achieved with an array of older and newer β-lactams with good PBP2A affinity.[233,276] This approach is driving the development of new anti-MRSA compounds,[277] which recently generated some novel molecules of the cephalosporin (e.g., ceftobiprole and ceftaroline) and carbapenem classes.[278,279]

Glycopeptides

As a general rule, current *glycopeptides* (e.g., vancomycin) are less bactericidal than β-lactams against MSSA. Therefore they should not be used as first-line treatment against β-lactam–susceptible organisms.[280] However, vancomycin is still a gold standard against severe MRSA infections, recently enriched by daptomycin, novel anti-MRSA β-lactams (which are discussed later), and combinations of vancomycin or daptomycin plus β-lactams to take advantage of the so-called "seesaw effect" (also discussed in "Daptomycin" and "Alternatives Treatments" later).

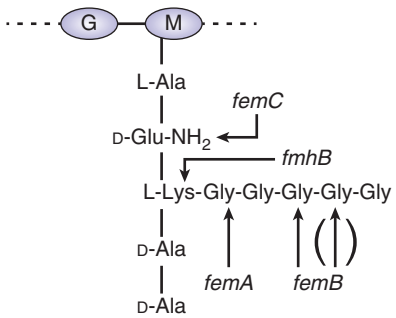

FIG. 194.13 Peptidoglycan precursor required for wall assembly by PBP2A. To be functional, penicillin-binding protein 2A (PBP2A) requires that the cell provide fully decorated precursors, containing both pentaglycine side chain and amidated glutamine. Inactivation of *femB, femA,* and *fhmB* genes blocks addition of pentaglycines and thus decreases expression of methicillin resistance even though PBP2A is present in bacterial membrane. Inactivation of *femC* has a similar effect. *(Modified from Berger-Bächi B. Expression of resistance to methicillin.* Trends Microbiol. *1994;2:389–393.)*

Two types of resistance to glycopeptides were reported in clinical isolates of *S. aureus,* namely "intermediate" and "high-level" resistance. According to the 2012 guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS])[281] the vancomycin MIC breakpoints for *S. aureus* are as follows: ≤2 mg/L for susceptible isolates; 4 to 8 mg/L for intermediate-resistant isolates; and ≥16 mg/L for high-level–resistant isolates. Both resistance phenotypes result from different mechanisms and may be of different clinical and epidemiologic relevance.

Therapeutic Monitoring of Vancomycin in Adult Patients

Although CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org/clinical_breakpoints/) agree on the ≤2 mg/L susceptibility breakpoint, several reports and meta-analyses have indicated that infections due to *S. aureus* with vancomycin MICs >1 mg/L but ≤2 mg/L (referred to as high MICs) result in a greater mortality than infections due to *S. aureus* with vancomycin MICs ≤1 mg/L (referred to as low MICs).[282,283] Therefore, special attention was given to pharmacokinetic and pharmacodynamic parameters that could help predict treatment efficacy.[284] A consensus review proposed the following guidelines for vancomycin treatment and monitoring in severe infection, based on twice-daily administration of the drug:

From the therapeutic point of view:

1. The trough concentration of vancomycin in the serum is the best indicator of efficacy and prevention of resistance selection.
2. Trough levels of vancomycin should be between 15 and 20 mg/L.
3. Obtaining peak levels is not indicated.
4. Drug dosages should be adapted to the patient's body weight and renal function. A loading dose of 25 to 30 mg/kg IV must be considered, followed by 15 to 20 mg/kg IV q12h depending on the renal function and drug dosage monitoring.

From the toxicity point of view:

1. Unlike original preparations of vancomycin (in the late 1950s), ototoxicity is no longer an issue if vancomycin is given in monotherapy.
2. Nephrotoxicity at high doses in monotherapy has been reported.
3. Vancomycin-induced nephrotoxicity should be considered if two to three consecutive measurements of renal function are abnormal during therapy.
4. Peak concentration of vancomycin does not seem to be a good correlate of toxicity.

Applying appropriate vancomycin dosage might explain the results of a more recent study reporting no difference in the mortality rate of patients infected with low or high vancomycin MIC *S. aureus.*[285]

Intermediate Resistance to Glycopeptides

Intermediate-resistant *S. aureus* isolates were originally described in Japan and in the United States[286,287] but are ubiquitous. The first isolate,[286] called Mu50, was recovered from a 4-month-old child with MRSA sternal wound infection after cardiac surgery. The infection did not respond to vancomycin treatment. The organism had an MIC of vancomycin of 8 mg/L, as detected with standard broth dilution methods. At that time, the CLSI defined staphylococci for which the MIC of vancomycin was 4 mg/L or less as susceptible, for which the MIC was 8 to 16 mg/L as intermediate, and for which the MIC was 32 mg/L or more as resistant.[29] Therefore, the Mu50 isolate was defined as a vancomycin (or glycopeptide)-intermediate *S. aureus* (GISA).

