concluded that vitamin C supplementation did not reduce the incidence of common colds but did produce a consistent reduction in the duration of cold symptoms. A reduction in symptom days in adults (8%; 95% CI, 3%–12%) and in children (14%; 95% CI, 7%–21%) was summarized in a Cochrane review.[36] In contrast to most other dietary antioxidants, vitamin C appears to be safe even at high levels of consumption.

Trials have sought to assess the role of vitamin C in sepsis. A retrospective before-after study of 47 control subjects and 47 patients examined intensive care unit (ICU) data 7 months before and 7 months after a protocol that administered a combination of vitamin C, hydrocortisone, and thiamine during severe sepsis and septic shock. The treatment arm was associated with a lower hospital mortality (8.5% vs. 40.4%; $P < .001$).[37] Although the results are promising, randomized controlled studies are currently being performed to assess whether these findings can be confirmed before this protocol can be incorporated into the care of patients with sepsis.

Vitamin B_6

Vitamin B_6 (pyridoxine) plays an essential role in nucleic acid and protein synthetic pathways. Not surprisingly, it appears to be essential for optimal lymphocyte function, maturation and growth, antibody production, cytokine production, and natural killer (NK) cell activity.[13,14] Increased consumption of vitamin B_6 enhanced lymphocyte proliferation and cytokine production.

Folate

Folate deficiency is associated with reduced numbers of circulating lymphocytes, poor lymphocyte proliferation, impaired Th1 immunity including delayed-type hypersensitivity responses, and impaired NK-cell activity. Supplementation studies with immune response end points are few, but supplementation has improved age-related declines in NK-cell function.[13]

Vitamin B_{12}

Similar to folate, vitamin B_{12} is essential to cell reproduction including lymphocyte proliferation. Vitamin B_{12} deficiency is associated with reduced production of antibody to pneumococcal polysaccharide; however, whether repletion of vitamin B_{12} reverses this defect has not been addressed, and no causal relationship has been established.

Trace Metals

Zinc

Zinc is a dietary trace mineral that plays a critical role in the structure of cell membranes and in the function of cells of the immune system. Zinc is required for the activity of hundreds of enzymes associated with carbohydrate and energy metabolism, protein synthesis and degradation, nucleic acid synthesis, heme biosynthesis, and carbon dioxide transport.[13]

Zinc deficiency occurs most commonly in association with starvation, PEM, and malabsorption syndromes. In the developed world, zinc deficiency is seen primarily in children and elderly adults, although it is estimated that a larger proportion of North Americans may be at risk. Zinc deficiency has been documented in association with conditions of relative immunocompromise including pregnancy, alcoholism, kidney disease, burns, inflammatory bowel disease, and HIV infection.[13] Clinical manifestations of zinc deficiency include growth impairment, delayed sexual maturation, hypogonadism, impotence, oligospermia, alopecia, dysgeusia, night blindness, impaired wound healing, skin abnormalities, and impaired immunity.

Clinical trials have examined the role of zinc in immune system modulation during infection and other illnesses. For children living in developing nations, zinc supplementation limited growth stunting and reduced the duration and intensity of diarrheal illness, acute lower respiratory tract infections, and pneumonia.[38] Children receiving zinc supplements had higher $CD3^+$ and $CD4^+$ lymphocyte counts and higher $CD4^+/CD8^+$ ratios in peripheral blood and improved cell-mediated immunity compared with control subjects. Zinc supplementation also reduced the incidence of clinical disease caused by *Plasmodium falciparum*.[38] In patients with sickle cell disease, zinc supplementation increased IL-2 production and decreased microbiologically confirmed infections and hospitalizations.[39]

A number of studies have evaluated the role of zinc in protecting against the common cold. Postulated mechanisms include zinc-mediated interference with rhinoviral protein cleavage and assembly of viral particles and protection of plasma membranes against lysis by cytotoxic agents such as microbial toxins and complement; some of these effects may be due to correction of subclinical zinc deficiency. It has also been suggested that the common cold symptoms sneezing and nasal discharge may be reduced in intensity by elevations in intranasal zinc salts through production of a "chemical clamp" on trigeminal and facial nerve endings.[40] A meta-analysis of eight published randomized trials found no clear benefit for the use of zinc gluconate lozenges in the treatment of the common cold.[41] An individual patient data meta-analysis of three randomized placebo-controlled trials of zinc acetate at doses of at least 75 mg/day showed earlier recovery from cold symptoms: 70% versus 27% recovery by day 5.[42] One possible mechanism that could account for the discordant results is that acetate has lower affinity for zinc than gluconate, leading to a higher available concentration of the zinc cation. Data are mixed/inconclusive on the efficacy of zinc to prevent otitis media or as adjunctive treatment for pneumonia in children.[43,44] The benefit of zinc acetate lozenges, 75 mg/day, in treating the common cold appears to be significant, given the lack of proven alternative therapies and the minimal side-effect profile. As such, zinc acetate may be considered to have a role in the early treatment of cold symptoms.

Selenium

Selenium is essential for the function of selenium-dependent proteins, which play critical roles in the redox regulation of key enzymes, transcription factors, and receptors. Beyond its role as an antioxidant, selenium may have additional immune properties that contribute to the maintenance of normal immune function.[13] Selenium is ubiquitous in the soil and enters the diet through both plant and animal sources. Dietary intake varies depending on geographic region. Overt selenium deficiency is rare and is limited to certain regions of China. However, the effects of relative selenium deficiency on disease susceptibility and disease progression remain only partially characterized and are a subject of intense ongoing studies of both hosts and pathogen (see "Host Nutritional Status and Pathogen Virulence").

Selenium deficiency has been shown to decrease the production of free radicals and killing by neutrophils, IL-2 receptor affinity and expression on T cells, T-cell proliferation and differentiation, and lymphocyte cytotoxicity. In vitro, selenium deficiency results in enhanced neutrophil adherence to endothelial cells, an early event in the inflammatory response. In both mice and humans, supplementation with selenium has been shown to increase lymphocyte proliferative responses, IL-2 receptor expression, and macrophage and cytolytic T-lymphocyte–dependent tumor cytotoxicity. Even at plasma selenium levels associated with normal dietary intake in the United States, supplementation with 200 μg of selenium per day has considerable immunoenhancing effects, although an upper limit is likely because megadose therapy may be associated with reduced immunity.[45] There appears to be risk in selenium supplementation in terms of immune function in patients without selenium deficiency, so widespread supplementation should be avoided.

Iron

Iron deficiency is the most common trace element deficiency worldwide. It is estimated to affect 20% to 50% of the world's population including infants, children, and women of childbearing age in tropical regions.[46] The effects of iron deficiency are seen in multiple systems of the human body including the immune system.[13] In animal and human studies, iron deficiency has been associated with impairments in cell-mediated immunity, reductions in neutrophil activity with decreased myeloperoxidase activity and bactericidal activity, and diminished NK-cell activity.[13,47] Iron deficiency has been shown to impair lymphocyte and neutrophil functions in children, although no resultant increase in susceptibility to infection has been described. Whereas iron deficiency and infection often coexist in developing nations, cause-and-effect relationships have not been established in most cases. The exceptions to this include heavy infestations of hookworms leading to subsequent gastrointestinal blood loss and *Helicobacter pylori* likely causing decreased absorption and in some cases occult gastrointestinal blood loss.[48]

Many of the immune abnormalities associated with iron deficiency appear to be reversible with iron replacement, but this has been difficult to demonstrate in human studies. Studies in laboratory animals have demonstrated reversible, deleterious effects of iron deficiency on measures of functional immunity,[47] even in mildly iron-deficient animals.

Most clinicians routinely replace iron in documented iron deficiency to avoid anemia and associated morbidities. However, controversy exists regarding possible deleterious effects of iron supplementation in some settings. Many microorganisms require trace elements such as iron and zinc for survival and replication in the host and may increase in pathogenicity with supplementation. Iron deficiency appears to protect against severe malaria,[49,50] and oral iron supplementation has been associated with increased infection rates.[51,52] Furthermore, parenteral iron supplementation has been shown in human and animal studies to be harmful when administered during infection,[47] and certain pathogens such as *Yersinia enterocolitica* and *Vibrio vulnificus* thrive in the setting of iron and iron-chelating agents.[53] Therefore administration of iron, particularly intravenous iron, or iron-chelating agents such as deferoxamine should be delayed in subjects with active infection.

Fatty Acids

Three major groups of dietary fatty acids—oleic acid, linoleic acid, and linolenic acid—serve as precursors for the biosynthesis of polyunsaturated fatty acids (PUFAs). Metabolic competition exists among these groups of fatty acids, and modification of dietary fatty acid intake can lead to alterations in the fatty acid composition of tissue lipids and changes in cellular responses.

PUFAs including arachidonic acid and eicosapentaenoic acid (EPA) can be enzymatically converted to eicosanoids. Extensive data suggest a strong modulatory role for fatty acids in various cellular responses including inflammation and immune function,[54] and there is growing evidence that they also act as second messengers or regulators of signal-transducing molecules.[54] Among the fatty acids, omega-3 (ω-3) PUFAs possess the most potent immunomodulatory activities; ω-3 PUFAs found concentrated in fish oil, EPA, and docosahexaenoic acid are more biologically potent than α-linolenic acid.

A number of clinical trials have assessed the benefits of dietary supplementation with fish oils in several inflammatory and autoimmune diseases in humans including rheumatoid arthritis, Crohn disease, ulcerative colitis, psoriasis, lupus erythematosus, and multiple sclerosis. Animal and clinical studies of acute respiratory distress syndrome and sepsis suggest that a high-fat diet containing EPA (fish oil), γ-linolenic acid (borage oil), and antioxidants can improve lung microvascular permeability, oxygenation, and cardiopulmonary function; reduce proinflammatory eicosanoid synthesis and lung inflammation; and improve survival (see "Surgical and Critically Ill Patients").[55,56]

OVERNUTRITION: OBESITY AND INFECTIOUS DISEASES

Obesity, defined as a BMI greater than 30, is epidemic in the United States and rapidly increasing globally (www.who.int/mediacentre/factsheets/fs311/en/index.html). The association of obesity with diabetes, cardiovascular disease, osteoarthritis, and many other chronic illnesses is well known, but the impact of obesity on infection and immunity is a relatively new field. Infection risk and outcomes for many syndromes are influenced by obesity but not in a uniform direction (Table 11.1). For example, it has been well documented that obesity was a major risk factor for adverse outcomes in hospitalized subjects during the 2009 pandemic H1N1 influenza outbreak. Additionally, obesity is a risk factor for surgical site infection, prosthetic joint infection, and hospital-acquired infections. However, and perhaps surprisingly, there is a strong and consistent association with *better* clinical outcomes in obese patients with community-acquired pneumonia compared with their nonobese counterparts.[57] Several excellent reviews have outlined the impact of obesity on infectious disease acquisition and outcome as well as postulated immune changes that may contribute to these clinical findings.[58,59,60]

The immune mechanisms affected by obesity are broad, involve all aspects of the immune response, and in general upregulate inflammatory responses while diminishing most cellular responses, but there are notable exceptions.[61,62,63,64] In humans, obesity has been associated with an increased inflammatory milieu that has been hypothesized to increase the production of short-term memory T-cell and B-cell responses but impaired memory responses (Fig. 11.1). This is consistent with the finding that obesity does not impair initial vaccine responses to influenza and other antigens but shortens the duration of protective immunity.[65,66,67,68]

At least in the case of 2009 H1N1 influenza, overexuberant inflammatory responses may have been a major driving factor in adverse outcomes and were likely mediated by the high leptin levels in obesity. Increased mortality in obese mice, similar to that seen in obese humans, is markedly attenuated by administration of antileptin antibodies.[69]

SPECIAL POPULATIONS: CLINICAL TRIALS OF NUTRITIONAL SUPPLEMENTATION TO REDUCE INFECTION RISK

To illustrate the general issues discussed to this point, three clinical populations of specific interest are highlighted: surgical and critically ill patients, patients with HIV/AIDS, and older adults. These populations are frequently encountered by infectious disease specialists, and research in these three groups has been of particularly high quality, with well-designed epidemiologic and interventional studies.

Surgical and Critically Ill Patients

Total Parenteral Nutrition Versus Enteral Nutrition[a]

Enteral and parenteral nutrition have been compared in a number of conditions in critically ill patients in randomized controlled trials; these have confirmed the utility of using enteral nutrition whenever possible. Furthermore, in acute pancreatitis, enteral nutrition was associated with reduced risks for death, multiple organ failure, systemic infection, and local septic complications as well as reduced length of stay.[70] For severe pancreatitis, the reduction in mortality for enteral versus parenteral nutrition was greater than 80% (RR, 0.18; 95% CI, 0.06–0.58).[70] Thus the adage of "if the gut works, use it" appears applicable across the breadth of critical care patient populations, and enteral nutrition should be used whenever possible. Total parenteral nutrition can be useful in patients for whom enteral nutrition cannot be applied. Data from an international survey of nearly 3000 critically ill patients suggested a strong inverse relationship between 60-day mortality and ventilator-free days with total daily calorie intake, particularly in patients with a BMI less than 25 or greater than 35, and this was true even in the 25% of patients who received total parenteral nutrition alone or in combination with enteral nutrition to achieve caloric goals.[71] Randomized controlled studies in both adult and pediatric critically ill patients show that delayed initiation of parenteral nutrition does not change overall mortality and leads to decreased rates of infection.[72,73]

Immunonutrition

Although the mechanism through which nutritional supplementation exerts positive effects on critically ill patients is uncertain, the reduced infection risk with enteral feeding is hypothesized to be related to the promotion of intestinal mucosal integrity, blunting of proinflammatory responses, and enhanced wound healing.[74,75,76] This hypothesis has given rise to the concept of "immunonutrition"—specific formulations to replace micronutrients that frequently become deficient in acute inflammatory states and to provide antiinflammatory nutrients that reduce infection risk.[74] Studies using glutamine, arginine, *N*-acetylcysteine, branched-chain amino acids, nucleotides, ω-3 PUFAs, antioxidant vitamins and trace elements, taurine, and various mixtures of these compounds as supplements have been investigated. Guidelines published by the Society of Critical Care Medicine and American Society for Parenteral and Enteral Nutrition in 2016 make a number of recommendations regarding immunonutrition. The guidelines recommend routine use of an immune-modulating formula (containing both arginine

[a]An outstanding reference with exhaustive and well-organized topical reviews of the concepts discussed in this section can be found at http://criticalcarenutrition.com/index.php?option=com_content&view=category&layout=blog&id=21&Itemid=10.

TABLE 11.1 Risk for Acquiring Infections and/or Adverse Outcome in Obese vs. Nonobese Patients

INFECTION/ SYNDROME	RISK FOR ACQUISITION[a]	RISK FOR ADVERSE OUTCOME[a]	COMMENT	REFERENCES
Respiratory Viruses				
Influenza (H1N1 2009 pandemic)	±	≫	Obesity clearly increases risk for morbidity and mortality in hospitalized patients with H1N1 2009 strain, but the limited data regarding acquisition do not suggest a big increase in risk. Elevated leptin, impaired CD4 and CD8 T-cell responses, and poor TLR-based responses in obese subjects may play a pathogenic role.	Multiple; reviewed in 61, 62, and 63
Influenza (non-H1N1)	±	>	Weaker database than for H1N1, but some data suggest hospitalization due to influenza more likely in obese patients, but obese patients not more likely to acquire influenza. Adequacy of vaccine response depends on outcome measured (seroprotection/ seroconversion [which is not impaired] vs. duration of antibody rise, memory cell production [which is impaired]).	65, 66, 67, 68
Respiratory syncytial virus	NR	> (pediatric data only)		132
Community-Acquired Bacterial Pneumonia				
	±	≪	Risk for acquisition data greatly confounded by associated comorbidities; once these are adequately controlled, there is little/no association of obesity with acquisition of pneumonia. There is a surprising and consistent *reduction* in mortality in obese patients vs. nonobese patients.	Risk: 133 (reviewed in 62) Outcomes: 134–138
Bacteremia and Sepsis				
	>	> (bacteremia) < (sepsis)	Bacteremia *without sepsis* on presentation is associated with increased mortality in obese patients in small studies. Although obese subjects have a greater risk for sepsis, when presenting with *sepsis, severe sepsis,* and *septic shock,* they fare *better* than nonobese subjects.	139–142
Surgical Site Infections				
	≫	NR	Consistent increase across general, colorectal, spinal, joint replacement, cesarean section, and other surgeries	Multiple; reviewed in 143–146
Bone and Joint Infections				
Osteomyelitis	NR	NR	Clearly associated with DM, but no data about obesity risk alone when appropriately controlling other comorbidities	
Septic arthritis	NR	NR		
Prosthetic joint infection	≫	NR	May be due to increased surgical time, underdosing of prophylactic antibiotics, etc.	58, 147–151
Urinary Tract Infection				
	>	NR	More prominent effect of obesity in male patients than in female patients	59, 60
Hospital-Acquired Infections[b]				
	≫	>	Worse outcomes in obese patients include longer length of stay and ventilator duration in addition to adverse clinical outcomes (e.g., mortality)	152–156
***Helicobacter pylori* Infection**				
	≫	>	Multiple studies demonstrate increased prevalence of *H. pylori* in obese individuals perhaps due to lack of *H. pylori* altering ghrelin expression and predisposing to obesity, rather than obesity predisposing to *H. pylori* infection	157–159
HIV Infection				
	NR	>	Greatly increased obesity prevalence, particularly among women with HIV; weight gain is substantial after initiating ART; obesity associated with increased risk for DM, cardiovascular disease, frailty, and multimorbidity	160–164

[a]≫, substantially greater risk; >, greater risk; ±, little difference; <, less risk; ≪, substantially less risk; NR, not reported.
[b]Consists of hospital-acquired bacteremia, catheter-related infection, pneumonia, urinary tract infection, and *Clostridioides difficile* (formerly *Clostridium difficile*) colitis.
DM, Diabetes mellitus; *ART,* antiretroviral therapy; *HIV,* Human immunodeficiency virus; *TLR,* Toll-like receptor.
Modified from data from references 165–167.

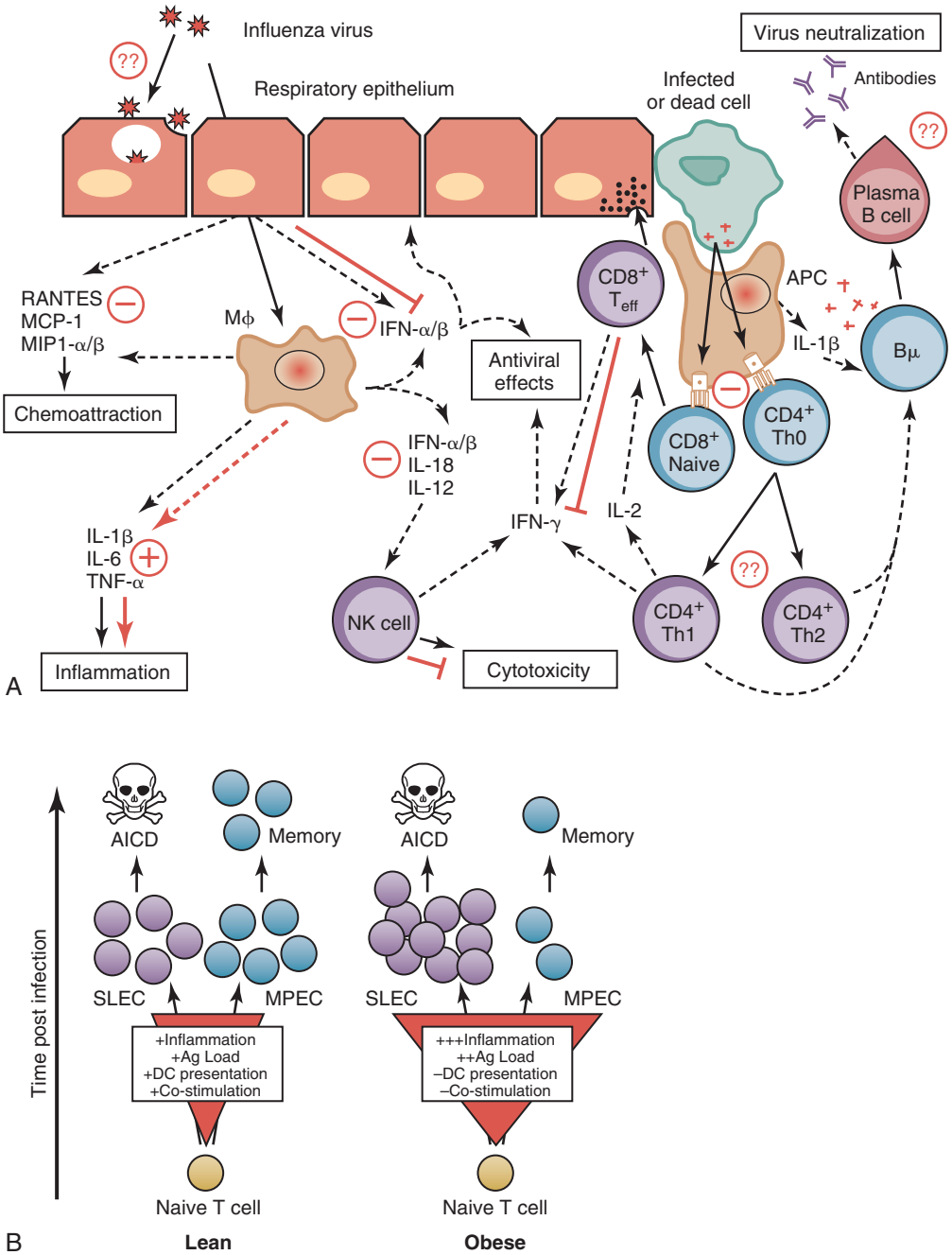

FIG. 11.1 Hypothesized alterations in immune responses to influenza in obese hosts. (A) Influenza infection and response processes modified by obesity are shown in red. (B) Net result of obesity is elevated levels of short-lived effector cells (*SLEC*) but impaired long-lived memory cells. *AICD,* Activation-induced cell death; *APC,* antigen-presenting cell; *DC,* dendritic cell; *IFN,* interferon; *IL,* interleukin; *MCP,* monocyte chemotactic protein; *MPEC,* memory precursor effector cells; *NK,* natural killer; *RANTES,* regulated on activation, normal T cell expressed and secreted; *TNF,* tumor necrosis factor. *(From Karlsson EA, Beck MA. The burden of obesity on infectious diseases.* Exp Biol Med (Maywood). *2010;235:1412–1424.)*

and fish oils) in patients with severe trauma and postoperative surgical ICU patients requiring enteral nutrition therapy and recommend the use of either arginine-containing immune-modulating formulations or EPA/docosahexaenoic acid supplement with standard enteral formula in patients with traumatic brain injury. The guidelines further recommend against adding supplemental enteral or parenteral glutamine routinely in critically ill patients and that immune-modulating formulas not be used routinely in patients with severe sepsis.[77]

A Cochrane review of 19 randomized controlled trials (with 1099 participants) assessing the effects of immunotherapy in postoperative outcomes in head and neck cancers found there were no differences in mortality or risk of infection, but there was a decreased risk in fistula formation in the immunotherapy arm (5.4% vs. 11.3% absolute risk).[78]

A multicenter randomized controlled trial comprising 301 patients on assisted ventilation in the ICU (including medical, surgical, and trauma patients) assessed the use of high-protein feeds with or without immunomodulators (glutamine, ω-3 fatty acids, and antioxidants) and found no difference in infection rates, but noted an increased 6-month mortality in the immunomodulator arm for the subgroup of medical patients (54% vs. 35%, $P = .04$).[79] These findings along with others showing trends toward increased 6-month mortality with glutamine supplementation[80] without clinical benefit[79,80,81] indicate serious safety concerns for medical patients receiving immunomodulating nutritional supplements that include glutamine. These findings have led to the update in guideline recommendations that had previously recommended glutamine supplementation for all critically ill patients.[82]

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

Recognition of malnutrition and principles of nutritional management are important aspects of the primary care of patients with HIV infection.[83,84] Severe malnutrition was one of the first recognized manifestations of advanced disease in HIV-infected patients, initially described as "slim disease" in early reports from Africa.[85] Wasting syndrome was later defined by the Centers for Disease Control and Prevention as a body weight loss equal to or greater than 10% with associated fatigue, fever, and diarrhea unexplained by another cause; however, any weight loss of more than 5% is associated with accelerated disease progression, impaired functional status, and increased mortality.[86] Early in the HIV epidemic, studies reported wasting in up to 20% of patients at the time of AIDS diagnosis in the United States. Despite advances in the management and treatment of HIV infection, wasting syndrome and other forms of malnutrition remain highly prevalent in HIV-infected patients.[87] Wasting is associated with increased mortality and remains a significant prognostic factor in advanced HIV disease.