The same author reported that a second *S. aureus* isolate, called Mu3, was responsible for vancomycin treatment failure in an adult patient with pneumonia.[288] Although the vancomycin MIC for this isolate was 4 mg/L, formally considered as susceptible at the time, Mu3 contained GISA subpopulations ($\leq 10^{-6}$ colony-forming units) that grew in the presence of 5 to 9 mg/L of vancomycin and were not detected with standard drug-susceptibility testing. The term *heteroresistant GISA* (hGISA) was coined to define the Mu3 phenotype. Since then, a number of cases of GISA and hGISA have been described worldwide and were associated with vancomycin treatment failures both in animal experiments and in human cases.[289] As mentioned earlier, CLSI and EUCAST breakpoints now classify isolates with a vancomycin MIC of

4 and 8 mg/L as GISA. Currently the term "heteroresistant" is applied to isolates with a vancomcyin MIC of 2 μg/mL or less but which harbor resistant colonies (see Chapter 30).

Intermediate glycopeptide resistance arises from pleiotropic chromosomal mutations that affect the structure of the wall peptidoglycan. In susceptible strains, glycopeptides inhibit cell wall assembly by binding to the D-ala-D-ala terminal of cell wall precursors and block both transpeptidation and transglycosylation. GISA harbors a thickened cell wall that contains an increased number of free non–cross-linked D-ala-D-ala terminals. This increased amount of free D-ala-D-ala is believed to act as a lure that traps glycopeptide molecules before they reach their target.[290]

Genomic analyses indicate that mutations in two-component sensing systems are involved, including *vraSR* (for vancomycin resistance–associated sensor/regulator),[291] *graSR* (for glycopeptide resistance–associated regulator),[292,293] and *walKR*.[294] *vraSR* is a cell wall stress response regulator that affects the expression of up to 100 genes, including cell-wall building enzymes such as PBP2 and MurZ.[291] *vraSR* dysfunction affects peptidoglycan sturdiness, which is compensated for by wall thickening. *graSR* affects the expression of up to 200 genes including the *dlt* operon and muropeptide resistance factor *mprF*. As mentioned in the "Immune Evasion" section, the *dlt* operon and *mprF* are involved in cell surface charge modulation by means of alanylation of teichoic acids and lysine decoration of membrane phospholipids, respectively.[142] This explains the phenomenon of GISA cross-resistance with AMPs and daptomycin, the antibacterial activities of which depend on membrane charges (see also discussion later).[295] *walKR* is a wall metabolism-associated regulon that is highly conserved in low GC gram-positive bacteria and senses bacterial wall changes—for instance, in response to antibiotic exposure—in order to adapt cell wall metabolism.[296] *walKR* mutations affect both vancomycin and daptomycin resistance by means of *mprF* deregulation, among other processes.[294,296]

Experimental work indicates that like SCVs, GISA strains preexist and are selected during therapy.[297] However, their low level of resistance and sometimes-heterogeneous phenotype make them hard to detect in the laboratory.[29] Pending the development of efficient automated systems, a convenient low-technology dual-antibiotic Etest strip assay containing vancomycin and teicoplanin performed remarkably well. It detected GISA and hGISA with a high sensitivity (95%) and specificity (94%), which was almost identical to much more cumbersome population analysis, which is not performed in routine laboratory testing.[298]