The cause of AIDS-associated wasting is multifactorial.[88,89] Wasting has been associated with decreased oral intake, malabsorption syndromes, endocrine dysfunction, and increased cytokine production. Reductions in food intake may be caused by disease-associated or drug-associated anorexia, central nervous system dysfunction, dysphagia, and odynophagia. Absorption may be impaired by infectious or drug-associated intestinal inflammation, dysfunction, and diarrhea. Endocrine abnormalities include alterations in thyroid and adrenal function and fluctuations in growth hormone levels. Production of proinflammatory cytokines leads to accelerated metabolic degradation of essential micronutrients, further compromising the HIV-infected host. The use of nutritional therapy throughout the course of HIV infection and with increased intensity during symptomatic infection can slow and perhaps reverse the compounding effects of nutrition-associated immunodeficiency.

PEM is the most common form of malnutrition seen in HIV disease worldwide, but alterations in the stores of fat-soluble and water-soluble vitamins and trace elements are also seen. Vitamin A deficiency has been associated with the progression of HIV disease, development of secondary infections, increased HIV-associated mortality, and increased maternal-fetal transmission.[90–92] Deficiencies of water-soluble vitamins appear to occur less frequently than deficiencies of fat-soluble vitamins, and only cobalamin (vitamin B_{12}) deficiency is associated with HIV disease progression.[93] Of the trace elements, deficiencies in iron, zinc, and selenium have been described. Zinc levels decline as HIV disease progresses, and zinc supplementation in HIV infection has been shown to improve immune responses.[94]

Selenium deficiency occurs more commonly in HIV-infected patients, as documented by low plasma and red blood cell levels of selenium, diminished activity of glutathione peroxidase, and low selenium levels in cardiac muscle of patients with AIDS. Plasma selenium concentrations may be reduced by 50% in patients with AIDS, with lesser reductions seen in patients receiving antiretroviral therapy.[95,96] The mechanism of selenium depletion in HIV-infected patients is poorly understood and is probably multifactorial. Declines in selenium levels have been documented even in the earliest stages of HIV infection,[97] and accelerated progression of HIV disease has been described in patients with selenium deficiency.[98]

Selenium supplementation trials in patients with HIV infection improved markers of oxidative stress, improved $CD4^+$ T-cell counts, and, in pregnant women in Tanzania, reduced mortality associated with diarrheal illness.[99] Based on the consistency of the literature, we recommend zinc and selenium supplementation in patients with HIV and deficiencies of these micronutrients.

Strategies to Combat Weight Loss and Wasting Associated With Human Immunodeficiency Virus

Appetite stimulants, hormone replacement therapy, and anticytokine therapies—all with or without exercise—have been used in small clinical trials to combat wasting syndrome in HIV-infected patients with variable success. Treatment with anabolic steroids, particularly intramuscular testosterone in testosterone-deficient men, may offer sustainable gains in muscle mass and improvements in mood and quality of life. A full examination of these strategies is beyond the scope of this review, but specific nutritional approaches are evaluated in the following paragraphs.

Macronutrient Supplementation

Macronutrient (calorie and protein) supplementation was evaluated in a Cochrane Collaboration Review by Grobler and colleagues.[100] Fourteen trials comprising 1996 HIV-positive participants including 245 children met the stringent criteria for inclusion; excluded were studies of more than one nutritional intervention, studies involving pregnant women, and studies examining total parenteral nutrition versus enteral nutrition. Total caloric and total protein intakes were enhanced by supplementation of 600 to 960 kcal/day, but body weight, fat mass, fat-free mass, $CD4^+$ count, and HIV viral load were unchanged.

As pointed out in the Cochrane Review, most studies were small, and combining studies is difficult given the varying formulations used. A meta-analysis showed that in studies in which underweight participants were targeted an increase in weight, BMI, and $CD4^+$ counts was detected with supplementation, whereas studies that recruited subjects regardless of baseline nutritional status did not show differences in these parameters.[101] Very little data exist in older age groups or in HIV-infected patients with lipodystrophy or obesity, in whom nutritional supplementation could result in increased rates of diabetes or cardiovascular disease. Patients with HIV who are underweight should be treated with macronutrient supplementation to improve their BMI and $CD4^+$ counts as an aid to improving their immune function.

Micronutrient Supplementation

Micronutrient deficiency is even more common than macronutrient deficiency in the developing world, and there is strong epidemiologic evidence linking micronutrient deficiencies to adverse outcomes in many infectious diseases including HIV infection. A Cochrane Systematic Review evaluating micronutrient supplementation in HIV-infected children and adults was published in 2005 and then updated in 2010 and again in 2017.[99] An important feature of this meta-analysis is that most of the studies were conducted in resource-poor countries where malnutrition is very common. The analysis included 33 studies involving 10,325 HIV-positive participants. Most participants came from multiple micronutrient supplementation studies. The nutrients studied were vitamin A, vitamin D, vitamin E, folinic acid, zinc, and selenium.

In essence, micronutrient supplementation had very little effect on morbidity, mortality, or surrogate markers of immune function in adults. We agree with the authors who note that trials have not revealed consistent clinically important benefits with routine multiple micronutrient supplementation in people living with HIV, but that should not lead to denial of supplementation of patients with micronutrient deficiencies.

Two important subgroups of HIV-positive patients are pregnant women and children. Nutritional indicators of adverse outcomes in pregnancy for HIV-infected women in Africa include a BMI of less than 21.8, a hemoglobin concentration of less than 8.5 g/dL, and weight loss or excessive weight gain during pregnancy.[102] One relatively large (N >1000) randomized trial of multivitamin/mineral supplementation in pregnant or lactating Tanzanian women demonstrated benefit for both mothers and infants.[92,103,104] Women experienced less AIDS-related progression of disease and mortality, fewer adverse pregnancy outcomes, and less diarrheal morbidity. Infants of mothers with impaired immunologic and nutritional parameters suffered less early childhood mortality if they were born to women in the multivitamin-supplemented group. A study from Malawi in iron-deficient children with HIV found that children given iron supplementation had improved hemoglobin and $CD4^+$ counts but had increased incidence of malaria.[105] Clinicians giving iron supplementation to children in malaria-endemic regions should consider these findings and encourage enhanced malaria prophylaxis including interventions such as bednets.

Older Adults

Older adults represent a population at significant risk for malnutrition and its related health problems. Malnutrition and decreased oral intake in older adults are often multifactorial. In the inpatient setting, "nothing

by mouth" orders, inability to self-feed, and increased caloric needs head the list. In the outpatient setting, depression, medications, dental or swallowing problems, and social issues (e.g., choices between food and medicine) are paramount. Studies using anthropometric measures and laboratory values estimate 40% to 60% of hospitalized older adults are malnourished.[106,107] A prospective study of non–terminally ill elderly patients discharged from an acute care hospital found a BMI less than or equal to 20 kg/m^2 to be associated with a markedly increased risk for death within 1 year.[108] Among long-term and subacute care residents, the prevalence of malnutrition is 15% to 72%,[109,110] and malnutrition is even prevalent in community-dwelling seniors (Table 11.2). Micronutrient deficiencies are also prevalent among older adults, with 10% to 30% having subnormal levels of some vitamins or minerals (see Table 11.2). A prospective study in France of 252 patients older than 70 years of age who were admitted for rehabilitation found that lower baseline energy intake and lower albumin, zinc, selenium, and vitamin C levels were risk factors for developing health care–associated infections. These associations were not significantly changed by adjusting for T-cell subset values.[110]

Nutritional Supplements in Older Adults: Effects on Immunity and Clinical Outcomes

Although malnutrition in elderly persons is clearly associated with impaired immunity and poor clinical outcomes, nutritional supplementation has not definitively been shown to reverse this trend. Many studies examined only immune response variables rather than clinical end points, and these have been reviewed.[14,111,112] Studies specifically addressing clinical end points are emphasized in the following paragraphs for the most well-studied entities: multivitamin/mineral supplements, vitamin E, and zinc.

Multivitamin and Trace Mineral Supplements

Studies of multivitamin/mineral supplementation for the prevention of infection that have been performed in both outpatient healthy elderly persons and long-term care residents are summarized in Table 11.3.[113–118] Overall, it has been difficult to show any clear benefit from multivitamin/mineral supplementation in community-dwelling older adults. In long-term care residents, a series of studies hints that trace mineral (zinc

TABLE 11.2 Prevalence of Nutritional Deficiencies in Older Adults Residing in Various Settings

NUTRIENT	LONG-TERM CARE OR HOSPITALIZED (% DEFICIENT)	COMMUNITY-DWELLING (% DEFICIENT)
Protein/calories	20–50	1–16
Vitamin A	1–2	ND
Vitamin B_{12}	ND	20
Vitamin D	50–85	50
Vitamin E	1–3	1
Zinc	30–50	10

[a]Data are pooled from multiple studies. See references 106, 107, 109, 115, 118–120, 168–171.
ND, No data.

TABLE 11.3 Randomized, Placebo-Controlled Multivitamin/Mineral Supplementation Trials in Older Adults

REFERENCE (Year)	NO. AND POPULATION STUDIED	STUDY DURATION	RANDOMIZED GROUPS	OUTCOME MEASURED	COMMENT
Girodon et al.[113] (1997)	81 NH residents	24 months	1. Daily MVI 2. Daily Zn^{2+}, Se 3. Daily MVI, Zn^{2+}, Se 4. Placebo	MD-confirmed respiratory or urogenital infection–related mortality	No mortality difference; lower infection with TM[a] or TM[a] + MVI, but not with MVI alone
Girodon et al.[114] (1999)	725 NH residents	24 months	1. Daily MVI 2. Daily Zn^{2+}, Se 3. Daily MVI, Zn^{2+}, Se 4. Placebo	MD-confirmed respiratory or urogenital infection–related mortality	No mortality difference; borderline reduction in infection (P = .06) and improved influenza vaccine responses with TM, but not with MVI alone
Graat et al.[115] (2002)	652 community-dwelling older adults	15 months	1. Daily MVI, Zn^{2+}, Se 2. Daily MVI, Zn^{2+}, Se, vitamin E 3. Vitamin E 4. Placebo	Self-reported respiratory tract infection	No difference in incidence of infection; significantly worse symptom severity in vitamin E recipients
Barringer et al.[116] (2003)	130 community-dwelling older adults (33 were >65 y; others had DM)	12 months	1. Daily MVI, Zn^{2+}, Se 2. Placebo	Self-reported infection confirmed by MD	Lower incidence of infection overall (P < .001) and in subset with type 2 DM (P < .001), but not in elderly subset (P > .2)
Avenell et al.[117] (2005)	910 community-dwelling older adults	12 months	1. Daily MVI, Fe, I, Zn^{2+}, Mn 2. Placebo	No primary care MD visits for infection, self-reported infection, quality of life	No significant difference in any parameter measured
Liu et al.[118] (2007)	763 NH residents	18 months	1. Daily MVI, Ca, Mg, Fe, I, Cu, Zn^{2+}, Se 2. Placebo	Infection control practitioner surveillance for all infections, hospitalizations, and antibiotic use	No significant difference except in post hoc analysis: after excluding subjects with dementia, infection risk was reduced in remaining NH residents (RR, 0.81; 95% CI, 0.66–0.99).

[a]Zinc and selenium supplementation.
Ca, Calcium; *CI,* confidence interval; *Cu,* copper; *DM,* diabetes mellitus; *Fe,* iron; *I,* iodine; *MD,* medical doctor; *Mg,* magnesium; *Mn,* manganese; *MVI,* multivitamin; *NH,* nursing home; *RR,* relative risk; *Se,* selenium; *TM,* trace minerals; *Zn^{2+},* zinc.

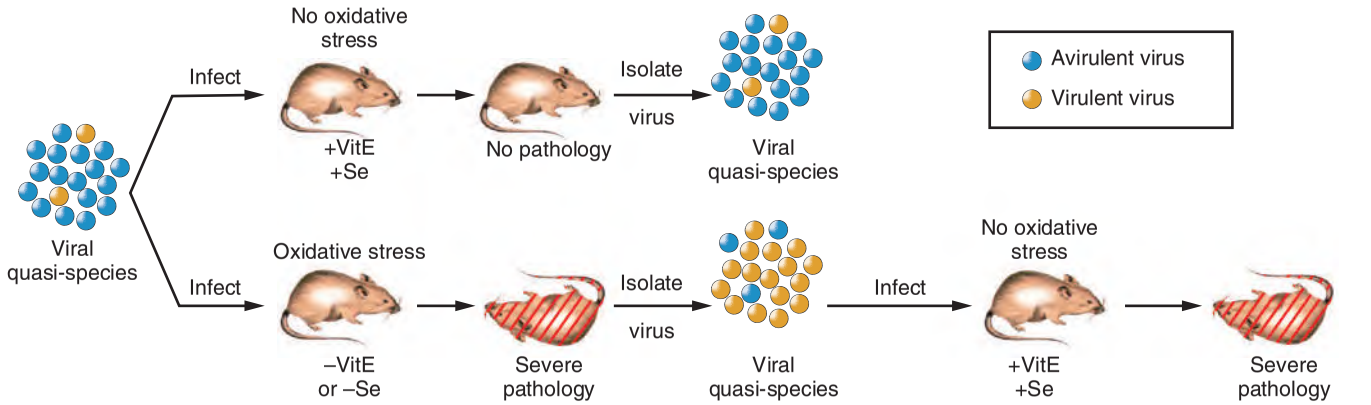

FIG. 11.2 Effect of malnutrition on virulence of a viral pathogen. Replication of relatively nonvirulent strains of a virus within a malnourished host (e.g., mouse with selenium [*Se*] deficiency) leads to hypermutation within the viral genome, resulting in the emergence of more virulent strains. Once the hypervirulent quasispecies emerge, they cause more serious illness even in nutritionally replete hosts. *VitE,* Vitamin E. *(From Beck MA, Handy J, Levander OA. Host nutritional status: the neglected virulence factor.* Trends Microbiol. *2004;12:417–423.)*

and selenium) supplementation may be more effective than multivitamins for reducing the incidence of respiratory tract infection, but there is little effect on urinary tract infection or pressure ulcers.[113,114] Furthermore, patients without dementia may benefit most from supplementation, based on a post hoc analysis of one study.[118]

Vitamin E

Vitamin E has been shown to enhance both humoral and cell-mediated immune responses in elderly individuals. Supplementation with vitamin E at 200 mg/day or 800 mg/day in healthy older adults improved delayed-type hypersensitivity responses and immunization responses to hepatitis B, but not to pneumococcal polysaccharide or tetanus.[34] However, in another study, the severity of symptoms due to infection was significantly *worse* in the vitamin E–supplemented group, with greater total illness duration and number of symptoms and more frequent fever and activity restriction (compared with subjects receiving placebo),[115] although it is possible that these findings represent enhanced immune responses in the vitamin E recipients. One additional study in long-term care residents showed varied benefit from vitamin E supplementation, depending on the outcome measured.[119] There was no difference in the number of overall days of respiratory tract infection, the primary end point of the study, but a lower proportion of subjects in the vitamin E group experienced one or more respiratory tract infections (RR, 0.88; 95% CI, 0.76–1.0), and the effect appeared to be most prominent for upper (vs. lower) respiratory tract infections. There was no effect on the amount of antibiotic use. A subanalysis of this study implicated low serum zinc levels as a risk factor for pneumonia in these subjects,[120] suggesting that trace minerals should be a focus of subsequent studies. Furthermore, there may be interactions between genotypes at specific loci (e.g., IL-10), sex, and vitamin E on risk for respiratory tract infection.[121] The same study suggested that specific single nucleotide polymorphisms of IL-1, IL-10, and interferon-γ have lower risk for respiratory infection regardless of vitamin E supplementation. There are also complex interactions of vitamin E supplementation in male smokers and risk for upper respiratory tract infections, pneumonia, or tuberculosis depending on duration and severity of prior smoking, activity level, and other vitamin supplementation.[122–125] Much work remains to determine people who may benefit or not from vitamin E supplementation for reducing infection risk.

It is clear that high doses of vitamin E should be avoided for most older adults. A meta-analysis of vitamin E supplementation trials demonstrated increased mortality for older adults receiving daily doses of 400 IU or greater.[126]

Zinc

As noted earlier, zinc has a role in immune function. Zinc deficiency is more common in elderly adults.[127] Zinc supplementation was included in all the clinical trials of vitamin E or multivitamin supplementation to prevent infection in older adults cited previously. Zinc supplementation was studied as the primary micronutrient provided to institutionalized elderly adults in an additional study,[128] and trends found were lower risk for overall infection, fever, and upper respiratory tract infections. An important insight regarding zinc and infection risk was noted in a reanalysis of a prior trial based on end-of-trial serum zinc level.[120] In that study, the incidence of pneumonia, duration of pneumonia, and use of antibiotics for pneumonia all were dramatically reduced in subjects with an end-of-trial serum zinc level of more than 70 μg/dL, whereas baseline serum zinc level was not associated with any of these outcomes. This indicates that the ability to increase serum zinc levels—perhaps due to unmeasured factors such as absorptive capacity, comorbidity, or zinc excretion—is a strong indicator of pneumonia risk, but the role of the zinc replacement itself remains to be defined.

HOST NUTRITIONAL STATUS AND PATHOGEN VIRULENCE

An important link of infection and malnutrition may not lie within the host's impaired response but instead within the pathogen itself, which may be altered by specific conditions present in the nutritionally impaired host. In selenium-deficient mice, infection with either coxsackievirus B or influenza virus led to the rapid development of mutations in the viral genome; in the case of influenza, mutations occurred specifically in the M1 and M2 matrix proteins. These mutations altered the virulence of influenza, increasing the severity of illness in subsequent hosts even if those hosts were adequately nourished (Fig. 11.2).[129,130] It appears that the redox status of the host is the key factor governing this process. In follow-up studies, Gay et al.[131] demonstrated that advanced age, characterized by a limited capacity to buffer oxidative stress (similar to selenium deficiency), could also induce the accelerated mutation rate and virulence change in coxsackievirus.

Although animal data support the model of enhanced mutation rates and augmented virulence shown in Fig. 11.2, there are sparse data in humans. There have been limited investigations of mutation rates in subjects with marginal selenium status.[129] Selenium supplementation was provided to a small group of human subjects before, during, and after administration of live oral poliovirus vaccine, and stool samples were collected to determine the mutation rate in the selenium-supplemented and control groups. The mutation rate of the oral poliovirus vaccine strain was significantly higher in the group that did not receive selenium supplementation, supporting the animal findings previously outlined. This observation led to the theory that hosts who are nutritionally deficient (e.g., selenium deficient) or who are oxidatively stressed (e.g., aged adults[131]) may provide an environment that induces a high mutation rate in infecting pathogens, leading to enhanced virulence (see Fig. 11.2).

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12 Evaluation of the Patient With Suspected Immunodeficiency

Steven M. Holland and John I. Gallin[a]

SHORT VIEW SUMMARY

Overview
- Infection is often the first and potentially the deadliest manifestation of congenital or acquired immunodeficiency.
- Infections involving common pathogens can be more prolonged, recurrent, or severe than in normals, or may be due to opportunistic organisms.
- A history of frequent infections and/or an unusual pathogen or site of infection should be a trigger for clinicians to consider that an immunodeficiency syndrome may be present; which may be either congenital or acquired.

Etiology
- The genetic disorders underlying the most common congenital problems have been gradually uncovered, providing strong tools for proper classification and leading to clinical approaches (see Table 12.2).

Infecting Agents
- The spectrum of infections associated with genetic immunodeficiencies is broad, including herpes simplex encephalitis, staphylococcal abscesses, *Burkholderia* bacteremia, mycobacterial osteomyelitis, severe human papillomavirus infections, disseminated coccidioidomycosis, and *Aspergillus* pneumonia, in addition to severe cytomegalovirus and *Pneumocystis,* among many others.
- However, particular infecting agents often are associated with specific immunodeficiency conditions (see Table 12.1), providing immediate clues about the probable basis of the illness.

Other Manifestations
- Immunodeficiency is also commonly associated with autoimmunity, reflecting the failure to protect against autoreactivity and invasion.

Diagnostic Approach
- Based on the type of clinical presentation, particular diagnostic tests can be used to determine the particular immunodeficiency present.
- A small battery of screening tests, emphasizing complete blood count, immunoglobulin levels and complement, and neutrophil function, will enable detection of most common disorders (see Table 12.3). Based on those findings and the clinical presentation, more specific functional assays can be undertaken (see Table 12.4).

Therapy and Prevention
- The clinical presentation, results of diagnostic testing, and characterization of the genetic lesion will dictate the therapeutic approach, and in some cases a preventive strategy.

The most common causes of immunodeficiency are iatrogenic and result from the widespread use of therapies that modulate the immune system, either by design or incidentally. With the expanding recognition, characterization, and—in an increasing number of cases—correction of immune abnormalities, making the correct diagnosis is of critical importance. Identification and cloning of disease-related genes has now made precise antenatal diagnosis and genetic counseling a reality. With successful stem cell transplantation and the emerging field of gene therapy, it is essential to use a sensible, problem-oriented approach to the patient suspected of having an immunodeficiency. What follows are some general principles involved in the consideration of whether a patient may have an immunodeficiency and how to proceed with a diagnostic evaluation before, or as an adjunct to, referral or discussion with a specialist.

INDEX OF SUSPICION

Concern about the immune status of a patient is usually raised on the basis of the frequency or severity of infections or the finding of an unusual infectious agent. Table 12.1 lists some infectious organisms and the affected limb of host defense implied by their isolation. Clearly, not every isolation of a herpesvirus or *Staphylococcus* implies an immunodeficiency in a specific patient. However, in the setting of abnormally frequent or severe infections or failure to thrive, isolation of these organisms from patients should make one consider possible underlying diagnoses. In contrast, identification of *Pneumocystis, Mycobacterium, Burkholderia cepacia, Aspergillus,* or *Nocardia* from a patient without a known immunodeficiency is sufficient grounds for pursuing the probability of an underlying defect.

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

Recurrent hematogenous neisserial infections indicate deficiencies in the late components of complement.[1] *Pneumocystis (carinii) jirovecii* pneumonia indicates T-cell abnormalities.[2] *B. cepacia* bacteremia strongly suggests chronic granulomatous disease.[3] Some specific immunodeficiencies are listed in Table 12.2, along with the gene defects, if known, and some pertinent findings. Recognition and appreciation of the genetic bases of these disorders have been critical to the development of therapy for them and are fundamental to the curative approaches now available.

INITIAL EVALUATION

The screening approach to a patient with suspected immunodeficiency is listed in Table 12.3. Careful attention to historical detail is critical. Age at onset of the illness is helpful: hyperimmunoglobulin E–recurrent infection syndrome (HIES or Job syndrome) often has an onset within the first days to weeks of life, whereas antibody deficiency states appear only after several months of life, when maternal immunoglobulin (Ig) levels have fallen.[4] Failure to thrive and diarrhea are important points in favor of a substantial problem but are not specific in terms of etiology. Birth history should include the condition and time of separation of the umbilical stump because stump separation is abnormally delayed in leukocyte adhesion deficiency type 1 (LAD1).[5] The past medical history should note the administration of vaccines, especially for measles, mumps, and rubella, and Calmette-Guérin bacillus (BCG), difficulties with which are suggestive of dysfunctional T-cell or monocyte immunity. A dental history can be quite informative because patients with abnormalities of phagocytic defense often have gingivitis with periodontal disease[6] (Fig. 12.1); patients with Job syndrome usually have prolonged retention of the primary teeth,[7] and patients with nuclear factor kappa B (NF-κB) essential modulator (NEMO) deficiency often have conical or widely spaced teeth. Specific questioning regarding parental consanguinity is critical, even if uncomfortable.

TABLE 12.1 Selected Pathogens Associated With Immunodeficiency Diseases

PATHOGEN	HISTORY	HOST DEFENSE AFFECTED	CLINICAL EXAMPLES
Pneumocystis (carinii) jirovecii, Cryptococcus neoformans, herpesviruses	Disseminated infections, opportunistic infections, persistent viral infections	T cells	Severe combined immunodeficiency, acquired immunodeficiency syndrome
Haemophilus influenzae, Streptococcus pneumoniae, Giardia lamblia, Campylobacter spp., enteroviruses	Recurrent respiratory infections with encapsulated organisms, chronic diarrhea, aseptic meningitis	B cells	Common variable immunodeficiency, X-linked agammaglobulinemia
Staphylococcus aureus, Burkholderia cepacia, Serratia marcescens, Aspergillus spp., *Nocardia* spp.	Gingivitis, aphthous ulcers, recurrent pyogenic infections, delayed umbilical stump separation	Phagocytes	Chronic granulomatous disease, Chédiak-Higashi syndrome, leukocyte adhesion deficiency
Nontuberculous mycobacteria, BCG, *Salmonella, Histoplasma capsulatum, Coccidioides immitis*	Disseminated infections, multifocal osteomyelitis	Monocytes	Interferon-γ/IL-12 pathway defects, STAT1, NEMO, GATA2
S. pneumoniae, Neisseria meningitidis, S. aureus, herpes simplex virus	Recurrent meningitis, bacteremia, lack of fever	TLR pathway	MyD88, IRAK-4 defects, TLR defects
Neisseria spp.	Recurrent bacteremia, recurrent meningitis	Complement	Late complement component deficiency
S. aureus, H. influenzae, S. pneumoniae, Candida albicans	Eczema, kyphoscoliosis, pathologic fractures, pulmonary and cutaneous infections mucocutaneous candidiasis	T cells, phagocytes	Hyperimmunoglobulin E recurrent infections (Job) syndrome

BCG, Calmette-Guérin bacillus; *GATA2,* a hematopoietic transcription factor; *IL-12,* interleukin-12; *IRAK-4,* interleukin-1 receptor–associated kinase 4; *MyD88,* myeloid differentiation (primary response) protein 88; *NEMO,* nuclear factor kappa B essential modulator; *STAT1,* signal transducer and activator of transcription 1; *TLR,* Toll-like receptor.

TABLE 12.2 Congenital Immunodeficiencies

CLINICAL DISEASE	AFFECTED GENE PRODUCT[a]	CHROMOSOMAL LOCATION	INHERITANCE	FUNCTIONAL DEFECT	IMPORTANT FINDINGS	REFERENCES
T Cells						
X-linked SCID	Interleukin (IL)-2 receptor gamma common chain	Xq13-21.1	X	T-cell proliferation, antibody production	Lymphopenia, hypogammaglobulinemia	2, 16
Adenosine deaminase (ADA) deficiency	Adenosine deaminase	20q13-ter	AR	T-cell functions, antibody production	Absent ADA activity, lymphopenia, hypogammaglobulinemia	14
Purine nucleoside phosphorylase (PNP) deficiency	Purine nucleoside phosphorylase	14q13.1	AR	T-cell functions	Absent PNP activity, low CD3 cells, increased NK cells, low uric acid	15
Defective major histocompatibility complex (MHC) molecules	RF-X	19q13	AR	Cell-mediated immunity	B cells normal, Ig normal or low, absent MHC molecules	24
IL-2 deficiency	Nuclear factor–activated T cells (NFAT)	?	AR	Cell-mediated immunity, antibody production	Lymphopenia, hypogammaglobulinemia	20–23
Reticular dysgenesis	Adenylate kinase 2	1p34	AR	Leukocyte mitochondrial metabolism	Pancytopenia and deafness	11–13
DiGeorge syndrome, velocardiofacial syndrome CATCH 22	TBX-1 and contiguous gene products	22q11.21-q11.23	AD	Anomalous development of third and fourth pharyngeal pouches	Thymic aplasia, parathyroid aplasia, cardiac anomalies, abnormal facies	8, 26
Ataxia-telangiectasia	Ataxia-telangiectasia mutated (ATM)	11q22.3	AR	DNA repair, T cells	Low IgA, low CD3 and CD4 cells, malignancies	27
Wiskott-Aldrich syndrome	Wiskott-Aldrich syndrome protein (WASP)	Xq11-11.3	X	T cells and platelets	Eczema, thrombocytopenia, low platelet volume, low IgM, high IgA, IgE	28–33
B Cells						
X-linked agammaglobulinemia	B-cell progenitor kinase (BTK)	Xq22	X	B cells	Very low antibody levels	2, 34, 35

Continued

TABLE 12.2 Congenital Immunodeficiencies—cont'd

CLINICAL DISEASE	AFFECTED GENE PRODUCT[a]	CHROMOSOMAL LOCATION	INHERITANCE	FUNCTIONAL DEFECT	IMPORTANT FINDINGS	REFERENCES
Immunodeficiencies with hyper-IgM	CD40 ligand (CD40L, gp39)	Xq26	X	B cells, T cells, monocytes	High IgM, low IgG, IgA, poor T-cell responses	36, 37
	NF-κB essential modulator (NEMO)	Xq28	X	Neutrophils	High or normal IgM, low IgG, broad spectrum of infections	38
	CD40	20q12	AR	Monocytes	High or normal IgM, low IgG, poor T-cell function	39
	Activation-induced cytidine deaminase (AID)	12p13	AR	B cells	Lymph nodes present, normal-to-high IgM, low IgG	40
X-linked lymphoproliferative syndrome (Duncan syndrome)	SLAM-associated protein (SAP)	Xq25	X	EBV response	Low EBNA antibody, uncontrolled cellular activation	42–44
Common variable immunodeficiency	ICOS and others	2q33 and others	AR	Antibody synthesis T-cell function	Low IgG, poor antibody response, low IgA common	45, 142
IgA deficiency	IgA	?6p21.3	AR	IgA	Associated with other immunodeficiencies	45, 46
Phagocytes						
Chronic granulomatous disease (CGD)				Bacterial and fungal killing defective in all forms of CGD	Infections with catalase-positive microbes, granulomas, and reduced superoxide generation	76–78, 143
X-linked CGD	gp91*phox*	Xp21.1	X 70% of CGD			
Autosomal recessive CGD	p22*phox*	16q24	AR <5% of CGD	—	—	
	p47*phox*	7q11.23	AR 25% of CGD	—	—	
	p67*phox*	1q25	AR <5% of CGD	—	—	
	p40*phox*	22q12.3	AR <1% of CGD	Less severe superoxide impairment	Predominantly colitis	
	EROS	17q25.3	AR <1% of CGD	Less severe superoxide impairment	Predominantly colitis	
Severe chronic neutropenia and cyclic neutropenia	Neutrophil elastase (ELANE)	19p13	AD	Neutropenia	Cyclic hematopoiesis, cycle ≈21 days; some cases of severe chronic neutropenia	49–54
	Growth factor independent 1 (GFI1)	1p22	AD	Neutropenia	Severe neutropenia	51
	HAX-1	1q21.3	AR	Neutropenia	Kostmann syndrome, severe chronic neutropenia	53
MonoMAC; dendritic cell, myeloid, and NK-cell lymphopenia (DCML); Emberger syndrome; familial acute leukemia/myelodysplasia	GATA2	3q17	AD haploinsufficiency	Monocytopenia, B and NK lymphopenia, macrophage dysfunction	Warts, dysplastic megakaryocytes, hypoplastic bone marrow, cytopenias, pulmonary alveolar proteinosis	124
Warts, hypogammaglobulinemia infections, and myelokathexis (WHIM)	CXCR4	2q21	AD	Impaired chemokine receptor activity	Impaired neutrophil exit from the marrow (myelokathexis), low IgG, warts	144
Chédiak-Higashi syndrome	Lysosomal transport protein (LYST)	1q43	AR	Chemotactic defect neutropenia	Giant granules in neutrophils, oculocutaneous albinism	57–59
Leukocyte adhesion deficiency type 1	CD18 (ITGB2)	21q22.3	AR	Absent leukocyte integrins	Chronic leukocytosis, delayed umbilical cord separation, recurrent infections	5, 61, 63, 64
Leukocyte adhesion deficiency type 2	Sialyl-Lewis X (due to mutations in GDP-fucose transporter-1 [FUCT1])	11p11.2	AR	E-selectin ligand, fucose metabolism	Short stature, mental retardation, Bombay blood type	64, 65

TABLE 12.2 Congenital Immunodeficiencies—cont'd

CLINICAL DISEASE	AFFECTED GENE PRODUCT[a]	CHROMOSOMAL LOCATION	INHERITANCE	FUNCTIONAL DEFECT	IMPORTANT FINDINGS	REFERENCES
Leukocyte adhesion deficiency type 3	KINDLIN-3 (FERMT3)	11q12	AR	Signaling from integrin ligation	Bleeding diathesis, leukocytosis	66
Neutrophil-specific granule deficiency	CCAAT enhancer binding protein (C/EBPε)	3q21	AR	Neutrophil granule products	Absent neutrophil-specific granules, absent defensins (primary granules)	67–72
Myeloid differentiation primary response gene 88	MyD88	3p22	AR	Signal transduction through the Toll-like receptors, the IL-1 receptor, and others	Severe pyogenic infections in childhood	98
IL-1 receptor–associated kinase-4 deficiency	IRAK-4	12q12	AR	Signal transduction through the Toll-like receptors, the IL-1 receptor, and others	Severe pyogenic infections in childhood	97
Toll-like receptor 3	TLR3	4q35	AR	TLR3 signaling	Recurrent HSV encephalitis	99
Myeloperoxidase deficiency	Myeloperoxidase (MPO)	17q21-q23	AR	Catalysis of superoxide to hydrogen peroxide	Absent MPO, usually unassociated with infections	73
Interferon-γ (IFN-γ) receptor 1 deficiency	IFN-γR1	6q23-q24	AR AD	Absence of IFN-γ binding Overaccumulation of defective receptor	Recurrent nontuberculous mycobacterial and *Salmonella* infections Nontuberculous mycobacterial osteomyelitis	102–104 106, 107
IFN-γ receptor 2 deficiency	IFN-γR2	21q22.1-q22.2	AR	Absence of IFN-γ signaling	Recurrent nontuberculous mycobacteria	105
IL-12 receptor β1 deficiency	IL-12Rβ1	19p13.1	AR	Absence of IL-12 signaling	Recurrent nontuberculous mycobacterial and *Salmonella* infections	109, 110, 112
IL-12p40 deficiency (IL-12p40)	IL-12p40	5q31	AR	Lack of IL-12 leading to low production of IFN-γ	Recurrent nontuberculous mycobacterial and *Salmonella* infections	111
Signal transducer and activator of transcription 1 (STAT1)	STAT1	2q32.2	AD AR AD	Reduced IFN-γ receptor signaling Absent IFN-γ and IFN-α/β receptor signaling Gain of function; reduced IL-17 producing T cells	Severe disseminated nontuberculous mycobacteria and BCG Disseminated fatal mycobacterial and viral infections Chronic mucocutaneous candidiasis; disseminated histoplasmosis or coccidioidomycosis	114 113 115–118
Hyper-IgE–recurrent infection syndrome (Job syndrome)	STAT3	17q21	AD	Reduced STAT3-dependent signaling (IL-6, IL-17)	Extremely high IgE, eczema, facial, dental and bony abnormalities, pneumatocele formations	7, 119, 121–123
Complement ***Classical Pathway***						
C1q deficiency C1r deficiency C1s deficiency C2 deficiency C3 deficiency C4A deficiency C4B deficiency C5 deficiency C6 deficiency C7 deficiency C8 deficiency C9 deficiency	C1q C1r C1s C2 C3 C4A C4B C5 C6 C7 C8 C9	1p36.3 12p13 12p13 6p21.3 19p13.3 6p21.3 6p21.3 9q34.1 5p13 5p13 1p32 5p13	AR AR AR AR AR AR AR AR AR AR AR AR	Antibody-dependent complement lysis is depressed in all forms of classical complement component deficiencies.	Low CH_{50} is seen with all forms of classical complement component deficiency. Individual components are very low or absent. Autoimmune disease is common in early-component deficiencies (C1-C4). Bacteremia and meningitis are common in all types of complement deficiencies.	1

Continued

TABLE 12.2 Congenital Immunodeficiencies—cont'd

CLINICAL DISEASE	AFFECTED GENE PRODUCT[a]	CHROMOSOMAL LOCATION	INHERITANCE	FUNCTIONAL DEFECT	IMPORTANT FINDINGS	REFERENCES
Alternative Pathway						
Properdin deficiency	Properdin	Xp11.4-23	X	Antibody-independent complement lysis is depressed in alternative complement component deficiencies	More severe susceptibility to infection than classical component deficiencies	
Factor H deficiency	Factor H	1q32	AR			
Factor I deficiency	Factor I	4q25	AR			

[a]The affected gene product is not always the gene in which the lesion has occurred. The genetic lesion may disable a regulatory gene required for expression or function of the affected gene product.

AD, Autosomal dominant; *AR,* autosomal recessive; *BCG,* Calmette-Guérin bacillus; *CATCH 22,* cardiac defects, abnormal facial features, thymic hypoplasia, cleft palate, and hypocalcemia; CH_{50}, total hemolytic classical complement; *EBNA,* Epstein-Barr virus nuclear antigen; *EBV,* Epstein-Barr virus; *ELANE,* elastase neutrophil expressed; *GATA2,* a hematopoietic transcription factor; *GDP,* guanosine diphosphate; *HAX-1,* HCLS-1 (hematopoietic cell-specific Lyn substrate 1)–associated protein X-1; *HSV,* herpes simplex virus; *ICOS,* inducible costimulator; *Ig,* immunoglobulin; *IRAK-4,* interleukin-1 receptor–associated kinase 4; *MonoMAC,* monocytopenia and mycobacterial infections; *MyD88,* myeloid differentiation (primary response) protein 88; *NF-κB,* nuclear factor kappa B; *NK,* natural killer; *RF,* regulatory factor; *SCID,* severe combined immunodeficiency; *SLAM,* signaling lymphocyte activation molecule; *STAT1,* signal transducer and activator of transcription 1; *TLR3,* Toll-like receptor 3; *X,* X-linked inheritance.

TABLE 12.3 Screening Evaluations for Immune Defects

History

Medications and treatments
Relatedness of parents, umbilical stump separation, age of onset, dental history
Frequency, severity, distribution, types of infections
Vaccination history, especially handling of live vaccines and antibody response to vaccination
Causative infectious agents

Physical

Weight and height
Hair: sheen, pigmentation
Abdomen: organomegaly
Skin: dystrophic scars, telangiectasis, eczema, warts
Oropharynx: thrush, ulcers, gingivitis, secondary tooth eruption
Facies: nasal size, eye slant, philtrum
Skeleton: kyphoscoliosis, fractures

Routine Laboratory Values
Complete Blood Count

Differential: lymphopenia, neutropenia, monocytopenia
Peripheral smear: giant granules, specific (secondary) granules
Platelet count: thrombocytopenia
Erythrocyte sedimentation rate: usually elevated in infection

Chemistries

Serum calcium
Serum uric acid
Liver functions

Immunoglobulins

IgA, IgM, IgG, IgE
Isohemagglutinins
Antibody titers (tetanus, pneumococcus, etc.)

Complement

Total hemolytic complement (classical CH_{50}, alternative AH_{50})

Radiography

Plain chest films: kyphoscoliosis, pneumatoceles, scarring

Physical examination can yield findings diagnostic or highly suggestive of lesions in specific arms of the immune system. Facial anomalies, including hypertelorism, shortened philtrum, and down-slanting palpebral fissures, are encountered in DiGeorge syndrome,[8] whereas characteristic facies with a broad nose and a triangular mandible are seen in Job syndrome.[7,9] Hair with a silvery sheen and irregular melanin distribution is seen in Chédiak-Higashi syndrome.[10] Cutaneous signs of immune defects include telangiectasia over the bulbar conjunctivae and skin in ataxia-telangiectasia, the severe eczema that accompanies Job syndrome, or the dystrophic scarring seen in LAD1.

FIG. 12.1 Severe periodontal disease in leukocyte adhesion deficiency type 1 as shown by gum recession.

The initial laboratory examination should consist of a complete blood count with differential, platelet count, examination of the peripheral blood smear, and erythrocyte sedimentation rate. Chédiak-Higashi syndrome and neutrophil-specific granule deficiency can be detected on peripheral smear, whereas severe neutropenias and Wiskott-Aldrich syndrome can be largely excluded by normal neutrophil or platelet counts, respectively. An Ig profile with total IgA, IgM, and IgG levels will help detect cases of IgA deficiency and hypogammaglobulinemia. In cases in which eczema is a prominent feature or Job syndrome is suspected, measurement of IgE levels is indicated. The humoral immune system is functionally interrogated by preimmunization and postimmunization antibody levels. Testing of total hemolytic complement (CH_{50}) gives a quick assessment of the functional integrity of the classical component of the complement cascade from C1 through the membrane attack complex (C5 to C9), whereas the alternative pathway hemolytic complement (AH_{50}) tests the alternative pathway of complement activation. Plain radiographs of the chest can demonstrate pulmonary scarring, pneumatoceles, and parenchymal destruction, often encountered in phagocyte defects and Job syndrome. Scoliosis, osteoporosis, rib and long bone fractures, and their sequelae are frequently seen in Job syndrome.[7,9]

LYMPHOCYTE IMMUNE DEFECTS

T Cells and Cell-Mediated Immunity

The initial manifestations of congenital T-cell defects usually, but not always, occur within the first few months of life and include severe mucocutaneous candidiasis, recurrent persistent respiratory infections, diarrhea, and failure to thrive. A broad spectrum of onset and severity is seen. Reticular dysgenesis (adenylate kinase 2 deficiency) appears within the first weeks of life and is characterized by pancytopenia, infection, deafness, and early demise.[11–13] The lymphocyte enzymopathies (adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency) tend to occur after several months of life, when lymphocyte counts begin to fall because of accumulation of the toxic metabolites deoxyadenosine triphosphate and deoxyguanosine triphosphate, respectively.[14,15] The occurrence of *P. (carinii) jirovecii* pneumonia, disseminated BCG infection after vaccination, persistent poliovirus infection after oral polio vaccination, or persistent respiratory virus infection should initiate consideration of a defect in lymphocyte function.[16] Graft-versus-host disease, either acquired from in utero transfer of maternal lymphocytes or through transfusion of unirradiated blood, may be the underlying cause of cutaneous eruptions, transaminase elevations, or malabsorption and diarrhea and is a strong indicator of defective T-cell immunity.[16]

In many forms of T-cell abnormality, B-cell function is also compromised, and both cell-mediated and humoral deficiencies occur, a state referred to as severe combined immunodeficiency (SCID).[16] This syndrome tends to be recognized after maternal antibody levels wane and recurrent bacterial infections begin. The finding of low Ig levels in association with marked lymphopenia in the appropriate setting should lead to the consideration of severe combined immunodeficiency.[2,16]

The most direct and simplest assessment of T-cell immune status is the determination of lymphocyte number, which is obtained in the screening differential count. Circulating lymphocyte numbers range from about 7000 cells/μL in infants to 4000 cells/μL in children and about 2000 cells/μL in adolescents and adults through old age.[17] Of these lymphocytes, roughly half are T cells, with the remainder split between B cells and natural killer (NK) cells. Severe quantitative abnormalities of lymphocytes are relatively uncommon; low absolute lymphocyte numbers are encountered in the great majority of cases of SCID, and in several other congenital immunodeficiencies (see Table 12.2). The lymphocyte number is now detected as part of newborn screening through the analysis of T-cell receptor excision circles (TRECs)—small, stable DNA circles that reflect thymic output and are detectable with use of 50 μL of dried blood on the Guthrie card.[18] In almost all of the United States, newborns are screened and low TRECs are confirmed with flow cytometry; those with very low T-cell numbers are referred for evaluation in the first days of life, before severe infections begin. Acquired immunodeficiency syndrome (AIDS); high plasma corticosteroid levels (iatrogenic or endogenous); obstructed lymphatic circulation (e.g., intestinal lymphangiectasia or protein-losing enteropathy); severe systemic illness (e.g., carcinomatosis, miliary tuberculosis); systemic lupus erythematosus; sarcoid, cytotoxic, or immunosuppressive therapy; and severe right-sided congestive heart failure can also cause lymphocytopenia. These latter causes are much more common than the congenital ones.

T-lymphocyte function is initially and most easily assayed in vivo through testing of type IV cell-mediated immunity or delayed-type hypersensitivity (DTH) (Table 12.4). DTH is elicited by the intradermal injection of an antigen to which the subject has been exposed. Antigen-specific $CD4^+$ T cells are recruited, and in turn, these T cells recruit macrophages, with resulting interstitial fibrin deposition and induration. Erythema and edema are seen early after the injection but are not indicative of DTH. Frank induration, which is the best marker of effective DTH, is best appreciated between 24 and 48 hours and then gradually falls off.

In the screening evaluation for immune defects, it is important to consider the patient's experiences, such as immunizations, previous infections, and regional exposures, in the selection of antigens. It is useful to test several different antigens simultaneously for the determination of T-cell responsiveness, such as mumps, *Candida, Trichophyton,* streptococcal antigens, and tetanus. The demonstration of intact DTH confirms the presence of functional $CD4^+$ T cells and excludes most of the severe congenital defects in cell-mediated immunity. DTH can also be evaluated in vitro, as antigen-specific T-cell proliferation or cytokine response. Of importance, DTH can be preserved until relatively late into human immunodeficiency virus (HIV) infection. Therefore the presence of DTH should not be considered evidence against HIV infection per se. Selective anergy to the antigens of the infecting organism has been observed in active visceral leishmaniasis, lepromatous leprosy, and active tuberculosis. These specific defects typically reverse after successful treatment of the underlying infection. Complete anergy to a battery of antigens is a relatively nonspecific finding in terms of etiology, insofar as the differential diagnosis includes all the entities that cause lymphopenia. However, anergy is an indication for further evaluation of cell-mediated immunity.