Full Resistance to Glycopeptides

Full vancomycin resistance (MIC ≥16 mg/L for *S. aureus*) has been known for more than two decades in *Enterococcus* spp.[299] In these organisms, glycopeptide resistance results from the acquisition of either Tn*1546* or Tn*1547,* two transposons that encode for a series of genes that modify the D-ala-D-ala terminal of the bacterial peptidoglycan precursor, the very target of glycopeptide compounds, to D-ala-D-lactate. The modified D-ala-D-lactate–containing precursor has a low affinity for glycopeptides and therefore confers resistance. Tn*1546,* which encodes the so-called VanA resistance phenotype, could be transferred to *S. aureus* experimentally.[300] Thus, the recent emergence of fully vancomycin-resistant *S. aureus* (VRSA) that expresses the VanA phenotype among human clinical isolates is not astonishing. Only 14 cases were described in the United States until 2015,[301] but these organisms must be taken seriously. First, most patients had evidence of previous MRSA and enterococcal infection, but not all had received vancomycin. Thus, transfer of the transposon may occur by more generalized triggering effects, perhaps involving unrelated antibiotics. Second, the VRSA phenotype may be missed with routine automated antibiotic susceptibility testing[302] and could be more prevalent than observed. Third, new cases were described in hospitals in the Middle East.[303] Fourth, bloodstream isolates of both methicillin-susceptible and methicillin-resistant, but fully vancomycin-resistant, have been described in Brazil. The conjugative plasmid carrying the *vanA* cluster has been identified, and in vitro transfer demonstrated.[304,305] Finally, a few isolates of VanA-positive MRSA were recovered in a screen of river surface water in Turkey.[306] Taken together, this information indicates that VRSA is pending and warrants constant attention in the diagnostic laboratory.

Daptomycin

Daptomycin is a relatively new lipopeptide that was approved in the United States and elsewhere for *S. aureus*–complicated SSTI, bacteremia, and right-sided infective endocarditis.[236] It is increasingly used as a replacement for vancomycin against MRSA. Daptomycin is an amphiphilic molecule that requires calcium to solubilize as octamer-micelles in liquid phases.[307] Because of its large size, it cannot traverse the outer membrane of gram-negative bacteria, which are naturally resistant to the drug. In gram-positive organisms, it diffuses through the peptidoglycan toward the plasma membrane, where the calcium ions disperse and leave the lipid moiety of daptomycin to interact with the plasma membrane and destabilize its electric potential.[308] Daptomycin is highly bactericidal, but its activity is dose dependent and the dosage of the drug must be large enough to ensure supra-MIC tissue levels. Standard recommendations are 4 to 6 mg/kg IV q24h, but 8 or even 10 mg/kg q24h have been used in severe infections without notable side effects.[309–311] Prospective observational studies and large retrospective series indicate that daptomycin may also be considered for use against left-sided infective endocarditis due to MRSA,[310,312] but approval by official agencies is still pending. Daptomycin should not be used against airway-acquired pneumonia because it is inactivated by alveolar surfactant.[313]

Mutants with decreased daptomycin susceptibility (MIC >4 mg/L) were recovered both in the laboratory and in clinical samples.[236,314] As mentioned in the "Intermediate Resistance to Glycopeptides" section earlier, daptomycin resistance is mediated by mutations in or deregulation of *mprF* (via *vraSR* or *graSR*), which increases the charge of staphylococcal plasma membrane, and is often associated with intermediate vancomycin resistance.[315,316] This cross-resistance raises caution in antibiotic use.

Interesting to note, however, is the fact that daptomycin-resistant mutants have a decreased fitness that may hamper their virulence and dissemination.[317] Moreover, like intermediate vancomycin resistance, daptomycin resistance is associated with the seesaw effect, characterized by a resensitization to β-lactams related to a general perturbation of the cell-call building machinery and an alteration of PBP expression.[318,319] Taking advantage of the seesaw effect opens ways to alternative antibiotic combination strategies.

Protein Synthesis Inhibitors

The MLS_B antibiotics and the oxazolidinone linezolid are discussed in this section. The tetracycline derivatives glycylcycline tigecycline and omadacycline are addressed in the subsequent section "Alternative Treatments."

MLS_B Antibiotics

MLS_B antibiotics comprise separate classes of molecules (i.e., macrolides, ketolides, lincosamides, and streptogramin B) that all bind to the bacterial 50S ribosomal subunit and block protein synthesis. Resistance proceeds by any of the three classic mechanisms: modification of the bacterial drug target, modification-inactivation of the drug itself, and decreasing intracellular accumulation of the drug.

Ribosome modification and drug efflux are the most frequent resistance mechanisms in *S. aureus*.[320] Ribosome modification is mediated by the *erm* gene (for erythromycin methylase), which encodes a methylase that adds one or two methyl groups to the 23S rRNA of the 50S ribosomal subunit. This inflicts a steric alteration that greatly decreases the affinity of the drug for its target. The *erm* determinants belong to a family of methylase genes preferentially located on mobile elements such as transposons (e.g., Tn*554* and *ermA*) or plasmids (e.g., pE194 and *ermC*). An additional sophistication in *S. aureus* is that the expression of *erm* may be inducible or constitutive.[320] In the case of the inducible form, the *erm* product is synthesized only in the presence of inducing drugs. Thus, the bacterium does not spend worthless metabolic energy in the absence of antibiotic pressure. Among MLS_B drugs, only macrolides are good *erm* inducers. However, once induced, the gene product confers cross-resistance to the other members of the group, including the newer ketolides, lincosamides, and streptogramin B, but not streptogramin A. Moreover, mutations that result in constitutive *erm* expression, and hence, global MLS_B resistance, occur at high frequency (10^{-7} to 10^{-8}). Therefore, lincosamides (e.g., clindamycin) should be used with great caution against *erm*-inducible isolates (i.e., resistant to erythromycin

but susceptible to lincosamides and streptogramin B) because the drug might select for constitutive MLS_B mutants, which are resistant to the whole group of compounds.[321]