TABLE 12.4 Directed Tests of Immune Function

T Cells
FACS (lymphocyte subsets, cytokine receptors)
Delayed-type hypersensitivity: mumps, *Candida,* tetanus, *Trichophyton*
Lymphocyte enzymes (adenosine deaminase and purine nucleoside phosphorylase)
Cytokine production and response
In vitro proliferation: stimulation with antigen, lectin, antibody, allogeneic cells, protein kinase C stimulants, and calcium ionophores
B Cells
FACS
Antigen challenge, recall or new
In vitro antibody production
Phagocytes
FACS for leukocyte adhesion molecules
Dihydrorhodamine oxidation, nitroblue tetrazolium reduction
Superoxide generation, hydrogen peroxide generation
Adherence
Staphylococcal killing
Chemotaxis
Phagocytosis
Complement
Assay of individual complement components, functional or quantitative

FACS, Fluorescence-activated cell sorting.

Fluorescence-activated cell sorting (FACS) analysis allows rapid enumeration and characterization of lymphocyte, monocyte, and neutrophil subsets.[19] Lymphocyte subset determination, specifically for $CD4^+$ T cells, is standard in the management of HIV infection, where it provides guideposts for initiation and monitoring of antiviral therapy. With the recent identification and cloning of many immunodeficiency-related genes and the development of specific antibodies, FACS is able to confirm or exclude specific immunodeficiencies.

Evaluation of T-cell function in vitro requires laboratory personnel skilled in the isolation, preparation, and stimulation of peripheral blood mononuclear cells. Stimulation is typically done on unseparated peripheral blood mononuclear cells and therefore represents the product of both lymphocyte and monocyte contributions. Proliferation is usually determined as some measure of lymphocyte division after stimulation with cell membrane–binding lectins (e.g., phytohemagglutinin [PHA], concanavalin A [ConA], pokeweed mitogen [PWM]), direct stimulants of cellular signaling pathways that bypass the need for membrane components (phorbol myristate acetate and the calcium ionophore ionomycin), antigens (purified protein derivative [PPD], *Candida,* tetanus), cytokines (interleukin [IL]-2), or allogeneic cells (mixed lymphocyte reaction).[19] Significantly low proliferation in vitro can result from lymphocytopenia; absent or impaired cell surface receptors, such as the CD3 or IL-2 receptor complexes[20]; antigen-specific anergy; IL-2 deficiencies[21–23]; or major histocompatibility complex abnormalities.[24]

At least four important $CD4^+$ T-cell subsets are distinguished: Th1 cells produce interferon-γ (IFN-γ) and IL-2 and are the predominant cells in the DTH response; Th2 cells produce IL-4, IL-5, IL-10, and IL-13 and regulate the differentiation of B cells and eosinophils; Th17 cells produce IL-17 and IL-22 and regulate neutrophil number and

epithelial responses to infection, including *Candida* and epithelial inflammation; and T regulatory cells (Tregs) control and regulate T-cell and B-cell responses and are essential for keeping T-cell responses in line. Defects in Tregs lead to aberrant inflammation, such as the immunodysregulation-polyendocrinopathy-enteropathy–X-linked (IPEX) syndrome. All of these cell types also regulate one another, opening new areas for immune defects and therapeutic manipulations of their relationship. Cytokine levels in the supernatants of stimulated and unstimulated cells can be readily determined and may demonstrate states of either deficiency[21–23] or excess.

DiGeorge syndrome is caused by anomalous development of the third and fourth pharyngeal pouches, with agenesis of the thymus and parathyroids and subsequent immunodeficiency and hypocalcemia. Neonatal tetany is an expected manifestation.[8] The defect in DiGeorge syndrome is due to interstitial deletions in chromosome 21, including mutations in the *TBX-1* gene.[25,26] Ataxia-telangiectasia is associated with low IgA and low $CD3^+$ and $CD4^+$ T-cell levels, progressive ataxia, and oculocutaneous telangiectasia. These patients usually experience recurrent bacterial respiratory infections and are at increased risk of malignancy because of abnormalities in DNA repair.[2,27] Wiskott-Aldrich syndrome is characterized by eczema, elevated IgE, thrombocytopenia with small platelets, and recurrent opportunistic infections resulting from mutations in the Wiskott-Aldrich syndrome protein (WASP).[28–31] Mutations in WASP also have been found to cause isolated X-linked thrombocytopenia[32] and X-linked neutropenia.[33]

B Cells and Humoral Immunity

Almost 70 years have elapsed since the first clear description of an immunodeficiency syndrome, X-linked agammaglobulinemia (XLA), by Colonel Bruton.[34] The clinical features of isolated Ig defects are distinct from those of T-cell or combined defects. The age at onset for congenital deficiencies is between 6 months and 2 years, and the initial infections are predominantly with encapsulated bacteria. The thymus gland is present and apparently normal in XLA, but peripheral lymphoid tissues, such as tonsils and lymph nodes, are typically absent.[35] B cells and plasma cells in the circulation and the periphery are rare despite normal numbers of pre–B cells in the bone marrow. These patients have elevated T-cell numbers, normal T-cell subsets, and intact T-cell functions. Although they have significant problems with bacterial infections, patients with XLA also have severe difficulty with persistent, disseminated echovirus infections, especially in the central nervous system. IgG, IgA, and IgM levels are extremely low, as are isohemagglutinins. No antibody to either new or previously administered antigens is detectable. In contrast, patients with any of the syndromes that can give rise to one of the hyperimmunoglobulin M syndromes (the X-linked deficiency of CD40 ligand [CD40L or CD154][36,37]; X-linked ectodermal dysplasia with immunodeficiency [NEMO][38]; autosomal recessive CD40 deficiency[39]; autosomal recessive, activation-induced cytidine deaminase deficiency[40]; phosphatidylinositol 3-kinase catalytic delta [PI3KCD][41]; and others) may have preserved lymphoid tissue mass, hepatosplenomegaly, normal numbers of B cells and T cells, and variable neutropenia. Autoimmune phenomena such as Coombs-positive hemolytic anemia and thrombocytopenia are common. IgM is often normal to elevated, and IgG and IgA are usually quite low; isohemagglutinins may be elevated.[35] The X-linked lymphoproliferative disorder—XLP or Duncan syndrome—occurs in males, typically after infection with Epstein-Barr virus (EBV), and can cause problems ranging from aplastic anemia to hypogammaglobulinemia to a full-blown immunodeficiency syndrome, including hemophagocytic lymphohistiocytosis (HLH), which can be fatal.[42] The causative gene product associates with the signaling lymphocyte–activation molecule (SLAM) and is called the SLAM-associated protein (SAP), which is critical for T-cell signaling.[43,44]

The initial test for the integrity of the humoral arm of immunity is determination of levels of isohemagglutinins (see later, this section) and levels of IgG, IgA, and IgM. Normal Ig levels are relatively low in infancy and childhood and increase with age. If Ig levels are appropriate, XLA can be excluded. Low Ig levels may be seen in the first year of life in the transient hypogammaglobulinemia of infancy.[2,35] However, these infants usually have detectable isohemagglutinins and can mount antibody responses to new antigenic challenges. Despite family histories notable for relatives with immunodeficiencies, patients with transient hypogammaglobulinemia of infancy tend to normalize their Ig levels over the first 2 years of life. In contrast, depressed levels of Ig in an adult with recurrent bacterial sinopulmonary infections raise the possibility of common variable immunodeficiency (CVID).[45] CVID is a heterogeneous group of diseases that share the features of hypogammaglobulinemia and an increased susceptibility to chronic enteric infections with *Giardia lamblia, Campylobacter,* and disseminated echovirus infections, in addition to sinopulmonary bacterial infections. Patients with CVID often have low isohemagglutinin levels and abnormal DTH and fail to make antibody to new antigens. IgG subclass analysis may show selective defects in IgG1 and IgG3 or in IgG2 and IgG4, in addition to defects in IgA.[45] However, the value of checking IgG subclasses is debated.[46]

Isohemagglutinins are IgM antibodies directed against blood group A and B antigens, which occur in all healthy people, except those with blood group AB. By the age of 3 years, 98% of patients with blood groups A, B, or O have isohemagglutinins with a titer of at least 1:16. Isohemagglutinin levels are determined in blood banks as a prerequisite to transfusion. Challenge with antigen is probably the simplest and most effective method for determining the functional integrity of the humoral immune system. Polysaccharide pneumococcal vaccinations examine the response to polysaccharide antigen, whereas tetanus challenge is more specific for peptide responses. Serum titers should be checked before and 3 to 4 weeks after immunization. Use of a recall antigen allows testing of anamnestic responses. Novel or rare antigens, such as bacteriophage ΦX174, keyhole limpet hemocyanin, or rabies vaccine, make it possible to test antibody responses even during Ig administration.[47]

PHAGOCYTE IMMUNE DEFECTS: NEUTROPHILS

The clinical findings of patients with neutrophil disorders often share common features: gingivitis, periodontal disease, and oral ulceration.[6] Cutaneous infections with *Staphylococcus aureus* are recurrent and can be severe. In neutrophil disorders characterized by inadequate inflammation (neutropenia, LAD1, Chédiak-Higashi syndrome, neutrophil-specific granule deficiency), infections can extend locally and subcutaneously with little reaction until marked destruction has taken place. Visceral and, especially, sinopulmonary involvement helps distinguish neutrophil defects from other syndromes in the differential diagnosis. Hepatic abscess is a frequent manifestation of chronic granulomatous disease (CGD) and is most often caused by *S. aureus,* an organism rarely encountered in the liver in patients with normal neutrophils.

Neutropenia

A neutrophil count of less than 500 cells/μL carries a profound risk of bacterial and fungal infection.[48] Although this principle was first extensively documented and is still most frequently displayed in patients undergoing combination chemotherapy, its importance has been confirmed in patients with genetic disorders that affect neutrophil number. Cyclic neutropenia is an autosomal dominant disease characterized by relatively regular 21-day oscillations in the levels of blood neutrophils, monocytes, eosinophils, lymphocytes, platelets, and reticulocytes.[49] The defects are usually the result of mutations in the elastase neutrophil expressed *(ELANE)* gene.[50] Severe chronic neutropenia (SCN) can also be caused by mutations in *ELANE* and in several other genes, most notably *HAX-1* (HCLS-1 [hematopoietic cell-specific Lyn substrate 1]–associated protein X-1).[51] Patients are usually first seen in childhood and have recurrent episodes of fever, malaise, mucosal ulcers, and occasionally, life-threatening infections associated with periods of profound neutropenia (<200/μL).[49] Neutrophil number is the defect, but function is normal. The diagnosis is suspected in children with recurrent stomatitis, gingivitis, cutaneous infections, lymphadenopathy, and fever and should be entertained in patients with intermittent neutropenia, especially if a periodicity of about 21 days can be documented on serial blood draws (the mechanisms behind the periodicity are unknown). Molecular diagnostics should be pursued to confirm the specific gene involved. In SCN (also known as Kostmann syndrome[52]), neutrophil counts are consistently low from birth and without periodicity.[53] Of

interest, some cases of SCN are also caused by mutations in *ELANE.*[54] Neutropenic syndromes are typically treated with recombinant granulocyte colony-stimulating factor; myeloid malignancies are increased in these populations.[55] Adult-onset cases have also been described, with an associated clonal proliferation of large granular lymphocytes ($CD3^+$/$CD8^+$/$CD57^+$).[56]

Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome is a rare autosomal recessive disorder caused by defects in the lysosomal transport protein LYST, encoded by the *CHS1* gene.[57,58] It is characterized by recurrent bacterial infections, partial oculocutaneous albinism, photophobia, nystagmus, and peripheral neuropathy.[10] Many patients die in childhood from infection. An aggressive "lymphoproliferative" phase, with diffuse organ infiltration and death, develops in about half of the patients who survive into adolescence. Patients who live into adulthood may develop a severe peripheral neuropathy.[10] Microscopic examination reveals giant abnormal granules in neutrophils, melanocytes, hair, Schwann cells, the central nervous system, and other granule-containing cells.[10] In neutrophils, the granules are formed mainly by fusion of azurophilic or primary granules to each other and, to a lesser extent, by fusion to specific or secondary granules.[59]

Features of Chédiak-Higashi syndrome include central and peripheral nervous system involvement with peripheral neuropathy, myopathy, autonomic dysfunction, and leptomeningeal involvement.[10] Low intelligence has been noted in some series.[10] The lymphoproliferative "accelerated phase" is prevented by bone marrow transplantation, whereas the neuropathic features are not.[60] The phenotypic diagnosis of Chédiak-Higashi syndrome is easily made by inspection of the peripheral smear for giant lysosomes or microscopic examination of hair for characteristic melanin clumps.

Leukocyte Adhesion Deficiency

Leukocyte adhesion to endothelium and other leukocytes is mediated by several sets of molecules, among which are the integrins and the selectins. Defects in either of these two intercellular adhesion pathways can lead to overlapping clinical phenotypes. LAD type 1 is a rare autosomal recessive disorder involving one set of the leukocyte integrins—the molecules required for leukocyte adherence to endothelium, other leukocytes, and bacteria.[5,61] Deficiency of the integrin component CD18 leads to a corresponding deficiency of the complexes with which it partners—leukocyte function–associated antigen 1 (LFA-1), cell surface glycoprotein Mac-1, and p150,95, resulting in abnormalities of cellular adhesion. The absence of a marginated pool of neutrophils leads to chronic leukocytosis. Patients with LAD1 are prone to both recurrent infections and dysregulated inflammation. The infections are due to failure of neutrophil response. However, the failure of neutrophils to penetrate the tissues leads to increased macrophage expression of IL-23 and its downstream effector, IL-17, leading to exacerbated local inflammation.[62]

LAD1 defects can be severe or moderate, depending on the degree of CD18 deficiency.[5] Severe deficiency (<0.5% of normal protein expression) is characterized by delayed umbilical stump separation, umbilical stump infection, persistent leukocytosis in the absence of active infection (>15,000/μL), and severe destructive periodontitis with associated loss of dentition and alveolar bone, which is IL-17 mediated. Septicemia and recurrent infections of the skin, upper and lower airways, bowel, and perirectal area are common and usually due to *S. aureus* or gram-negative rods, most notably *Pseudomonas* species. Infections tend to be necrotizing and ulcerative but demonstrate almost no tissue neutrophils at histopathologic evaluation. Patients with moderate deficiency (3%–30% of normal expression) tend to be diagnosed later in life, have normal umbilical stump separation, and have fewer life-threatening infections. Leukocytosis is still the rule, as are delayed wound healing and periodontal disease. Although patients with a moderate form of the disease are less ill and tend to live past childhood, deaths from infection have been reported in young adults.[5]

The severe form shows grossly defective granulocyte and mononuclear cell mobilization in vivo and diminished neutrophil migration in response to the bacterial chemoattractant f-Met-Leu-Phe in vitro, despite normal numbers of receptors.[5] Histologic sections of infected tissues show mononuclear cells but very few neutrophils. Granulocyte adherence to glass, plastic, nylon, wool, and other LAD granulocytes is greatly reduced and not stimulated by exposure to f-Met-Leu-Phe or phorbol myristate acetate. The absence of CD18 also leads to the absence of the inactivated C3b (iC3b) receptor CR3. Therefore, complement-mediated phagocytosis is severely impaired, whereas IgG-mediated phagocytosis is normal. Although viral infections are not usually special problems in LAD, antibody-dependent cellular cytotoxicity by patient cells is also diminished. The diagnosis is established by eliciting a thorough history, with special attention directed to consanguinity, evidence of depressed inflammation in the neonatal period, delayed umbilical stump separation, and recurrent infections. A dental history is helpful inasmuch as most of these patients have severe problems with gingivitis, periodontal disease, tooth loss, and alveolar bone erosion. Wounds often heal abnormally, with dystrophic, paper-thin scars remaining. The diagnosis is confirmed when FACS shows reduction or absence of the leukocyte adhesion molecule CD18 or its partners CD11a, CD11b, and CD11c; and sequence abnormality in the gene encoding CD18, *ITGB2.*

In LAD2, neutrophil adherence to endothelial cells is defective because of absence of the sialylated Lewis X antigen (CD15s) on the neutrophil surface, which is the binding site for E-selectin.[63,64] The patients have neutrophilia, recurrent pulmonary, periodontal, and cutaneous infections, abnormal chemotaxis, mental retardation, short stature, distinctive facies, and the Bombay (hh) blood phenotype. The defect is autosomal recessive and caused by mutations in guanosine diphosphate (GDP)-fucose transporter 1 gene *SLC35C1.*[63,65] This disease is also known as congenital disorder of glycosylation IIc (CDG IIc). LAD3 is characterized by recurrent infections, impaired neutrophil migration, and platelet dysfunction caused by mutation in the gene *KIND3,* which links surface ligation of receptors to cellular signaling.[66]

Neutrophil-Specific Granule Deficiency

Neutrophil-specific (secondary) granule deficiency is a rare autosomal recessive heterogeneous disease characterized by a profound reduction or absence of neutrophil-specific granules and their contents.[67] Associated abnormalities in the few patients reported include bilobed or trilobed neutrophil nuclei, absence of some neutrophil primary (azurophil) granule proteins, mononuclear eosinophils without eosinophilic granules, and dysfunction of platelet α-granules. The neutrophil-specific granule protein lactoferrin is diminished or absent in these patients' neutrophils, whereas production by lacrimal glands is normal.[67–69] Neutrophil-specific granule deficiency results from mutation in the transcriptional regulator *C/EBPε*, which controls early neutrophil granule biogenesis.[70–72] However, cases without *C/EBPε* mutation suggest that there may be several genes that can lead to the same defect.

Myeloperoxidase Deficiency

Myeloperoxidase (MPO, also called verdoperoxidase), the heme-binding protein that makes pus green, catalyzes the conversion of hydrogen peroxide to hypochlorous acid (bleach). MPO deficiency, the most common neutrophil disorder, affects about 1 in 2000 persons but is quite silent in most cases. Neutrophil function is affected by MPO deficiency in a variety of ways. The respiratory burst in MPO-deficient neutrophils is prolonged and, as a result, exaggerated amounts of hydrogen peroxide are produced.[73] Phagocytosis is normal to increased in MPO-deficient neutrophils, whereas bactericidal activity is somewhat slower than normal. Killing of *Aspergillus* conidia by MPO-deficient neutrophils is retarded, whereas the combination of MPO-deficient neutrophils with CGD neutrophils (see "Chronic Granulomatous Disease"), which are unable to generate hydrogen peroxide but do produce MPO, results in normal killing of *Aspergillus* conidia.[74] Pathologic sequelae of MPO deficiency are brought out only in the presence of other impairments of host defense, such as diabetes mellitus. A very few MPO-deficient diabetic patients have had severe yeast infections. Results of the dihydrorhodamine oxidation assay, which is used for CGD diagnosis, can be falsely abnormal in MPO deficiency.[75]

Chronic Granulomatous Disease

CGD is caused by deficient function of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is responsible for the

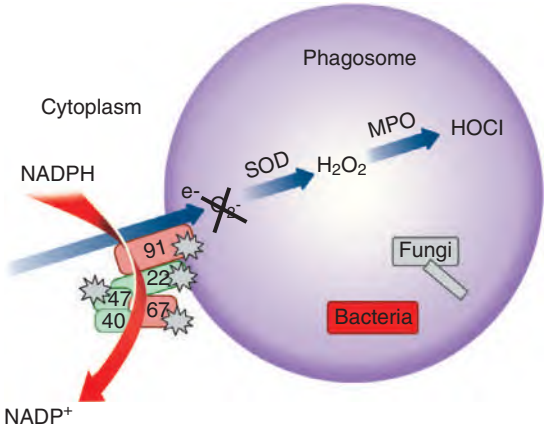

FIG. 12.2 The phagocyte respiratory burst. The neutrophil oxidative burst is mediated by the structural components of the reduced nicotinamide adenine dinucleotide phosphate *(NADPH)* oxidase: the membrane-bound components gp91*phox* and p22*phox* and the cytosolic factors p47*phox,* p67*phox,* and p40*phox.* In the setting of cellular activation, these factors coalesce on the phagosome membrane and catalyze the transfer of an electron from NADPH to molecular oxygen. This, in turn, creates superoxide, which is converted to hydrogen peroxide (H_2O_2) and then to hypochlorous acid (*HOCl,* bleach). The genetic absence of any of these structural components causes chronic granulomatous disease. *MPO,* Myeloperoxidase; *$NADP^+$*, oxidized nicotinamide adenine dinucleotide phosphate; *SOD,* superoxide dismutase.

respiratory burst and the generation of phagocyte superoxide, hydrogen peroxide, and hypochlorous acid (Fig. 12.2). Five distinct gene defects converge on this point in phagocytic cell oxidative metabolism, and their defects lead to recurrent life-threatening infections with bacteria and fungi and dysregulated inflammation.[76] The frequency is greater than 1 in 200,000 persons.[77] Clinically, CGD is quite variable; the time of onset may occur from infancy to late adulthood, although it is diagnosed in most patients while they are toddlers and young children. However, CGD is diagnosed in a significant number of patients later in life.[76–78] Children with CGD tend to be short and small for their age, even in the absence of overt infections, but often achieve the height predicted by their parents' height.[79]

Pulmonary, cutaneous, lymphatic, and hepatic infections are frequent. Osteomyelitis, perianal abscess, and genitourinary obstruction are also common.[76–78] The microbiologic features of infections associated with CGD are remarkable for their relative specificity: *S. aureus, B. cepacia* complex, *Serratia marcescens, Nocardia* spp., and *Aspergillus* spp. account for the overwhelming majority of infections in North America and Europe (Fig. 12.3). Where exposure is common, BCG, tuberculosis, and *Salmonella* infections are also prominent. As in other neutrophil abnormalities, the most common offender in CGD is *S. aureus.* Whereas the typical liver abscess in an immunologically normal patient involves enteric organisms and is liquid and easily drained, the liver abscesses encountered in patients with CGD are dense, caseous, and staphylococcal (Fig. 12.4). In the absence of antibiotic prophylaxis, lung, skin, and bone infections are also usually staphylococcal. However, *Aspergillus* spp. and some of the rarer fungi, such as *Exophiala dermatitidis*[80] and *Paecilomyces* spp.,[81] are encountered in CGD. Infections with *Nocardia* spp., *Chromobacterium violaceum, S. marcescens,* and *B. cepacia* are seen frequently in patients with CGD and strongly suggest the diagnosis.[3,76–78,82] Bony involvement can occur by direct extension in the case of *Aspergillus* or hematogenously, as in the case of *Staphylococcus* and *Nocardia.*[83] Infection with *Aspergillus nidulans* is especially aggressive and requires more intensive therapy than does infection with *Aspergillus fumigatus.*[84] Antibiotic prophylaxis has altered the frequency of infections in CGD and has reduced the frequency of staphylococcal infections in particular.[3,85] Therefore, infections outside the liver or lymph nodes occurring in CGD patients who have been taking antibacterial prophylaxis should not be presumed to be staphylococcal. The rate of fungal infections in CGD is lower than that for bacterial infections and has apparently

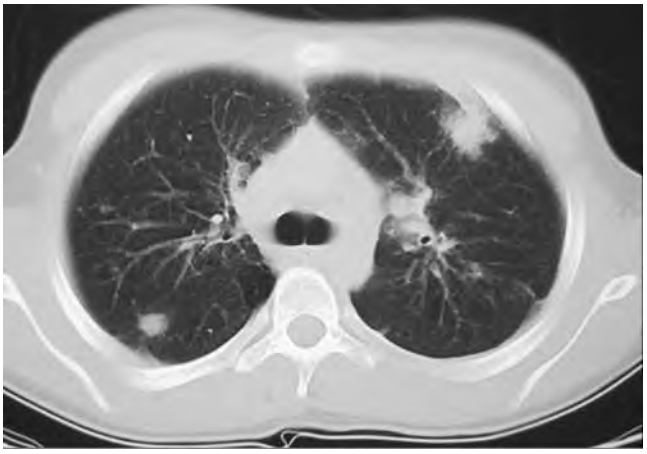

FIG. 12.3 Multifocal asymptomatic *Aspergillus fumigatus* pneumonia in a patient with chronic granulomatous disease.

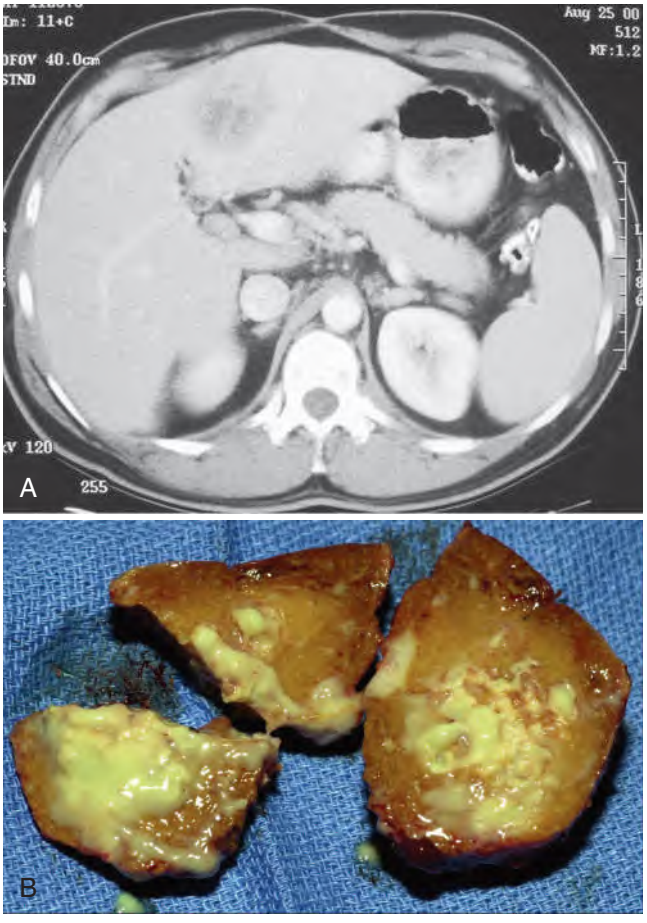

FIG. 12.4 Staphylococcal liver abscess in a patient with chronic granulomatous disease. (A) It is large, diffuse, and multiloculated. (B) The excised abscess shows a dense fibrocaseous mass that is typically not amenable to catheter drainage and requires surgical removal.

not changed in the setting of prophylactic antibiotics.[85] Itraconazole prophylaxis has reduced the frequency and severity of fungal infections in patients with CGD.[86]

The granulomas that occur in CGD are presumed to originate from an inflammatory response to infectious or irritative foci (e.g., sutures) that fails to eradicate the infection or irritation. This may be because hydrogen peroxide, a moiety missing in CGD phagocytes, is involved in the degradation of inflammatory mediators, such as leukotrienes and complement factors.[87,88] This persistent inflammatory reaction may

lead to exuberant and often obstructive granulomatous lesions. The gastrointestinal and genitourinary tracts are frequently involved by granulomatous lesions in CGD, sometimes as the site of the initial complaint, sometimes asymptomatically. Malabsorption, abundant lipid-laden histiocytes in bowel biopsy specimens, and pigmented histiocytes and granulomas in bowel biopsy material are sometimes difficult to distinguish from Crohn disease.[89] Esophageal, jejunal, ileal, cecal, rectal, and perirectal involvement occurs in greater than 40% of patients with CGD, affecting patients with X-linked more than autosomal disorders.[90] Gastric outlet obstruction is an especially common manifestation and may be the initial feature of CGD. In a comprehensive review of genitourinary manifestations, Walther and colleagues[91] found that 38% of patients with CGD had some kind of urologic event, including bladder granulomas, ureteral obstruction, and urinary tract infection. The diagnosis of CGD is made by assays of superoxide or hydrogen peroxide production, such as dihydrorhodamine oxidation or nitroblue tetrazolium reduction. Mothers of boys with the X-linked form of CGD are typically carriers of a defective X chromosome. Therefore, by lyonization, a certain proportion of the mother's cells will fail to produce superoxide and thus will give a characteristic mosaic pattern at testing, so that by performing a functional test on the mother's blood, one can determine whether a boy has the X-linked form of CGD. X-linked carriers of CGD have an increased incidence of discoid lupus.[92]

IFN-γ is a cytokine with neutrophil- and monocyte-macrophage–stimulating properties that has reduced the frequency and severity of infections in patients with CGD.[93] Although granulocyte transfusions supply only a small portion of the body's normal neutrophil output in a day, they may be effective in helping to clear severe infections. Transfused granulocytes produce superoxide, which can be used by the intact MPO system in CGD neutrophils, thereby allowing the biochemical defect to be bypassed. The granulomatous complications of CGD pose a special problem in management. Although these patients are already somewhat immunocompromised, the judicious use of corticosteroids in conjunction with antibiotics has been successful in opening and maintaining the patency of hollow viscera in patients with CGD.[90] Furthermore, adjuvant steroid therapy coupled with antibiotic therapy for staphylococcal liver abscess obviates surgery and has shown better overall survival than standard surgical therapy.[94,95]

DEFECTS AFFECTING PHAGOCYTE SIGNALING

Among the critical ways through which myeloid, lymphoid, and other cells sense pathogens are preformed receptors on the cell surface or in vesicles. The Toll-like receptors (TLRs) are important mediators of this sensing that signal through intracellular molecules, including myeloid differentiation (primary response) protein 88 (MyD88), IL-1 receptor–associated kinase (IRAK), and NF-κB[96] (Fig. 12.5). Defects in any of the genes along this innate immune pathway lead to severe infections. Mutations in the *IRAK4* or *MyD88* gene lead to severe, recurrent pyogenic infections in infancy and childhood that improve with age, perhaps because of acquired immunity.[97] Mutations in the gene *IKBKG* (NF-κB essential modulator, NEMO) often have a profound defect that encompasses pyogenic in addition to mycobacterial, viral, and *Pneumocystis* infections, depending on the specific mutation.[98] Many of these patients also have mild-to-moderate forms of ectodermal dysplasia, reflecting the fact that morphogenic pathways also signal through NF-κB.[38] Mutations in specific TLRs confer highly pathogen-specific susceptibility, such as TLR3 and herpes simplex virus (HSV) encephalitis[99] and TLR4 and meningococcemia.[100]

PHAGOCYTE AND LYMPHOCYTE DEFECTS AFFECTING MONONUCLEAR CELLS

Although tuberculosis is highly virulent in humans and infects more than one-third of the globe's population, infections with nontuberculous mycobacteria (NTM) are much less common; when these infections are disseminated, they bespeak substantial immunocompromise. If HIV infection and iatrogenic causes are excluded, one is left with patients with intrinsic immune defects, some of which have recently been characterized at a genetic level.

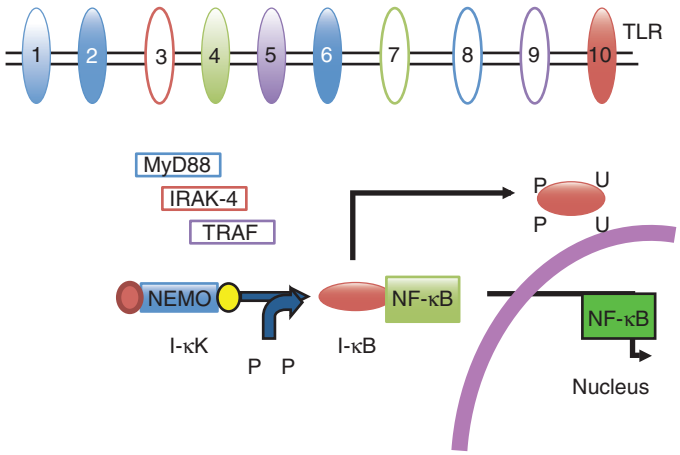

FIG. 12.5 Toll-like receptor *(TLR)* signaling. The TLRs signal through a variety of molecules, mostly culminating in nuclear factor kappa B *(NF-κB)* activation. NF-κB is held latent in the cytoplasm by inhibitor of kappa B kinase *(I-κB),* and it is released only after I-κB is phosphorylated *(P)* and ubiquitinated *(U).* Mutations have been identified in myeloid differentiation (primary response) protein 88 *(MyD88),* interleukin-1 receptor–associated kinase 4 *(IRAK-4),* and NF-κB essential modulator *(NEMO),* which predispose to severe bacterial infections, predominantly with encapsulated bacteria. Mutations in NEMO are also highly associated with mycobacterial infections and *Pneumocystis.* TLRs signal in response to bacterial, fungal, and viral products. Mutations in TLR3 are associated with recurrent herpes simplex virus encephalitis. Mutations in TLR4 are associated with meningococcemia. Polymorphisms in other TLRs have been shown to affect infection susceptibility to tuberculosis, *Legionella,* and other agents. Shaded TLRs are surface displayed. Open TLRs are maintained in intracellular vesicles. *TRAF,* Tumor necrosis factor receptor–associated factor.

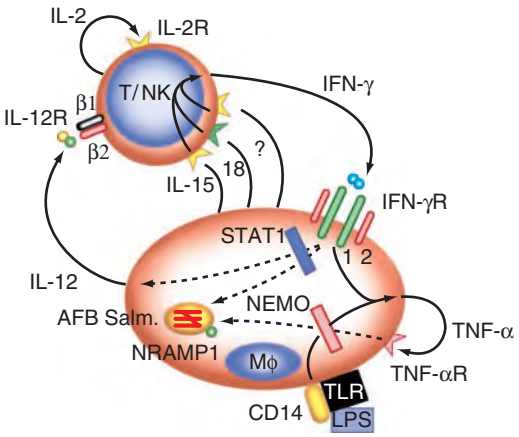

FIG. 12.6 The interacting cytokine and receptor pathways that regulate the resistance to, and killing of, mycobacteria and *Salmonella.* Lesions resulting in severe disseminated mycobacterial disease have been described in the interferon-γ *(IFN-γ)* receptors, signal transducer and activator of transcription 1 *(STAT1),* interleukin *(IL)*-12p40, IL-12 receptor *(R)* β1, and the signal transducing molecule nuclear factor kappa B essential modulator *(NEMO). AFB,* Acid-fast bacilli; *CD14,* cluster of differentiation antigen 14; *LPS,* lipopolysaccharide; *MΦ,* macrophage; *NK,* natural killer cell; *NRAMP1,* natural resistance–associated macrophage protein 1; *Salm., Salmonella; T,* T-cell; *TNF-α(R),* tumor necrosis factor-α (receptor).

IFN-γ is produced by lymphocytes and acts through its cognate receptor on monocyte-macrophages to facilitate the killing of intracellular pathogens, such as mycobacteria, *Salmonella,* and dimorphic yeasts (Fig. 12.6).[101] Therefore, it is expected and observed that severe, disseminated infections with NTM, such as *Mycobacterium avium* complex, develop in humans with defects in either the ligand binding (IFN-γ receptor 1) or the signal transducing (IFN-γ receptor 2) chains of the IFN-γ receptor.[102–105] These infections often have their onset in childhood, and if BCG is given

to these infants, it often disseminates. Patients with complete defects of the IFN-γ receptors have autosomal recessive defects, characteristically fail to form granulomas, and have very high mortality from their *M. avium* complex infections. They are also susceptible to recurrent disseminated infections with the intracellular pathogen *Salmonella*. Patients with partial defects in IFN-γ receptor 1 have an intermediate phenotype with severe but curable mycobacterial infections and retain the ability to form granulomas.[106] The autosomal dominant form of IFN-γ receptor 1 deficiency is the most common; patients present in childhood and later, typically with treatable nontuberculous mycobacterial infections involving bone. In these patients, a nonfunctional IFN-γ receptor 1 overaccumulates on the cell surface because of a common mutation in the intracellular domain that impairs receptor recycling.[107,108] The diagnosis of complete recessive (receptor absent) or partial dominant (defective receptor increased) IFN-γ receptor 1 deficiency is most easily accomplished by examination of the cell surface for IFN-γ receptor 1 expression by flow cytometry. However, demonstration of protein-positive IFN-γ receptor 1 deficiency and all cases of IFN-γ receptor 2 deficiency must still be performed by molecular assays.

IL-12 is a monocyte-macrophage product that acts on lymphocytes to drive the production of IFN-γ. As expected, defects in IL-12 signaling resulting from receptor (IL-12Rβ1, IL-12Rβ2)[109] or ligand (IL-12p40)[110] defects result in disseminated infections with NTM and *Salmonella*. However, because patients with IL-12 defects have some residual IFN-γ production, granuloma formation is preserved, and these patients tend to be less severely affected than those with complete IFN-γ receptor defects.[111,112] Treatment of IL-12– or IL-12 receptor–deficient patients with disseminated infections can be greatly aided by the use of IFN-γ. The diagnosis of IL-12 receptor deficiency currently requires functional or molecular assays.

Signal transducer and activator of transcription 1 (STAT1) mediates most signals from the IFN-γ and IFN-α receptors. Three discrete kinds of STAT1 defects occur: recessive complete loss of function, dominant negative loss of function, and dominant gain of function. Severe complete recessive STAT1 defects ablate both IFN-γ and IFN-α signaling, leading to fatal mycobacterial and viral infections.[113] In contrast, autosomal dominant mutations in STAT1 affect primarily IFN-γ receptor signaling, resulting in treatable disseminated nontuberculous mycobacterial infections in childhood.[114] Surprisingly, dominant gain-of-function mutations lead to accentuated STAT1 signaling, resulting in impaired formation of IL-17–producing T cells in some cases, which leads to chronic mucocutaneous candidiasis.[115,116] Other complications of the gain of function mutations include disseminated histoplasmosis, coccidioidomycosis, progressive multifocal leukoencephalopathy,[117] and IPEX-like syndrome.[118] This latter set of infections and complications may in part reflect IFN-γ excess.[117]

Hyperimmunoglobulin E–Recurrent Infection Syndrome (Job Syndrome)

Job syndrome is a rare disorder characterized by recurrent infections (typically of the lower respiratory system and skin), eczema, extremely elevated levels of IgE, and eosinophilia resulting from dominant negative mutations in the *STAT3* gene.[119,120] Most patients have facial abnormalities, including a prominent, protruding, triangular mandible and a broad, somewhat bulbous nose.[4,7,9] Failure of primary teeth to fall out is common and results in a frequent need for dental extractions to allow eruption of normal secondary teeth.[7,121] Moderate scoliosis develops in most patients. Many also have abnormalities of bone formation and metabolism, osteopenia, fractures, and craniosynostosis.[7,9,119,120] Job syndrome occurs spontaneously in all racial and ethnic groups and is transmitted as an autosomal dominant trait.[4,7,119,120]

Patients are usually noted within the first days to months of life to have severe eczema, mucocutaneous candidiasis, and cutaneous, sinus, or pulmonary infections, predominantly with *S. aureus* or *Haemophilus influenzae*. Pneumatoceles are often noted by adolescence, and these, in turn, provide a hospitable site for subsequent infections with *Aspergillus* and *Pseudomonas aeruginosa* (Fig. 12.7). Otitis media and externa are relatively common, as are intertriginous infections and breast abscesses. Infections occur less frequently in bones and joints and very infrequently in the liver, kidneys, and gastrointestinal tract. Documented sepsis is rare. Recurrent "cold" abscesses of the skin are commonly due to staphylococci. The inflammatory response in these patients is impaired, in part because of impaired IL-6 signaling and diminished circulating IL-17–producing T cells.[122] Therefore, STAT1 gain-of-function and STAT3 loss-of-function mutations overlap in having defects of IL-17–producing T cells, and thus mucocutaneous candidiasis, but differ because of STAT3's extensive role in somatic development. Pathogens recovered from patients with Job syndrome include *S. aureus, H. influenzae, Aspergillus* spp., *P. aeruginosa, Streptococcus pneumoniae,* group A streptococci, *P. (carinii) jirovecii*, *Cryptococcus neoformans, Histoplasma capsulatum*, and *Candida albicans*.[4,7,9,119]

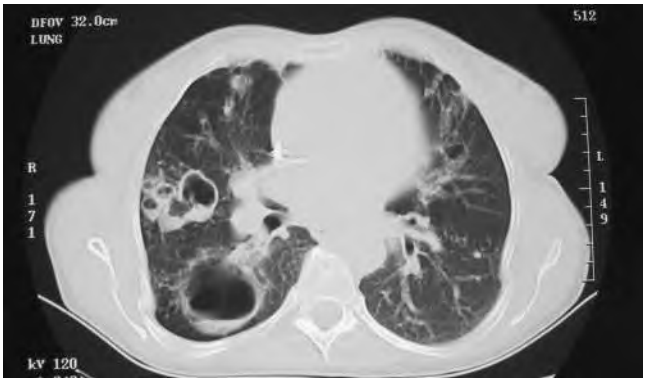

FIG. 12.7 Multifocal postinflammatory pneumatoceles in a patient with hyperimmunoglobulin E–recurrent infection (Job) syndrome.

The syndrome is defined by marked elevations of IgE (>2000 IU/mL), with levels of greater than 50,000 IU/mL reported.[4,7,9] Levels may start out elevated in cord blood and then climb through infancy and childhood, but they may also decline into the normal range over time.[7] Therefore, authentic cases do at times lack elevated IgE. Chronic leukopenia with borderline neutropenia has been observed in several patients.[9] Mild-to-moderate eosinophilia is the rule, although exceptions do occur.[7] No correlation between IgE levels and the degree of eosinophilia or clinical disease has been made.[7,9] The diagnosis should be suspected in the setting of combined immunologic and somatic features, including extremely elevated IgE, eczema, recurrent sinopulmonary infections, recurrent skin abscesses, failure of primary dental deciduation, scoliosis, characteristic facies, and a positive family history. Protein-positive heterozygous mutations in *STAT3* correlate well with clinical scores.[119,123]

GATA2 Deficiency

Haploinsufficiency of the primitive hematopoietic transcription factor GATA2 underlies several clinical syndromes, including monocytopenia and mycobacterial infections (MonoMAC); dendritic cell, monocyte, B, and NK lymphoid (DCML) deficiency; familial myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML); and Emberger syndrome (primary lymphedema with MDS).[124–129] These syndromes encompass immunodeficiency, bone marrow failure, leukemia, pulmonary alveolar proteinosis (PAP), and lymphedema. *GATA2* mutations also account for significant percentages of pediatric neutropenia, idiopathic CD4 lymphocytopenia, and aplastic anemia.[130,131] The age at presentation ranges from early childhood into late adulthood, for reasons that are still unclear.

Peripheral blood counts are normal when patients are asymptomatic but decline as complications mount. Profound B and NK lymphocytopenia and monocytopenia are strongly suggestive of GATA2 deficiency and should prompt genetic testing.

NK dysfunction and cytopenia underlie the susceptibility to human papillomavirus, cytomegalovirus, and varicella-zoster virus. Systemic IFN-α improves NK-cell cytotoxicity in vitro and may be beneficial for patients with refractory human papillomavirus.[132] EBV-positive mesenchymal tumors, as seen in advanced HIV/AIDS, indicate a profound host defect that may also be NK-cell mediated. Likewise, the deficit

and dysfunction of monocytes and macrophages may underlie susceptibility to intramacrophagic pathogens, including NTM and *H. capsulatum*. GATA2 also regulates alveolar macrophage phagocytosis, dysfunction of which may underlie PAP.

Complement-Mediated Immunity

Deficiencies in complement components clinically manifest as recurrent systemic bacterial infections. Bacteremia and meningitis are common in all the complement deficiencies.[1] Pneumonia is common in the early classical pathway (C1, C4, C2) and alternative pathway (factors I and H, properdin, C3) defects. Late-component defects (C5 to C9) are associated with recurrent *Neisseria* bacteremia and meningitis. Surprisingly, the bacteremia associated with late-component defects occurs at a much later age (average, 17 years) than does meningococcal bacteremia in the healthy population. Although these patients also have much higher rates of relapse and reinfection than do healthy people, their mortality from the infection is lower than normal. Patients with deficiencies of the early components of complement (C1, C4, C2, and C3) tend to have considerably higher rates of collagen-vascular disease, such as systemic and discoid lupus erythematosus, than do either healthy populations or patients with late-component defects.

Except for properdin deficiency, which is X-linked, complement deficiency states are inherited as autosomal recessive disorders. Heterozygotes have 50% of normal levels, whereas homozygous-defective persons tend to have very low levels, if any, of the affected component. Screening for the presence of classical complement deficiencies is best accomplished with use of the test for total hemolytic complement (the CH_{50} assay). This test examines the integrity of the classical pathway of the complement system by determining the ability of complement in patient serum to lyse antibody-coated sheep erythrocytes in vitro. Cell lysis leads to the release of hemoglobin, which can be determined spectrophotometrically. Specific classical complement component defects can be detected with a modified CH_{50} assay that uses purified proteins and selectively omits the one to be assayed so that patient serum must supply the missing factor.[19] Direct determinations of immunologically reactive protein, including members of the alternative pathway, can be performed with enzyme-linked immunosorbent assays or diffusion assays. These types of direct assay systems do not offer functional data but can help quantify apparent functional defects. The alternative complement pathway is similarly screened using the AH_{50} assay.

Acquired Immunodeficiencies

The most common immunodeficiencies are acquired after birth and are not clearly traceable to an immune genetic basis. Like other immunodeficiencies, they are best approached through a thorough history and physical examination to search for associated findings and to guide diagnostic testing. Special attention to the infecting organisms can point to underlying abnormalities in host defense. AIDS is caused by HIV, which induces progressive $CD4^+$ T-cell depletion.[133] A syndrome that manifests with opportunistic infections, as AIDS does, but is unassociated with HIV infection is idiopathic $CD4^+$ lymphopenia.[134] The diagnosis is made by excluding all other known causes of immunodeficiency, including HIV, and determining that the $CD4^+$ T-cell count is 300/μL or less. Certain malignancies, particularly hematopoietic and lymphoid malignancies, result in immune dysfunction by causing a deficiency in immune effector cells or dysregulation of such activities as antibody synthesis, and they are associated with severe or opportunistic infections. Drug therapy can be complicated by rare or idiosyncratic reactions, such as aplastic anemia with chloramphenicol or drug-induced neutropenia.[135] During chelation of iron overload with deferoxamine, the available circulating iron makes the intravascular environment more hospitable for certain bacteria, notably *Yersinia enterocolitica*.[136–138] Splenectomy, especially after trauma, predisposes to overwhelming infection with encapsulated organisms, such as *S. pneumoniae* and *Capnocytophaga canimorsus* (previously biogroup DF-2 [dysgonic fermenter 2]),[139] and parasites, such as *Babesia microti* and *Plasmodium* spp.[140] Severe thermal injury is associated with selective degranulation of neutrophil-specific granules, decreased chemotaxis, and profound susceptibility to infection.[141]

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The complete reference list is available online at Expert Consult.

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C Epidemiology of Infectious Disease

13 Applied Epidemiology for the Infectious Diseases Physician

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WHAT IS EPIDEMIOLOGY?