Newer drugs of the ketolide subfamily, including telithromycin and solithromycin, have a greater ribosomal affinity, are poor *erm* inducers, and have lower MICs than erythromycin for *S. aureus.*[322] However, they are still affected by constitutive expression of *erm* and thus are not useful against these types of organisms, which include the majority of HCA-MRSA isolates.[322–324]

In the laboratory, the MLS_B resistance phenotype is detected with the disk diffusion D-test in which erythromycin and clindamycin disks are placed at a distance on a plate inoculated with bacteria and the diffusion of erythromycin toward the clindamycin disk induces clindamycin resistance. As a result, the zone of inhibition around the clindamycin disk takes a D shape.[321] In contrast, constitutive MLS_B resistance yields no inhibition zone at all around the clindamycin.

Drug Efflux

Active macrolide efflux has been reported in both streptococci and staphylococci.[325,326] In *S. pyogenes* and *Streptococcus pneumoniae,* efflux is mediated by the *mefA* and *mefE* genes, respectively, which are members of the major facilitator transporter and export only macrolides (M-resistance phenotype). *S. aureus* and CoNS may contain *msrA,* which belongs to the complex ABC-transporter (adenosine triphosphate [ATP]–binding cassette) set of genes[325] and confers resistance to both macrolides and streptogramin B (MS-resistance phenotype). In contrast to major facilitators, ABC transporters use ATP hydrolysis as a source of energy for active efflux. The *msrA* complex is located on a plasmid and is frequently observed in MLS_B-resistant CoNS. It can be transferred into *S. aureus,*[325] but its clinical relevance for MLS_B resistance is unclear because it is rarely detected in clinical isolates.[327] Of note, lincosamides (e.g., clindamycin) are not subject to efflux by these pumps.

Constitutive MLS_B resistance, associated or not with drug efflux, is extremely frequent (>90%) in HCA-MRSA.[324] Therefore, use of MLS_B drugs should never be considered against such organisms. The only exception is the quinupristin-dalfopristin combination (streptogramin B and A; see "Alternative Treatments"). In contrast, only 5% of CA-MRSA isolates are reported as clindamycin resistant and mostly are of the inducible phenotype.[153,328] Thus, clindamycin remains a therapeutic option against CA-MRSA.

Oxazolidinones

The oxazolidinone linezolid prevents initiation of protein synthesis by binding to the 23S rRNA of the 50S ribosomal subunit, near its interface with the 30S subunit. It is active only against gram-positive bacteria and is essentially bacteriostatic. It is approved in the United States for complicated SSTI and nosocomial pneumonia from susceptible organisms, including MRSA. Although originally controversial, linezolid was shown to be superior to vancomycin against MRSA nosocomial pneumonia (daptomycin cannot be used in pulmonary infections) in a randomized double-blind study.[329] A systematic review on infective endocarditis from multiresistant bacteria, including 18 MRSA and vancomycin-intermediate *S. aureus* (VISA) isolates, reported a success rate of about 60%, which suggests that compassionate use of linezolid might be an option in such complicated situations.[330] Moreover, evidence-based reviews indicate that it is equal to vancomycin in a number of clinical situations[331] including osteomyelitis in children.[332]

One asset of linezolid is that it can be administered orally and thus is useful for outpatient therapy. Another is that, like clindamycin, it inhibits the secretion of TSST-1 and other toxins and should be considered against toxin-associated infections, including CA-MRSA hemorrhagic pneumonia.[268,333] On the other hand, linezolid is not suitable for long-term (>28 days) therapy because prolonged treatment may be associated with thrombocytopenia, sometimes peripheral or optic neuropathy, and lactic acidosis.[334]