The word *epidemiology* originates from the Greek *epi-* (meaning "surrounding"), *demos* (meaning "people"), and *logos* (meaning "the study of"). Thus epidemiology literally refers to the study of population occurrences. We now define epidemiology as *the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems*.[1] Historically, the discipline of epidemiology reaches back in time over 2500 years and includes contributions from thinkers such as Hippocrates, John Graunt, James Lind, and William Farr. However, John Snow, an English anesthesiologist, is widely considered the "father of field epidemiology." His famous study identified a single water pump on Broad Street as the cause of a cholera epidemic in the Golden Square of London. In 1854, twenty years before the invention of the microscope, Snow mapped the distribution of households with cholera cases during the epidemic and recognized that the cases were clustered around a particular water pump, the Broad Street pump. To confirm his suspicion that contaminated water from this pump was causing the cholera epidemic, Snow removed the handle of the pump, and the outbreak ended.[2]

The field of epidemiology has expanded from the investigation of infectious disease outbreaks, to the study of injuries and violence, and to the examination of any exposure that influences health outcomes, including molecular and genetic factors.[2] Epidemiology is used in many ways, such as for evaluating a population's health, informing individual decisions, and establishing causality.[2] Using rigorous statistical methods, epidemiology can answer complex questions, such as whether risk patterns correlate with exogenous factors or whether a public health program modifies risk.[2] For infectious diseases clinicians, the epidemiologic factors associated with a case or cluster of cases can be used to great benefit, potentially decreasing the time to diagnosis, limiting the need for extensive testing, and tailoring the use of broad-spectrum antibiotics. Epidemiologic data may also be used to inform policies or guidelines that affect groups or individuals, such as choosing which populations to vaccinate, setting speed limits on a highway, or determining how many times a week to exercise.[2]

WHAT CAN EPIDEMIOLOGY TELL THE INFECTIOUS DISEASE CLINICIAN?

Epidemiology asks the clinician to think of the patient's community and exposures, as well as the individual patient. Is my patient's respiratory infection part of an outbreak of influenza in the community and, if so, what can be done to identify and control it? Interaction with the local health department or hospital infection control staff in this instance might have a broader impact than treating a single patient but will also require understanding how epidemiologic questions are formulated and answered.

THE EPIDEMIOLOGIC TRIAD MODEL: AGENT, HOST, AND ENVIRONMENT

To better inform clinical and public health decisions, epidemiologists (and clinicians) try to find causal links between exposures and health outcomes. If this is an influenza outbreak, how is it being spread, and how severe are the illnesses? The epidemiologic triad, or triangle, is one of the simplest models used to describe disease causation.[2] In this model disease arises from the interaction between three components: an external "agent," a susceptible "host," and an "environment" that supports the transmission of the agent from its source to the host (Fig. 13.1).[2] By tradition the agent represents an infectious microbe, such as a virus, bacterium, or parasite. However, this component of the model does not sufficiently describe all diseases; some can be chronic processes with multiple contributing causal factors. Host susceptibility can depend on an individual's genetic makeup, immune status, and host defenses, such as skin integrity, gastric acidity, ciliary function, and other factors. Host immunity, acquired immunodeficiency, and principles of immunization are discussed in Chapters 4 to 6, 118, and 316. Environmental factors that promote agent transmission to a susceptible host include the presence of animals and other reservoirs of infectious agents, vector populations, crowding, poor sanitation, climate, and geography.[2] A detailed understanding of the epidemiology, pathogenesis, and ecology of a disease is required to construct optimal epidemiologic triads (agent, host, and environment).

THE SUFFICIENT-COMPONENT CAUSE MODEL OF EPIDEMIOLOGY

A second causation model that considers the multiple factors that contribute to disease is called the "sufficient-component cause model."[3] In this model each disease pathway has "component causes" or factors that contribute to disease. Different combinations of component causes are considered "sufficient causes" of disease when they complete the causal pathway. A component cause that is present in every combination of factors in the causal pathway is considered a "necessary cause" because the pathway cannot be complete without it. For example, human papilloma virus (HPV) infection is a necessary cause of cervical cancer because persons must be infected with this virus to develop HPV-associated cervical cancer. However, HPV infection is not a sufficient cause of cervical cancer because not all individuals infected with HPV develop cervical cancer.

OTHER METHODS TO CHARACTERIZE INFECTION TRANSMISSION

Multiple classifications exist to describe modes of disease transmission or the ways in which an agent leaves its natural environmental reservoir to infect a susceptible host. One classification distinguishes "direct" from "indirect" transmission.[2] Direct transmission includes direct contact and droplet spread (short-range aerosols produced by sneezing, coughing, or talking), whereas indirect transmission includes airborne, vehicle-borne, and vector-borne (mechanical or biologic) spread.[2]

DESCRIPTIVE EPIDEMIOLOGY

Descriptive epidemiology is used to characterize events in terms of person, place, and time and might answer the following questions: What health risks exist for this population? Who is getting influenza this winter?

Who are the population members at risk? Is the influenza strain circulating in nursing homes, hospitals, or schools? Which geographic locations are associated with increased risk? How has the level of risk changed over time? Case reports and case series are types of descriptive studies conducted in clinical settings.[4] Case reports describe the clinical features of a disease, along with demographic and historical details relevant to its presentation. Case series include the presentation, clinical features, and other significant facts pertaining to multiple patients with similar health conditions. Case reports (and series) often represent the first or novel presentations of a condition and can be very valuable from both a clinical and an epidemiologic perspective. For example, ribavirin was recently shown to be effective in a series of patients with chronic hepatitis E, not an original indication.[5] Descriptive epidemiology may also assist in recognition of an emerging disease or in generating hypotheses about causal factors and factors that increase the risk of disease.[2]

ANALYTIC EPIDEMIOLOGY

When one would like to more definitely establish a relationship between an exposure and a disease, analytic epidemiology is required. Hypotheses about the relationship are normally formulated, and a study design is selected based on the available data, the research question, ethical considerations, feasibility, and issues of validity and efficiency. Analytic epidemiologic studies fall into two categories: experimental and observational[2] (Table 13.1). An observational study has no intervention but attempts to link the exposure to the disease passively. In an experimental study the exposure is actively managed and the outcome measured prospectively. Precision, validity, and bias should all be considered when designing an epidemiologic study but are covered elsewhere.[2]

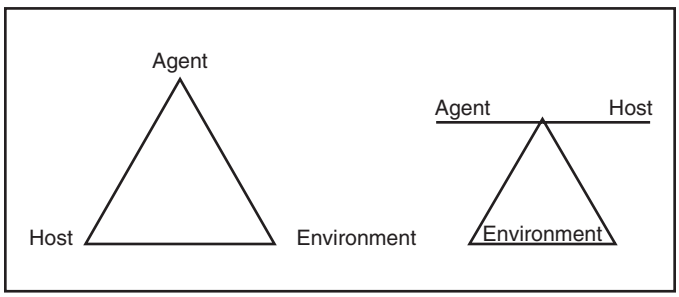

FIG. 13.1 **Epidemiologic triangle (triad).**

EXPERIMENTAL STUDIES

In an experimental study an investigator assigns similar groups of individuals (clinical trial) or communities (community trial) to different exposures or interventions and then follows the groups over time to ascertain the effects of the exposure.[2] A classic example of a clinical trial is a vaccine trial, where one group of individuals is assigned to receive an immunization to prevent a particular disease and another group of individuals to receive a placebo. There are numerous examples of vaccine trials, including the definitive demonstration of the effectiveness of influenza immunization in children.[6] After a defined period of time, the investigator monitors each participant to diagnose any occurrence of the disease in question, the investigator compares the rates of infection between the vaccine and placebo groups to determine if the vaccine successfully protected recipients from the disease. In a community trial, groups of individuals undergo randomization to receive an intervention, and investigators ascertain community health outcomes after a period of time relevant to the intervention and health outcome under investigation. Community trials are ideal for evaluating broad-based interventions, such as case management algorithms and health-related policies. An example of a community trial is testing the utility of long-lasting insecticide-treated bed net distribution on malaria incidence in endemic areas.[7]

OBSERVATIONAL STUDIES

Cohort Studies

The most common types of observational study designs are cohort and case-control studies. Cohort studies are conceptually similar to experimental studies. The investigator evaluates the exposure status of participants and then follows them over time to evaluate the incidence of one or more health outcomes in each exposure group. If a health outcome is significantly greater in the exposed group relative to the unexposed group, then the exposure is considered to be associated with the disease.

TABLE 13.1 Epidemiologic Study Design Strengths and Weaknesses

STUDY DESIGN	ADVANTAGES	DISADVANTAGES	METHOD	MEASURE OF ASSOCIATION
Ecologic	Very inexpensive Very fast Hypothesis generation	Unit of study is the population Risk of ecologic fallacy No temporal relationship Cannot control for confounders	Evaluates population-level occurrences	Prevalence ratio
Cross-sectional	Very inexpensive Very fast Individualized data Hypothesis generation Can control confounders	No temporal relationship Not good for rare diseases Not good for diseases of short duration	Evaluates two factors for all persons in a population	Prevalence ratio, prevalence odds ratio
Case-control	Inexpensive Fast Good for rare diseases Can assess multiple exposures	Cannot directly measure risk Can only evaluate one outcome Can introduce bias through control selection Prone to recall bias Can be hard to demonstrate temporality	Sampling conducted with respect to outcome status, and exposure evaluated retrospectively	Odds ratio
Cohort	Establish temporal relationships Individualized data Can control confounders Assesses multiple exposures Assesses multiple outcomes Can calculate incidence Can nest other studies within the cohort study	Expensive Time consuming (prospective) Not good for rare diseases Requires high follow-up rates	Sampling conducted with respect to exposure status, and outcome evaluated prospectively or retrospectively	Relative risk
Clinical trial	Controls bias via randomization Controls confounders via selection criteria Can assess multiple exposures (depending on number of arms) Can assess multiple outcomes	Expensive Ethical concerns for certain exposures of interest Participants may not represent the general population Must consider differences in adherence	Randomization of participants to exposure, then outcome evaluated prospectively	Relative risk

Cohort studies differ from experimental studies in that the investigator does not assign individuals to an exposure group; rather, the exposure group is determined passively. Cohort studies can be performed prospectively or retrospectively. In a prospective study the exposure status is established in advance, and the participant is followed over time to determine the occurrence of the outcome of interest. In a retrospective study both the exposures and the outcomes have already occurred at different points in time. The investigator collects the retrospective data and compares the rates of the outcome in the exposed and unexposed groups. A recent example of a well-designed cohort study described the frequency and distribution of pregnancy outcomes after Zika virus infection.[8]

Case-Control Studies

In contrast, case-control studies evaluate a single health outcome in relation to one or more exposures. For a case-control study, subjects with the health outcome of interest (e.g., became infected) are enrolled as well as control subjects without the health outcome of interest (e.g., did not become infected). The exposure histories of both sets of subjects are then compared, with the control group providing an estimated baseline or expected amount of exposure for the population of interest. If exposures are high among subjects with the health outcome of interest relative to the exposures expected based on evaluation of the control group, then the outcome is determined to be associated with that exposure. A recent case-control study from Peru demonstrated a link between latent tuberculosis infection and acute myocardial infarction.[9]

Cross-Sectional and Ecologic Studies

Cross-sectional and ecologic studies are also observational study designs. Cross-sectional studies examine the relationship between an exposure and an outcome among individuals in a defined population at a single point in time, like a snapshot. An example is determining the prevalence of syphilis in a population of prisoners in Brazil to decide if an intervention is required.[10] Ecologic studies evaluate associations using population-level exposures and outcome rates, rather than using individuals as the unit of analysis. Both cross-sectional and ecologic study designs have important limitations that make them less scientifically rigorous than other study designs.[11,12] Most important, they do not establish temporal sequences between exposures and outcomes, which limits the ability to determine causal inferences. In ecologic studies the lack of individual-level information contributes to what is known as the "ecologic fallacy," meaning that individual-level relationships between exposures and outcomes cannot be inferred from population-level associations.[12,13] In other words, the individuals with the exposure may not be the same individuals who developed the disease, even though a particular population has high rates of the exposure and outcome.[12]

DETERMINING THE APPROPRIATE EPIDEMIOLOGIC STUDY DESIGN

When determining which study design is most appropriate, it is important to consider the strengths and weaknesses of each (see Table 13.1). Experimental studies are ideal for evaluating an intervention for a particular outcome with a high degree of validity, such as when an intervention is expected to have a small effect or when a highly controlled setting with randomized participants is needed to minimize bias and confounding.[12] Only randomization, preferably blinded, can reduce the effect of unidentified factors or confounders that can potentially bias the outcome. The disadvantages of experimental studies include high cost and ethical considerations related to exposing participants to certain risks. Observational studies can be used to evaluate a wider range of exposures, including preventions, treatments, and causes of disease. The primary limitation of observational studies is the inability to control for confounding factors. Cohort studies are useful for investigating rare exposures, multiple outcomes, and outcomes for which little is known about exposure. Compared with other study designs, case-control studies generally take less time, require fewer resources, and can provide information about multiple exposures when little is known about the etiology of a particular health outcome.[12] In addition, case-control studies are more efficient than cohort studies for studying rare diseases and diseases with long incubation or latency times because they do not require long-term prospective follow-up.[12] Case-control studies are also more efficient when the population of interest is dynamic or when the study requires expensive or hard-to-obtain exposure data.[12] Although caution must be exercised with temporal inference, cross-sectional studies are useful because they can be performed in a short time period and yield highly generalizable results when based on large samples from the general population. Likewise, ecologic studies are rapid, inexpensive, and simple, and they can provide useful information about the context in which populations live.[13]

BASIC BIOSTATISTICS

A basic understanding of biostatistics is required to interpret medical literature or to plan appropriate epidemiologic studies. This section will cover measures of disease frequency, measures of association, and significance of association only. For more extensive reading on biostatistics, please refer to the classic references 2, 12, and 14. For the use of biostatistics in clinical trials see Chapter 52.

Measures of Disease Frequency

Measuring the frequency of disease in a population is the first step in characterizing the distribution and determinants of disease in populations.[12] To conceptualize disease occurrence, it is important to consider the size of the underlying population, the number of persons affected, and the length of time that the population is observed.

Ratios, Proportions, and Rates

These are the three types of calculations used to describe disease occurrence. A ratio is one number divided by another number. For example, the maternal mortality ratio is a ratio of two unrelated numbers, the number of maternal deaths per 100,000 live births. Proportions, known as fractions or percentages, also consist of one number divided by another, but the numerator must always be a subset of the denominator. Measures of disease occurrence are often written as rates. A rate also consists of one number divided by another number, but the denominator always includes time. The attack rate of a disease represents the proportion of individuals who developed illness divided by the total number of individuals exposed to a pathogen. For example, annual incidence rates for Lyme disease are calculated by state per 100,000 persons.[15]

Incidence

The two most basic measures of disease frequency are incidence and prevalence. Incidence is the number of new cases of disease that occur in an "at-risk" population over a specified period of time. Incidence takes into account how many persons are at risk in a particular population. For example, women are not at risk for prostatitis. Thus the incidence of prostatitis in a population would not include women in the denominator. The time period during which a population is followed is also important for incidence calculations. Incidence rate refers to the number of new cases of disease that occur during person-time observation, where time is included in the denominator. Incidence rate calculations include the amount of time that each individual contributes to the denominator before being diagnosed with the disease of interest. Cumulative incidence refers to the number of at risk individuals who become diseased during a specified period of time. It can be expressed as a proportion of the at-risk population and therefore ranges from 0% to 100%, or 0 to 1.[12]

Prevalence

Although incidence measures the frequency of the development of new disease, prevalence measures the frequency of existing disease in a population. Prevalence is defined as the number of persons with disease in a population divided by the total number of persons in that population. Unlike incidence, the prevalence numerator includes all persons with the disease, regardless of when it developed, and the prevalence denominator includes everyone in the population, whether or not the individuals are at risk. There are two different prevalence measures: point prevalence and period prevalence. Point prevalence refers to the proportion of the population with the disease at a specific point in time, such as a particular date. Period prevalence refers to the proportion of

		Outcome?		
		Yes	No	TOTAL
Exposed?	Yes	a	b	a+b
	No	c	d	c+d
		a+c	b+d	a+b+c+d

FIG. 13.2 Construction of a standard two-by-two table.[14]

the population with the disease over a specified period of time, such as a calendar year. Period prevalence includes the number of persons with disease present at the start of the time period, in addition to the number of persons who developed the disease during the specified period.[2] Prevalence depends on the rate at which new cases of disease develop (incidence rate), in addition to the duration of illness. The duration of disease begins when a person acquires the disease, and it ends when a person is cured or dies.

Measures of Association

Measures of association, or measures of effect, quantify the strength of the statistical association between exposures and health outcomes of interest. The respective measures of association for each type of study are shown in Table 13.1.

Two-by-Two Tables

To understand how to calculate measures of association, it is important to understand the basic construct of a two-by-two table. In many epidemiologic studies exposure and outcome data are dichotomous (e.g., "yes" or "no"). Thus the relationship between exposure and outcome can be cross-tabulated in a two-by-two table, which has two categories of exposure and two categories of outcome (Fig. 13.2[14]). By convention, outcome status is placed in columns, and exposure status is placed in rows. The four cells of the two-by-two table are labeled a, b, c, and d, and each letter refers to the number of persons with the outcome indicated by the column heading and the exposure status indicated by the row heading.

$$\text{Risk ratio (relative risk)} = \text{Risk}_{\text{exposed}} / \text{Risk}_{\text{unexposed}} = (a/[a+b])/(c/[c+d])$$

Risk ratio (RR) describes the excess risk of a health outcome in exposed persons compared with unexposed persons, who represent the background or expected risk. Mathematically, the RR is expressed as the risk in the exposed group divided by the risk in the unexposed group. A risk ratio greater than 1.0 indicates that risk is greater in the exposed group when compared with the unexposed group, whereas RR less than 1.0 indicates that the risk in the exposed group is less than in the unexposed group. This happens when the exposure protects against a health outcome, such as an immunization that protects against an infectious disease.[2]

$$\text{Odds ratio (cross-product ratio, relative odds)} = ad/bc.$$

Odds ratios (ORs) are measures of association used in case-control studies to approximate RR. The OR is also called the "cross-product ratio" because it is obtained by calculating the cross products of the cells in a two-by-two table, with "a" × "d" in the numerator and "b" × "c" in the denominator. In case-control studies, epidemiologists enroll a group of "cases" with the outcome and a comparable group of "controls" without the outcome. The number of individuals in each group is determined a priori, and the size of the populations from which the cases and controls arise is often unknown. This lack of population denominator data prevents direct calculation of the RR. However, the OR approximates the RR when the outcome of interest is rare.

Prevalence Ratio and Prevalence Odds Ratio

Cross-sectional studies usually measure the prevalence of a health outcome, rather than its incidence. Because prevalence is a function of both incidence and duration of illness, measures of association based on prevalent cases reflect the effect of the exposure on both incidence and duration, or survival. The prevalence ratio and the prevalence OR, respectively, are the measures of association that correspond to the RR and the OR in a cross-sectional study. These ratios are calculated using the same formulas but recognizing that the data in the two-by-two table is prevalence data.[2]

STATISTICAL SIGNIFICANCE

P Values

To make hypotheses about causal relationships between exposures and outcomes, three alternative explanations for study results should be considered: bias, confounding, and random error. Bias is a systematic error in study design, measurement, or analysis that leads to a false association between the exposure and outcome. Confounding is a systematic mixing of effects between the exposure, outcome, and a third variable called a confounder, unmeasured or unrecognized, which distorts the measure of association between the exposure and outcome. Random error arises from chance and leads to a false association between the exposure and outcome that is uncontrollable and appears not to have an assignable cause. Random error can occur from mistakes in measurement or sampling variability that occurs by randomly selecting an unrepresentative population sample purely by chance.

Hypothesis testing is a method of evaluating random error in an epidemiologic study. The default assumption, or "null hypothesis," is that there is no relationship between exposure and the outcome. For example, eating raspberries this July did not increase the chances of getting cyclosporiasis. The compatibility between the data observed in the study with the null hypothesis is evaluated using a statistical test. Computing the test statistic yields a *P* value, which represents the probability of obtaining the observed or more extreme results by chance alone. The *P* value ranges from 0.0 to 1.0, with a small value indicating that the null hypothesis may need to be rejected. By convention, a *P* value cutoff of 0.05 is traditionally used to determine whether to reject the null hypothesis, which assumes that a 5% or lower chance of random error is acceptable when asserting that there is a true relationship between exposure and outcome. This cutoff is also called the alpha or significance level.

Confidence Intervals

Another way to quantify random error in studies is to estimate the confidence interval (CI). CIs quantify the variability around a point estimate. The width is determined by random error, in addition to an arbitrary certainty factor, usually set at 95%, although some studies use 90% or 99%. Using a 95% CI as an example, the strict interpretation is that 95 of 100 CIs would contain the true measure of association if a study were repeated 100 times to obtain 100 point estimates with 100 CIs. Wider CIs mean that there is a larger amount of random error relative to narrower CIs. In general, sample size affects CI width, and wider CIs indicate that a relatively smaller sample created the point estimate, whereas narrower CIs generally indicate point estimates created by larger samples. CIs can also be used to determine statistical significance. For example, if the null value for a measure of association (example: RR of 1.0) falls within the 95% CI, then the result is by convention not statistically significant. Some epidemiologists prefer using CIs to *P* values when determining statistical significance because CIs are expressed in the same units as the measure of association and contain the point estimate, whereas the point estimate cannot be estimated by examining a *P* value. Nevertheless, similar to *P* values, CIs do not account for bias and confounding, nor can they confirm that a given correlation is causal.

DISEASE SURVEILLANCE

Surveillance for infectious diseases is one of the most critical functions of a public health system, from the clinic or hospital level all the way to the national and global level. Surveillance can take many forms but generally entails the continuous and systematic collection and analysis

of data, and the subsequent reporting of any significant findings to effect change. There are many valid indications for implementing surveillance, including measuring baseline levels of disease, identifying hypotheses to conduct further research, monitoring the effectiveness of interventions, and detecting outbreaks. Perhaps most relevant for infectious diseases clinicians are the recognition and initial management of outbreaks, because it is often physicians who first recognize an increase in cases. A brief discussion on outbreak management is included in the following text.

Classification of Surveillance

Surveillance can be either active or passive and may collect data on incident cases, prevalent cases, or both. Active surveillance is usually more sensitive for discovering an outbreak but is more costly and labor intensive. Passive surveillance is often less sensitive but is also less costly and thus can be deployed on a broader scale.

Surveillance systems in the United States often follow a hierarchical reporting scheme, with local clinics, hospitals, or laboratories tabulating cases and reporting them to the local public health authorities. The collated data is then passed to the state-level public health officials, who in turn report to the Centers for Disease Control and Prevention (CDC). The CDC reports to World Health Organization (WHO) through the US government focal point, if necessary, about outbreaks of potential global significance. Reports of outbreaks are updated by the CDC on the Epidemic Information Exchange (Epi-X), a secure web-based reporting system (https://emergency.cdc.gov/epix/index.asp) limited to public health officials, as well as in the Morbidity and Mortality Weekly Report (MMWR), which is available to the public electronically at http://www.cdc.gov/mmwr/ and in hard copy. An international treaty, the International Health Regulations (IHR [2005]) governs what type of outbreak is reportable and to whom and under what time requirements it must be reported.[16] Each signatory country is responsible for controlling disease within its own borders. The IHR (2005) is designed to allow less-developed countries to request assistance from WHO and its response mechanism, the Global Outbreak Alert and Response Network (GOARN). The GOARN and WHO Outbreak News websites provide public information about current outbreaks of potential global concern.[17]

Some sentinel surveillance systems attempt to identify specific diseases using representative sites. For example, FoodNet is part of CDC's Emerging Infections Program and conducts surveillance for enteric pathogens in 10 state health departments in collaboration with the US Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) and the US Food and Drug Administration (FDA).[18] The 10 sites cover approximately 48 million persons (≈15% of the US population) and include Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, and Tennessee, and selected counties in California, Colorado, and New York.[18] This system has identified many outbreaks of gastrointestinal infections, such as the multistate outbreak of *Salmonella* Virchow in 2015–16.[19]

Disease surveillance is also conducted on a global scale. Perhaps the best example of this is the Global Influenza Surveillance and Response System (GISRS), which is a unique, laboratory-based global surveillance network to monitor seasonal and emerging influenza viruses to mitigate pandemic influenza spread.[20] The GISRS network, established in 1952, consists of 142 National Influenza Centers, 6 WHO Collaborating Centers, and 4 Essential Regulatory Laboratories. Influenza isolates are systematically collected throughout the year and compared with previously circulating strains to predict which strain is likely to be predominant in the following season. Different compositions of influenza vaccine (containing influenza A H3N2, A H1N1, and B components) can be selected for Northern and Southern Hemisphere seasons.[21]

Technology is revolutionizing disease surveillance, especially in the field of infectious diseases. Classic tools, such as the outbreak line list and population serosurveys, remain important for understanding and controlling disease, but increasingly the power of "big data" is being leveraged to more rapidly and precisely address the challenges posed by infectious diseases. Syndromic surveillance aims to identify unusual or novel diseases by monitoring for general clinical syndromes, such as febrile pneumonia. In response to the intentional anthrax outbreak of 2001, electronic disease (syndromic) surveillance was intensified in the United States with the goal of rapidly identifying any clusters of illness that were compatible with a biowarfare attack. The Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE) is used by federal (CDC, the National Syndromic Surveillance platform), state, and local public health entities to monitor novel and emerging infectious diseases, chronic illnesses, and other public health conditions of interest, such as mental and behavioral health disorders and environmental exposures.[22]

Novel methods of disease surveillance are continuously being developed and refined. Some reporting systems, such as ProMED mail, have been functioning since the 1990s and rely on a network of health care professionals and other interested parties (more than 70,000 subscribers in 185 countries) to report outbreaks around the world.[23] This email- and web-based system covers outbreaks affecting humans, animals, and plants, and it is moderated by a panel of public health experts who screen each report before release. Digital disease detection or surveillance is a new technology that relies on collating and analyzing diverse streams of data that are increasingly available in machine-readable format. The prototypical digital disease detection platform is HealthMap (http://www.healthmap.org/en), which harnesses the broad reach of the Internet by searching "online" news outlets and applying automated logic to sort "real" outbreaks from rumor.[24] The CDC and the Infectious Diseases Society of America collaborate on the Emerging Infections Network (https://ein.idsociety.org/), an email-based list-serv that connects practicing infectious diseases physicians from the United States and some other areas around the world to conduct surveillance for emerging infectious diseases.[25] The GeoSentinel Surveillance System (http://www.istm.org/geosentinel) is likewise a worldwide network of travel medicine clinics that reports illnesses among travelers.[26]

Machine learning and related deep-learning computer algorithms are synthesizing and analyzing unapproachably large data sets that surveillance systems can collect. This allows aberration detection within surveillance data streams and may even lead to improved prediction of epidemiologic trends or forecasting of disease events.[27] Better-quality data also allow more precise geospatial representation of disease, increasing the efficiency of identification, response, and control of outbreaks.[28]

The astute and vigilant clinician arguably remains the most important factor in outbreak surveillance. The first case of severe acute respiratory syndrome (SARS) was identified by a physician in Hanoi, Vietnam, who noticed an unusually severe, atypical pneumonia.[29] The first cases of acquired immunodeficiency syndrome (AIDS) were identified by a clinician who noted rare infections among a group of homosexual men.[30] Also, West Nile virus was discovered by an observant clinician in New York City, following an independent veterinary investigation reporting a die-off of birds.[31] It is important for clinicians to understand that as individual providers they may be seeing the proverbial "tip of the iceberg." Only by reporting these cases can an outbreak be appreciated as cases are examined in aggregate.

DISEASE PREVENTION

Prevention includes a wide array of interventions aimed at promoting health by reducing risks that lead to poor health outcomes or disease. *Primary, secondary,* and *tertiary* prevention are three terms that describe the points in the disease process targeted by an intervention.[2] Primary prevention aims to prevent the occurrence of disease. This is done by preventing exposure to hazards that cause poor health outcomes, that is, changing unhealthy or unsafe behaviors that lead to poor health outcomes and increasing resistance to poor health outcomes if exposure occurs. Examples of primary prevention include legislative measures that control health hazards, education about healthy behaviors, and immunization against diseases.[2] Secondary prevention aims to minimize the impact of a poor health outcome that has already occurred. This can be done by identifying and treating a health condition as soon as possible to prevent its progression, implementing strategies to prevent recurrence, and modifying activities to help persons return to their original state of health. Examples of secondary prevention include screening tests that identify diseases during their earliest stages, such as hepatitis C screening. Tertiary prevention aims to lessen the impact of a poor health outcome with lasting effects, such as chronic disease or injury leading to disability. This can be done by managing long-term

ailments to lessen disability, improve quality of life, and lengthen life expectancy. Early antiretroviral therapy for human immunodeficiency virus (HIV) infection is an example of tertiary prevention.

DISEASE CONTROL: QUARANTINE AND ISOLATION

There are many strategies to mitigate the spread of infections, and those relevant to hospital infection control are given in Chapter 298. Some strategies targeting public health intervention, namely quarantine and isolation, and a short primer on outbreak investigation are discussed as follows.

Quarantine (separating and restricting movement of individuals *exposed* to a communicable disease) and isolation (separating and restricting movement of individuals *suspected or confirmed* to be infected with communicable disease) are public health strategies used to prevent exposure of the public to individuals or populations infected with a communicable disease. In an increasingly connected world, public health officials must interact with multiple entities and many levels of government to balance public health and humanitarian efforts with economic priorities, such as international travel and trade. In the 2014 West African Ebola epidemic, for example, the CDC partnered with WHO, the International Organization for Migration, nongovernmental organizations, and domestic entities, such as the US Department of Homeland Security and state and local health departments, to prevent international transmission of Ebola while minimizing disruption of economic activities.[32] Domestically, in addition to communicating with travelers via travel notices and airport messaging, CDC teams provided training and equipped US entry-point personnel to conduct screening using body-heat sensing devices and questionnaires to identify symptomatic and potentially exposed travelers arriving in the United States. If a potentially exposed individual was identified through screening, the domestic quarantine strategy relied on measures such as active self-monitoring, controlled movement, and daily communication with public health officials (for high-risk individuals, such as health care workers) during the 21-day Ebola incubation period, with the aim of applying the least restrictive measures necessary to prevent potential transmission.[32]

OUTBREAK INVESTIGATION PRIMER

Outbreaks of infectious disease can arise anywhere in the world and pose a threat to local and global populations without respect for political borders, geographic separation, or cultural differences. Many current conditions facilitate this potential for rapid spread of infectious diseases, such as the globalization of the food supply, overuse of antibiotics, the growth of megacities with severe crowding, our increasing proximity to animals and vectors of disease, and even climate change.[33]

There have been numerous recent outbreaks that have seized the public's attention. The SARS epidemic of 2003 and the recent resurgence of measles in North America and Europe were watershed events and hastened the strengthening of the IHR. These were followed by multiple outbreaks of imported foodborne gastroenteritis caused by *Cyclospora* and *Salmonella,* as well as by MERS, Nipah virus, pandemic H1N1 influenza, and, most recently, Ebola virus disease and Zika virus syndrome.[33,34] The scope and impact of these outbreaks underscore the importance of maintaining an informed network of clinicians who understand the dynamics of an outbreak and how to investigate one. This section describes the key concepts involved in an outbreak investigation, discusses basic transmission dynamics, and provides a simple guide for conducting an outbreak investigation.

An outbreak is an increase beyond expectation in the number of cases of a disease or condition, occurring in a specific population in a defined geographic location and period of time.[14] The cases are epidemiologically related, although this linkage is often not initially evident and may only be discovered after thorough investigation. For example, in the 1981 multistate outbreak of *Salmonella muenchen*, the epidemiologic link was discovered only when case patients reported smoking marijuana more frequently than control patients. This rare *Salmonella* species was then isolated from marijuana samples found in the homes of patients with the illness.[35]

Outbreaks very often have an infectious origin, although some can be due to noninfectious agents, such as food intoxication or even hysteria. It is often difficult to define how many cases beyond expectation constitutes an outbreak, but even one case can indicate an outbreak if the disease has been eradicated (smallpox), eliminated (poliomyelitis in Europe), or is novel to humans (highly pathogenic H5N1 avian influenza in the Americas).

In the absence of timely control measures, outbreaks can spread and lead to epidemics or even pandemics. Epidemics are conceptually identical to outbreaks but are more widely disseminated in time and space, such as the cholera epidemic in London during the mid-1800s and SARS in Southeast Asia in 2003. Pandemics spread globally and may persist through months, years, or decades. Examples of pandemics include such historic scourges as bubonic plague in medieval days and influenza in 1918-19, as well as AIDS or the influenza A/H1N1 pandemic of 2009 more recently.

Many infectious diseases are endemic in certain settings and occur routinely. Malaria, dengue and enteropathogens that cause diarrheal disease in the tropics are all examples. Endemnicity does not preclude the occurrence of outbreaks, however, that may occur during point-source foodborne outbreaks when large pools of susceptible individuals are exposed at a single time. *Vibrio parahemolyticus* and Norovirus, for example, are endemic causes of sporadic gastroenteritis but can also lead to outbreaks when contaminated seafood is eaten raw.[36,37] Often, all that is required for an outbreak to occur is a sufficiently large, naïve population that is exposed to an infectious inoculum of the agent.

OUTBREAK EPIDEMIOLOGY

General

The methodology for conducting outbreak investigations and the study designs and methods used have been well described in the literature.[2,14,38–41] Reviews of published outbreaks suggest that foodborne transmission accounts for nearly half of all reported outbreaks. Most efforts to improve the standardized reporting of outbreaks have been focused on either foodborne disease or nosocomial infections.[42,43] The CDC collects and collates data on outbreaks in the United States and maintains a dashboard that includes visualizations and statistics through 2016. Outbreak data can be explored at the National Outbreak Reporting System, which is found at https://wwwn.cdc.gov/norsdashboard/.[44] A current list of ongoing outbreaks, both domestic and significant international, can be found at https://www.cdc.gov/outbreaks/index.html.

KEY OUTBREAK PRINCIPLES

Incubation Period

Incubation period is the time elapsed from exposure to a certain infectious agent until the development of symptoms and is often expressed as a range. In an outbreak investigation the incubation period is a key parameter that may help to discern between multiple potential etiologic causes. For example, symptoms of foodborne staphylococcal intoxication often appear 30 minutes to 6 hours after exposure, whereas the incubation period for salmonellosis is 6 to 72 hours. So, abrupt gastroenteric illness in multiple subjects who recently shared a common meal is less likely to be caused by an agent with a longer incubation period like *Salmonella*. However, it should not be mistakenly assumed that infectious agents with lengthy incubation periods cannot cause outbreaks because transmission of *Mycobacterium tuberculosis* during air travel has been demonstrated on several occasions.[45] Cases from outbreaks caused by agents with long incubation periods will typically appear over extended time periods, making their common origin less obvious. The routine application of genetic sequencing of pathogens may assist in defining the relatedness of cases that are separated by time and space.[46]

Epidemic Curves

Epidemic curves are histograms depicting the number of cases of a disease during the duration of an outbreak or epidemic.[14] When properly prepared, epidemic curves are key epidemiologic tools in the outbreak investigation that may aid in determining the transmission mode of the outbreak, the incubation period and possible period of exposure. The main mode of transmission can often be identified from epidemic curves (point source, person-to-person, or continued common source),

although on occasion two of these modes are seen at different stages during a single outbreak. The crucial step in preparing an epidemic curve is choosing a time interval unit that corresponds to a fraction of the median incubation period of the suspected agent (usually between ⅓ to ¼ of the incubation period) so that the epidemic curve demonstrates the details of the outbreak and clearly identifies the onset, peak, and tail of the outbreak.[2,14]

Transmission Modes

Point source outbreaks can occur after the exposure of a group of people to an agent during a single, short period of time. Cases often present in a single group during a short time period, corresponding to the range of the incubation period, unless there is secondary transmission. The epidemic curve demonstrates an abrupt onset and gradual descent with a single peak. Food and beverages are common point sources, and single-source outbreaks very often present as gastroenteric illness (Fig. 13.3).[47]

Person-to-person, or propagated, outbreaks are characterized by the presence of two or more clusters of cases in time separated by approximately one median of the incubation period, thus suggesting secondary transmission. The distance between clusters of cases usually becomes less clear as the outbreak progresses because the ranges of the incubation periods tend to blend into each other. Respiratory transmission is the most efficient mechanism of person-to-person transmission, the most classic example being the influenza virus. Measles, adenovirus, and pneumonic plague may also be transmitted in this manner. (Fig. 13.4)[48]

In continuous-source outbreaks, exposure to the agent can occur over an extended period. Therefore cases appear over longer periods of time, often substantially beyond the range of the incubation period. Cases sometimes present in several clusters, but the timing between clusters does not necessarily correspond closely to the median incubation period. Vector-borne and zoonotic infections are typical of this transmission pattern, but waterborne, respiratory, and nosocomial outbreaks have occasionally been described as continuous-source outbreaks as well. (Fig. 13.5)[49]

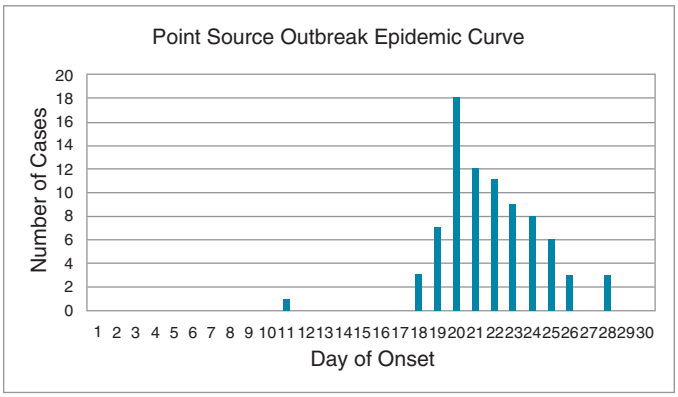

FIG. 13.3 Epidemic curve: single-point source outbreak.

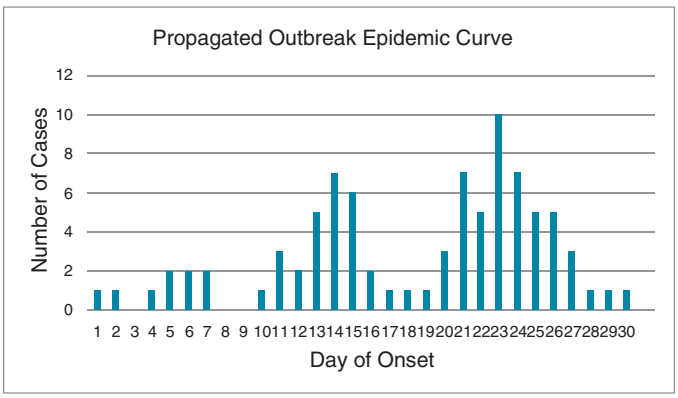

FIG. 13.4 Epidemic curve: propagated (person-to-person) transmission.

Chains of Transmission

Exploring the connection between initial waves of cases and those occurring later often reveals important interactions that can occur during propagated outbreaks with person-to-person transmission or a continuous common source. Clearly identified transmission waves can be observed, such as those related to cultural practices such as burial traditions in Africa[49] or the transmission of Nipah virus in a Bangladeshi community (Fig. 13.6).[48] Transmission chains can also provide hints about the incubation period of the agent, although these may be difficult to tease out of the data.

New molecular diagnostic tools are revolutionizing the study of outbreaks, especially in elucidating chains of transmission where the cases are separated by time and distance. Whole-genome sequencing of pathogens requires less time, money, and equipment than ever before in the United States and in many high-income settings.[46] Next-generation sequencing platforms are increasingly available in the field, as is the ability to interpret the massive volumes of data inherent to this technology. Genetic sequencing can be used to precisely determine transmission chains, relatedness of pathogens to known standards, and even characteristics such as antimicrobial resistance and virulence, and are extremely valuable tools for outbreak management.[46]

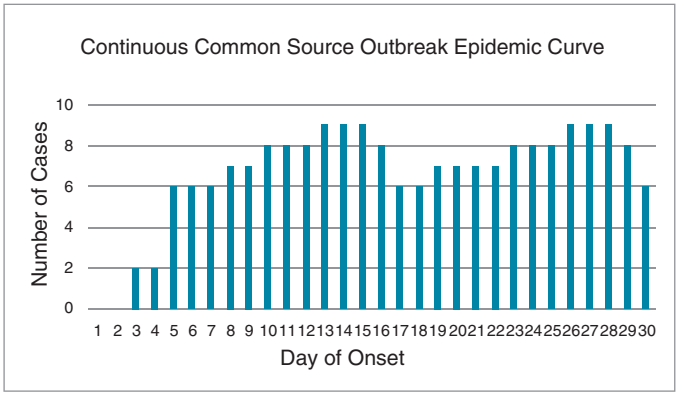

FIG. 13.5 Epidemic curve: continuous common source.

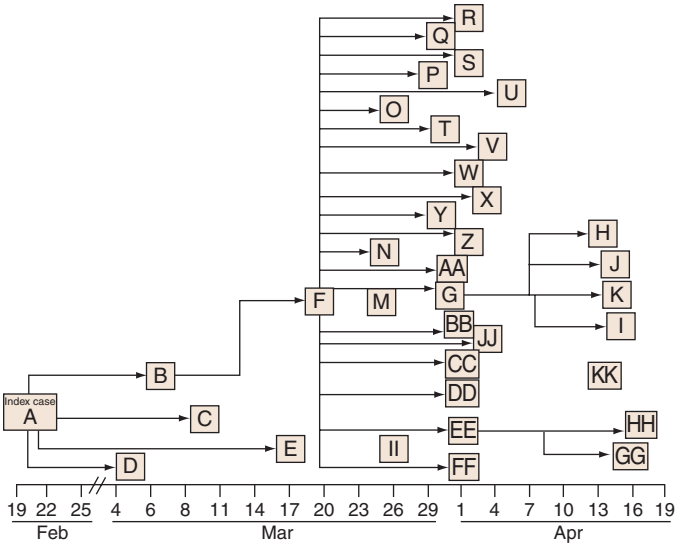

FIG. 13.6 Person-to-person chain of transmission of Nipah virus in a Bangladesh village with dates of onset of illness in 2004. Letters designate individual patients. Patients KK and II had no known contacts with any ill patients before onset of illness.

TYPES OF OUTBREAKS

Foodborne Outbreaks

Surveillance of foodborne outbreaks is routinely conducted in the United States and Europe but in fewer low-income countries where one might expect more frequent foodborne illness. The most recent comprehensive data are published by the CDC from 1998 through 2016. In the United States there were 19,991 outbreaks affecting 388,238 persons that led to 15,631 hospitalizations and 365 deaths.[50] For reference, this represents almost half of the total outbreaks[41] throughout this period in the United States. An etiologic agent is identified in approximately half of the foodborne outbreaks, and 91% of them were caused by an infectious agent, mainly viruses (54%) or bacteria (36%). Nearly all outbreak-causing foodborne viruses were Norovirus, and 52% of the bacterial causes were *Salmonella* spp.[51]

The primary factor leading to foodborne outbreaks is temperature mismanagement while preparing, cooking, or storing food. If food workers were involved in the food preparation, the outbreak was often associated with a single, nongloved worker who handled the implicated food or fecally contaminated foods that were improperly refrigerated.[52]

Nosocomial Outbreaks

Outbreaks in hospital settings often benefit from better infrastructure for diagnosis and investigation. There are a wide variety of pathogens that can lead to outbreaks in hospitals, from respiratory pathogens such as influenza or coronoviruses, to diarrheal pathogens such as *Clostridioides difficile* (formerly *Clostridium difficile*), to bloodstream infections such as multidrug-resistant gram-negative bacilli. Extensive work on infection control has led to the publication of the Outbreak Reports and Intervention Studies of Nosocomial Infection (ORION) guidelines for reporting nosocomial outbreaks.[44] An analysis of an open-access database of nosocomial outbreaks (www.outbreak-database.com) demonstrated that outbreaks occurred mainly in hospitals (83%) and more often in intensive care units (47%).[53] Surgical, neonatology, and internal medicine services accounted for similar proportions of the outbreaks. A source was identified in only 63% of outbreaks, often an index patient (40%), contaminated equipment or devices (21%), or the environment (19%). The mode of transmission was unidentified in 28% of outbreaks, and in the remainder it was primarily contact (60%). The three most frequent pathogens were *Staphylococci* (15%), *Pseudomonas* (8%), and *Klebsiella* (7%). Most of the staphylococci outbreaks (71%) were caused by methicillin-resistant *S. aureus*, and 24% of the *Klebsiella* outbreaks were due to extended spectrum β-lactamase–producing bacteria. Many smaller nosocomial outbreaks are likely excluded from this database due to publication bias in the literature, and most of the published cases come from high-income countries.[53]

Respiratory and Other Person-to-Person Outbreaks

The most typical outbreaks of respiratory illness are those caused by respiratory viruses with short incubation periods and potential for rapid dissemination in a population, such as influenza or adenovirus.[54] Viruses transmitted by droplet nuclei, such as measles and mumps, follow similar transmission patterns, although their incubation periods are often somewhat longer. Tuberculosis, on the other hand, is less frequently observed in outbreaks, probably because of its very lengthy incubation period and the consequent difficulty in establishing a clear epidemiologic link between index and secondary cases. On occasion, this link can be established, such as when there is a common airline flight or workplace.[51] Coronoviruses (CoV) such as SARS-CoV and, more recently, Middle East respiratory syndrome (MERS-CoV) have recently emerged as respiratory pathogens with epidemic potential. They are originally zoonotic in nature but can be transmitted person-person and in health care settings.[55]

Sexually Transmitted Outbreaks

Sexually transmitted diseases are typically less efficiently transmitted because an intimate relationship must be established. HIV has taught us that sexual transmission may be inefficient but was certainly sufficient to establish a global pandemic. The Internet and enhanced connectivity through virtual social networks has been cited as one mechanism that increases the probability of contact between cases and naïve, susceptible individuals.[56]

Zoonotic and Vector-Borne Outbreaks

Increased interaction between humans, animals, and their environment have led to a relentless surge of emerging and reemerging infections, most of them zoonotic or vector-borne in nature (see Chapter 317). No single common transmission pattern can describe these outbreaks because of their diversity. Point source exposures are often observed, such as in an outbreak of leptospirosis after a freshwater exposure during a military exercise in Guam.[57] Continuous common source exposures are also observed, especially for vector-borne diseases, such as dengue, malaria, or Zika virus.[58] Finally, the Ebola epidemic in West Africa in 2015–16 contributed much to the understanding of outbreak management, from the science of transmission, to the many social implications of infection control, to the mundane challenges of waste management.[59]

OUTBREAK INVESTIGATION AND RESPONSE

Conducting an investigation may help us to understand the mode of transmission of the disease, identify the etiologic agent and who may be at risk of infection, and ultimately prevent additional cases and reduce the overall morbidity and/or mortality rates. Conducting an outbreak investigation may also allow us to evaluate the sensitivity and specificity of a surveillance system, evaluate or implement intervention strategies (i.e., vaccination, social distancing, or removal of a point source) and contribute to the epidemiology and scientific knowledge of the disease.