Linezolid resistance has been reported episodically in clinical settings. It is primarily the result of mutations in the 23S rRNA gene.[335] Because staphylococci harbor six to seven copies of rRNA genes, mutation in only one of them does not yield high-level resistance at once. MIC increments are progressive, and MICs of such mutants are usually 4 to 8 mg/L compared with a baseline of 2 mg/L. However, plasmid-mediated high-level resistance was also detected in clinical isolates of *S. aureus* and of *S. epidermidis* (MIC, 8 and >257 mg/L, respectively).[336] The resistance gene *(cfr)* encodes a 23S rRNA methylase that confers cross-resistance to other drugs that bind at the same site, including chloramphenicol, lincosamides (i.e., clindamycin), and streptogramin A.[337] Plasmid-born *cfr* was recently reported in several isolates from livestock in Europe and from human patients in the United States and Ireland.[338,339] Nevertheless, the overall rate of linezolid resistance in *S. aureus* clinical isolates remains very low (<1%) even after more than 15 years of use.[340,341]

The newer semisynthetic tedizolid derivative, approved both in the United States and in Europe for SSTI, has 2× to 8× lower MICs than parent linezolid for MRSA and is not affected by *cfr*-mediated resistance.[342] It is noteworthy, however, that the improved in vitro efficacy is partially offset by a greater binding to plasma proteins—that is, 85% as compared with 30% for linezolid.[343] In two phase III trials of acute skin and skin structure bacterial infections, tedizolid 200 mg once daily for 6 days compared favorably with linezolid 600 mg twice daily for 10 days.[344]

Quinolones

Quinolones are an important class in the antiinfective armamentarium. They originated in the 1960s as a byproduct from the synthesis of antimalarial quinines. Fluorinated derivatives such as ciprofloxacin, norfloxacin, and ofloxacin appeared in the 1980s. They had low MICs (on the order of 0.01 mg/L) for most gram-negative pathogens. However, the MIC for gram-positive bacteria was relatively high (0.25–2 mg/L for *Staphylococcus* spp. and *Streptococcus* spp.)[345] and close to therapeutic concentrations in the serum of humans (2 mg/L for peak concentration of ciprofloxacin). Use of these borderline active drugs against MRSA facilitated the selection for resistant derivatives. The prevalence of quinolone resistance in HCA-MRSA has been around 90% for a long time and is close to 40% in CA-MRSA,[229,346] which makes older and newer quinolones mostly inappropriate against MRSA.

Mechanisms of Resistance

Known quinolone-resistance mechanisms result from chromosomal mutations (see Table 194.6). Plasmid-mediated resistance has been described in gram-negative pathogens and is associated with the *qnr* gene, which protects the quinolone targets.[347] A *qnr*-like gene has been described in *Enterococcus faecalis* and could confer resistance to *S. aureus.*[348] However, such a mechanism was not yet described in clinical isolates.

Quinolone resistance proceeds by two types of mechanisms, including overexpression of the efflux pump NorA[349] and structural mutations in the quinolone targets topoisomerase IV (*grlA* and *grlB*) and gyrase *(gyrA, gyrB)* genes.[350] Resistance is acquired stepwise. A first *grlA* mutation, which occurs at frequencies of 10^{-7} to 10^{-8}, produces a moderate increase in MIC (e.g., 0.5–2 mg/L of ciprofloxacin) that is still considered susceptible (<4 mg/L). However, this first mutation paves the way to a second mutation in the *gyrA* gene, which, combined with the *grlA* mutation, results in high-level resistance. Because the initial *grlA* mutation jeopardizes the efficacy of quinolones, it is critical to avoid selecting it at first, by ensuring appropriate drug levels in the blood and tissues.

Older quinolones readily select for such alterations, yielding highly resistant organisms after only a few serial exposures to the drug.[351] Quinolones with improved anti–gram-positive activity (levofloxacin, moxifloxacin, gatifloxacin, garenoxacin) are less selective. However, they may still select for higher resistance levels in bacteria that already acquired a first degree (*grlA* mutants) of ciprofloxacin resistance (MIC, 2–8 mg/L).

The newer agent delafloxacin might be more promising. Its modified chemical structure ensures dual binding to gyrase and topoisomerase IV, thus resulting in 10 to 100 times lower MICs then previous quinolones, including against *grlA* mutants, in addition to insensitivity to NorA-mediated efflux.[352] At the time of writing, delafloxacin is still investigational.[237]

Pharmacokinetic and pharmacodynamic criteria help predict quinolone efficacy and risk for resistance. Efficacy was predicted by peak drug-level/MIC ratios of 8 or more and ratios of area under the concentration-time curve/MIC of 100 or more.[353] With regard to resistance prevention, in vitro and in vivo experiments have suggested