A systematic, step-by-step approach to conducting an outbreak investigation is imperative for identifying the source of the outbreak and for controlling and preventing additional cases. This systematic approach can be divided into 13 distinct steps (Table 13.2), but these are not rigid in their order, and several steps are often accomplished simultaneously.[2,14]

1. Preparation for an outbreak should involve prestaging of standardized sample collection materials, personal protective equipment, and most important, the presence of trained personnel. It is also important to think about permissions for conducting the study, whether it is the Institutional Review Board (IRB) of the hospital or CDC, or jurisdictional approval if you are traveling to another state or country.
2. Conducting an outbreak investigation can be resource intensive. Before initiating the investigation, it is imperative to first determine whether or not an outbreak (or pandemic) is actually occurring. Several data sources may be available to help determine if the number of observed cases exceeds that of the expected baseline number (i.e., notifiable disease registries, death registries, hospital discharge summaries, etc.). It is important to recognize that new or improved diagnostic tests, a new or enhanced surveillance system, or simply increased awareness of a disease may artificially indicate that an outbreak is occurring.

TABLE 13.2 13 Steps to Conduct a Successful Outbreak Investigation

Prepare for field work.
Establish the existence of an outbreak.
Verify the diagnosis.
Construct a working case definition.
Find cases systematically and record information.
Perform descriptive epidemiology.
Develop hypotheses.
Evaluate hypotheses epidemiologically.
As necessary, reconsider, refine, and reevaluate hypotheses.
Compare and reconcile with laboratory and/or environmental studies.
Implement control and prevention measures.
Initiate or maintain surveillance.
Communicate findings.

3. Verifying the diagnosis often goes hand-in-hand with confirming the existence of an outbreak. It may be necessary to collect additional biologic samples and, if possible, request specialized diagnostic procedures or have the results confirmed at a secondary reference laboratory. However, in the event of an outbreak of a new pathogen, it may be necessary to rely on clinical diagnosis alone, as was the case in the early stages of the SARS epidemic. Furthermore, it is highly recommended to interview patients to gather additional clinical and epidemiologic features.
4. Enumerating the number of cases during an outbreak is only possible once a standard case definition has been established. This can be one of the most difficult and contentious components of an outbreak investigation. The case definition is generally based on clinical features, such as sudden onset of fever higher than 38°C, cough or sore throat, difficulty breathing, and so forth. The case definition is almost always restricted by person (i.e., children younger than years, no history of yellow fever vaccine), place (i.e., patients in a specific wing of a hospital, attendees of a county fair), and time (i.e., persons with illness onset within the previous 24 hours). The initial case definition is often quite broad, to capture all possible cases; however, as the investigation proceeds it generally becomes more refined and divided into subcategories, such as suspect (i.e., fever only), probable (i.e., fever with cough and shortness of breath, death with history of fever and cough), and confirmed (i.e., fever and cough with laboratory confirmation of influenza virus H5N1 infection).
5. Counting and tracking cases is most easily achieved by constructing a line listing of cases. Data in the line listing should include information such as symptom onset date, patient identification data, clinical data, demographic data, laboratory data, and some risk factor or epidemiologic data. (Fig. 13.7)
6. The next step, and often most revealing, is descriptive analysis of the epidemiologic data. The line listing is converted to visual depiction of the data, referred to as the epidemic curve (as described earlier), which is graphed with time on the x-axis (typically symptom onset data) and number of cases on the y-axis (Figs. 13.3 to 13.5). The epidemic curve can give clues about trend (i.e., person-to-person transmission or point/common source exposure), size of outbreak, and incubation period. The data can also be displayed in map form, revealing potential information such as common source exposure or clustering of cases. Global Positioning System (GPS)/Global Information Systems (GIS) are tools now commonly used in modern epidemiologic outbreak investigations.
7. The next step, albeit a process that likely begins from notification of the first case, is to begin to develop hypotheses about the cause of illness and/or source of infection. In general, conducting the initial descriptive epidemiologic analysis of the data will give us clues to better refine our hypotheses. Furthermore, it is important that the investigator speaks with an adequate representative sample of the initial patients to understand potential links between cases. It is important to remember that the working hypotheses must be testable with statistical methods (i.e., chi-square analysis, logistic regression, etc.).
8. After careful consideration and development of our initial hypotheses, we must evaluate these hypotheses with analytic methods. Two methodologies are typically used for outbreak investigations: cohort studies or case-control studies. These are described earlier under the "Analytic Epidemiology" section.
9. Once the hypotheses have been tested using conventional analytic epidemiologic methods, it may be necessary to refine the hypotheses by conducting additional studies (i.e., laboratory or environmental testing) to further support the conclusions. For example, identifying *Cyclospora cayetanensis* in the raspberry filling of a wedding cake would greatly increase your epidemiologic evidence that eating cake at the wedding was significantly associated with developing diarrheal illness.[60] If no conclusive results were initially revealed, a new hypothesis may be proposed.
10. The CDC recommends an explicit step to reconcile epidemiologic findings with the available laboratory and environmental data that have been collected. This is an important step but is often done in parallel with the hypothesis testing.
11. The primary purpose of conducting an outbreak investigation is to not only determine the source of the infection but also simultaneously control the spread of the outbreak and prevent additional cases. For example, during an outbreak of yellow fever it may be necessary to vaccinate any at-risk populations, apply insecticide, and educate the community regarding ways to reduce mosquito breeding sites. Planning for this step is often done in an empirical manner at first and refined as better data are accumulated.
12. As noted earlier, disease surveillance is one of the key components of a public health system and often is responsible for identification of an outbreak. It is important to remember to continue or even enhance surveillance during an outbreak, to assure that cases are not missed and the control measures that have been implemented are effective.
13. Finally, it is imperative to communicate all findings. Unfortunately, this last step is often overlooked. The results of the study should be presented to the stakeholders (i.e., hospital staff, local health authorities, scientific community) so that others can learn from the investigation and recommendations.

Case #	Patient Initials	Onset Date	Date of Death	Gender	Age	Case Status	Symptoms			Laboratory Values			Epidemiologic Data	
							Fever	Hematemesis	Bloody Diarrhea	Decreased Platelets	Ebola IgM	Ebola PCR	Contact With Other Cases	Bushmeat Hunter
1	JM	21-Aug	28-Aug	Male	39	C	+	+	+	+	+	+	–	+
2	DB	21-Aug	26-Aug	Male	39	C	+	+	+	+	+	+	+	+
3	WL	22-Aug	30-Aug	Male	40	C	+	–	–	–	–	+	+	–
4	VG	22-Aug	30-Aug	Male	27	P	+	+	–	–	NS	NS	+	–
5	JS	23-Aug	28-Aug	Male	42	C	+	+	+	+	+	+	+	–
6	KM	24-Aug		Female	38	S	+	+	–	–	TP	TP	–	–
7	MB	24-Aug		Female	40	S	+	–	–	–	TP	TP	–	–
8	BG	24-Aug		Male	25	S	+	+	+	–	TP	TP	–	–
9	HR	25-Aug	30-Aug	Male	28	C	+	+	+	+	+	+	+	+
10	MR	25-Aug		Female	26	S	+	–	–	–	TP	TP	–	–

NS No sample
TP Test pending
C Confirmed
P Probable
S Suspect

FIG. 13.7 **Example line list.**

Although infectious diseases do not necessarily respect or recognize boundaries and jurisdictions of cities, states, and countries, it is important to remember that local and state health departments, as well as national and international authorities have specific roles and responsibilities. An investigation of a nosocomial outbreak limited to an individual hospital or a diarrheal disease outbreak at a single daycare center would require coordination at the local health department level only. However, if it is determined in the course of the outbreak investigation that the source of the nosocomial outbreak was in multiple states (e.g., from contaminated blood products), the investigation would require coordination with other states and likely national authorities (i.e., CDC) to coordinate the investigation and response across borders. Similarly, if an outbreak was occurring in multiple countries (a pandemic such as SARS or influenza), then coordination would need to occur via WHO and the GOARN.[16,17]

Consequences of an Outbreak

Outbreaks of infectious disease have the potential to be sensational and may at times lead to significant public relations difficulties. The economic, social, or political disruptions that can result when the public learns about an outbreak may be a significant disincentive to reporting it in a timely fashion and may even lead, in some cases, to lack of cooperation with the outbreak investigation. This may occur at a local level, such as when a restaurant does not desire to be associated with an outbreak of foodborne disease for fear of losing customers, or on an international level, when a country does not report an epidemic for fear of decreased tourism or limited exportation of goods.[61] These sensitivities must be considered when investigating an outbreak, dealing with the media, and releasing public statements, with an effort to minimize sensationalism, but impart the correct information and secure the public's health.

The primary goal of any outbreak investigation is to control the disease within the affected population and to prevent the disease from spreading to other individuals or populations. Outbreak investigations are one of the most exciting aspects of epidemiology and are rewarding when they result in a tangible, positive outcome. Outbreak investigations can also be extremely stressful, such as when they are conducted under severe political and/or economic pressure and results are demanded on a short timeline. In these days of frequent pandemic threats, and with the reemergence of numerous diseases, careful preparation and a clear understanding of disease outbreaks is more essential than ever for timely and adequate disease control.

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14 Emerging and Reemerging Infectious Disease Threats

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SHORT VIEW SUMMARY

- Infectious disease threats (Table 14.1)—such as the acquired immunodeficiency syndrome (AIDS) pandemic, the severe acute respiratory syndrome (SARS) outbreak, the Ebola outbreak in West Africa, the Zika epidemic in the Americas, and major Ebola outbreaks in West Africa and the Democratic Republic of the Congo (DRC)—shock the world and spur renewed efforts to improve capacity to detect and control emerging and reemerging diseases.
- Some infectious diseases can cause or contribute to chronic diseases (Table 14.2), and some human genetic variants may influence disease susceptibility or severity (Table 14.3).
- This chapter identifies factors that favor disease emergence and spread, considers lessons learned from major outbreaks, and reviews infectious threats of current concern, including:
  - **Antimicrobial Resistance.** Drug-resistant infections are a major danger to global health and well-being. Urgent threats include strains of gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae, that are resistant to all available antibiotics. Other serious threats include multidrug-resistant tuberculosis, methicillin-resistant *Staphylococcus aureus,* and *Candida auris,* a multidrug-resistant fungus that causes health care–associated infections.
  - **Acute Respiratory Tract Infections.** Lower respiratory tract infections are the leading cause of acute incident disease and communicable disease mortality worldwide. Clinically important respiratory pathogens first recognized in the 21st century include human metapneumovirus, SARS coronavirus, human bocavirus, Middle Eastern respiratory syndrome coronavirus, and pandemic influenza A(H1N1). Public health authorities continue to monitor novel influenza A viruses with pandemic potential, such as avian influenza A(H5N1) and A(H7N9) viruses (Figs. 14.1 and 14.3).
  - **Enteric Diseases.** Diarrheal diseases are the second leading cause of acute incident diseases and communicable disease mortality worldwide. Pathogens that cause enteric disease are transmitted through food, water, and person-to-person contacts, as well as through environmental or zoonotic exposures. Enteric diseases of current concern include infections caused by noroviruses, by *Clostridioides difficile* (formerly *Clostridium difficile*), and by multidrug-resistant strains of *Campylobacter* and *Salmonella.* Other public health issues related to enteric diseases include the reemergence of cholera, which caused a major outbreak in Haiti in 2010 following an earthquake, and the identification of new food vehicles for disease transmission during investigations of multistate foodborne outbreaks in the United States (Table 14.4).
  - **Vector-Borne Diseases.** Vector-borne pathogens are transmitted by blood-feeding arthropods, typically insects and ticks. Mosquito-borne examples include viruses carried by *Aedes* mosquitoes that cause dengue, Zika, yellow fever, and chikungunya diseases, as well as the protozoan parasites carried by *Anopheles* mosquitoes that cause malaria. Tick-borne diseases of public health concern include Lyme disease, caused predominately by the bacterium *Borrelia burgdorferi,* the most common vector-borne disease in the United States; Rocky Mountain spotted fever, caused by *Rickettsia rickettsii;* and the recently discovered Heartland virus and Bourbon virus diseases.
  - **Ebola and Marburg Hemorrhagic Fevers.** Ebola and Marburg viruses—whose natural reservoir is thought to be fruit bats—cause rare, severe, and often-fatal diseases in humans and nonhuman primates. Since 2000, outbreaks of Ebola or Marburg virus have occurred multiple times in central and East Africa, in the Democratic Republic of the Congo, in Sudan, and in Uganda. The first Ebola outbreak in West Africa—and the largest outbreak of Ebola to date, involving major urban settings—began in Guinea in 2014 and spread to neighboring Sierra Leone and Liberia, causing more than 11,000 deaths. Secondary outbreaks occurred in Nigeria, Senegal, Mali, and the United States, illustrating in a profound way that infectious diseases do not recognize borders. During 2018–2019, the second largest Ebola outbreak ever reported occurred in the DRC.
- Global health initiatives to address emerging and reemerging diseases include the Global Alliance for Vaccines and Immunization (Gavi); the Global Fund to Fight AIDS, Tuberculosis and Malaria; the World Health Organization International Health Regulations of 2005; the Global Health Program of the Bill and Melinda Gates Foundation; the US President's Emergency Plan for AIDS Relief (PEPFAR); and the World Health Organization Global Action Plan on Antimicrobial Resistance (Table 14.5).

The rapid pace of microbial evolution enables pathogens to overcome human defenses, exploit human behaviors, and elude control efforts across a highly connected world.[1,2] Though the incidence of endemic diseases that are major killers of children and young adults has declined significantly since the 1990s—with fewer deaths due to respiratory tract and diarrheal diseases,[3,4] vaccine-preventable diseases,[5] and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), tuberculosis, and malaria[6–10]—new threats continue to emerge and spread. The dangers and uncertainties of emerging pathogens are epitomized by the 2014–16 epidemic of Ebola in West Africa and the 2015–17 epidemic of Zika in the Americas, which presented major challenges to global health,[11–14] as had severe acute respiratory syndrome (SARS) in 2003 and HIV/AIDS in the 1980s and 1990s.

As discussed in this chapter, emerging and reemerging infectious diseases of current concern include infections with drug-resistant pathogens, acute respiratory tract infections (see also Chapter 67), enteric diseases, vector-borne diseases (see also Chapter 296 [ticks]), and viral hemorrhagic fevers (see Chapter 164). Many emerging threats are zoonotic in origin, having "jumped" from animals to humans while continuing to be maintained in animal reservoirs.[2,15,16] Zoonotic pathogens (see also Chapter 317) are responsible for severe

human infections with avian influenza A(H7N9) virus in China and for recurring epidemics of Middle East respiratory syndrome (MERS) in Saudi Arabia; plague in Madagascar; and monkeypox in the Central African Republic, the Democratic Republic of the Congo, and Nigeria (Table 14.1). The recent Zika outbreak in the Americas—caused by a mosquito-borne virus—has focused attention on pathogens that can cross the placenta during pregnancy and cause birth defects[17,18] or congenital illness,[19–21] or both. Perinatal transmission issues of current concern in the United States (in addition to Zika virus) involve hepatitis C virus[20] and *Treponema pallidum* (the spirochetes that cause syphilis[21]).

Although vaccines have eliminated rubella in the Americas and eradicated smallpox worldwide, vaccine-preventable diseases such as measles can reemerge even in the setting of high-functioning immunization programs. In many parts of the world, weak primary health care systems and limited access to the most vulnerable populations result in many children being unimmunized. Most resurgences of vaccine-preventable diseases, such as the recurrent yellow fever outbreaks in South America and Africa, stem from low immunization coverage, but both reduced vaccine acceptance in some affluent countries and waning vaccine immunity in the setting of high vaccination coverage have emerged as threats to protecting communities from vaccine-preventable diseases.

INFECTIOUS CAUSES OF CHRONIC DISEASES

Several infectious organisms—including viruses, bacteria, parasites, and prions—have been linked to the development of chronic diseases such as cancer, arthritis, cirrhosis, gastric or peptic ulcers, and neurologic disorders[22] (Table 14.2). These diseases may be due to inflammation

TABLE 14.1 Examples of the Outbreaks, Pathogen Discoveries, and Other Notable Infectious Disease Events in a Recent Decade (2008–2017)

2008	Ebola-like outbreak in Zambia due to a previously unknown virus: Lujo hemorrhagic fever virus, an arenavirus related to Lassa fever virus, which is associated with rodents.
2008	Isolation in Australia of a new virus (transplant-associated arenavirus related to lymphocytic choriomeningitis virus) after three recipients of liver or kidney transplants from a single donor developed febrile illness and died.
2008	Increasing outbreaks and international spread of carbapenem-resistant Enterobacteriaceae, and first detection of New Delhi metallo-β-lactamase 1, a genetic element that can confer such resistance.
2009	Outbreak of severe fever with thrombocytopenia syndrome (SFTS) in China caused by a novel phlebovirus (the SFTS virus).
2009	Discovery of two novel tick-borne pathogens in the United States: the Heartland phlebovirus in Missouri and a pathogenic *Ehrlichia* species in Wisconsin and Minnesota.
2009–2010	Influenza pandemic caused by a new influenza virus strain, influenza A(H1N1)pdm09.
2009–2010	Locally transmitted dengue in Florida, representing the first cases acquired in the continental United States outside the Texas-Mexico border region since 1945.
2009 on	Emergence and global spread of *Candida auris,* the cause of multidrug-resistant health care–associated infections.
2010	Outbreak of cholera in Haiti.
2010 on	Rise in US cases of infection with hepatitis C virus (HCV)—including cases of congenital HCV—associated with the US opioid epidemic.
2011	Outbreak of Shiga toxin–producing *Escherichia coli* O104:H4 (STEC O104:H4) infections in Germany.
2011	Identification of a strain of gonorrhea (H041) resistant to all available antibiotics.
2011–2012	Influenza cases in the United States traced to a variant swine-origin influenza A(H3N2) virus carried by pigs exhibited at agricultural fairs.
2011–2014	Outbreaks of valley fever (coccidioidomycosis) in California.
2012	Ebola hemorrhagic fever outbreaks in Uganda and the Democratic Republic of the Congo and outbreak of Marburg hemorrhagic fever in Uganda.
2012	Outbreak of hantavirus pulmonary syndrome in Yosemite National Park, California.
2012	A novel rhabdovirus (Bas-Congo virus) identified by whole-genome sequencing as the cause of an outbreak of acute hemorrhagic fever in the Democratic Republic of the Congo.
2012–2013	Multistate outbreak of fungal meningitis in the United States caused by tainted steroid injections.
2012 on	Outbreaks of severe respiratory disease in Middle East countries and South Korea caused by a novel coronavirus (MERS-CoV) that belongs to the same viral species as the SARS coronavirus.
2013 on	Human infections with avian influenza A(H7N9) virus—an influenza virus strain with pandemic potential—reported in China.
2013 on	First cases of chikungunya reported in the Americas—on the Caribbean island of Saint Martin—with subsequent spread through North, Central, and South America.
2014	Marburg hemorrhagic fever outbreak in Uganda.
2014	Bourbon virus, the first human thogotovirus discovered in the Western Hemisphere, isolated from a resident of Bourbon County, Kansas, who died of the infection. Thogotoviruses are enveloped RNA viruses transmitted by ticks.
2014–2015	Outbreak of legionnaires' disease associated with the water system crisis in Flint, Michigan.
2014–2016	Outbreak of Ebola hemorrhagic fever in rural Guinea that spread to the capital cities of Guinea, Liberia, and Sierra Leone, causing a major global health crisis.
2014–2017	Outbreaks of bubonic and pneumonic plague in Madagascar.
2014 on	India was certified as polio-free in 2014. In the same year, however, WHO declared the international spread of poliovirus in other nations a Public Health Emergency of International Concern (PHEIC) under the International Health Regulations and issued Temporary Recommendations to prevent further spread.
2015	Outbreak of meningococcal meningitis in Niger caused by *Neisseria meningitides* serogroup C, the first detection of a large outbreak caused by this serogroup.
2015	Multistate measles outbreak in the United States likely started from an infected traveler who visited Disneyland.

Continued

TABLE 14.1 Examples of the Outbreaks, Pathogen Discoveries, and Other Notable Infectious Disease Events in a Recent Decade (2008–2017)—cont'd

2015	Community outbreak of HIV infection in a small town in Indiana, associated with injection drug use and the US opioid epidemic.
2015–2016	Lassa hemorrhagic fever outbreak in Nigeria.
2015–2016	Outbreak of yellow fever in Angola and the Democratic Republic of the Congo.
2015–2017	Outbreak of Zika virus disease in the Americas associated with birth defects.
2015–2017	Recurring outbreaks of monkeypox in the Central African Republic, the Republic of the Congo, and Nigeria.
2015–2017	Outbreaks of yellow fever in Brazil, including sporadic cases outside São Paulo and Rio de Janeiro.
2015 on	Emergence and global spread of the mobilized colistin resistance (*mcr-1*) gene, which confers resistance to colistin, a last-resort antibiotic for the treatment of gram-negative bacterial infections.
2016	Multistate outbreak of salmonellosis in the United States associated with exposure to pet turtles.
2016	Multistate outbreak of infections caused by *Elizabethkingia anophelis*—a bacteria commonly found in soil, river water, and reservoirs—in the United States.
2016–2017	Outbreak of cholera in Yemen, exacerbated by famine and war.
2017	Outbreaks of Marburg hemorrhagic fever in Uganda and Kenya and an outbreak of Ebola hemorrhagic fever in the Democratic Republic of the Congo.
2017	Outbreak of leptospirosis in Puerto Rico in the aftermath of Hurricane Maria.
2017	Multistate US outbreaks of Seoul virus infection associated with exposure to pet rats and *Campylobacter* infection associated with exposure to pet store puppies.
2017	Brucella RB51 infections in Texas and New Jersey, associated with drinking raw milk.
2017	Multistate foodborne outbreaks in the United States caused by contamination of papayas with *Salmonella;* of soft raw cheese with *Listeria;* and of soy nut butter with Shiga toxin–producing *Escherichia coli* O157:H7.

TABLE 14.2 Examples of Infectious Agents That Cause or Contribute to Chronic Diseases

PATHOGEN	CHRONIC CONDITION CAUSED/ EXACERBATED BY THIS PATHOGEN
Bacteria	
Borrelia burgdorferi	Antibiotic-refractory arthritis
Helicobacter pylori	Peptic ulcer disease Gastric carcinoma Mucosa-associated lymphoid tissue lymphoma
Viruses	
Epstein-Barr virus	Nasopharyngeal carcinoma (undifferentiated) Burkitt lymphoma Posttransplant lymphoproliferative disease B-cell lymphoma
Hepatitis B and C viruses	Cirrhosis Hepatocellular carcinoma
Human herpesvirus 8 (HHV-8)	Kaposi sarcoma
Human immunodeficiency virus	Lymphoma HIV-related neurocognitive disorders and peripheral neuropathy
Human papillomavirus	Cervical carcinoma Anogenital and oropharyngeal cancers
Human T-cell lymphotropic virus type 1 (HTLV-1)	Adult T-cell leukemia/lymphoma
Parasites	
Liver flukes	Cholangiocarcinoma
Schistosoma haematobium	Bladder cancer
Prions	
Variant CJD prion	Degenerative brain disorder

CJD, Creutzfeldt-Jakob disease; *HIV,* human immunodeficiency virus.

caused by chronic infection (e.g., ulcers caused by *Helicobacter pylori* infection[23]) or triggered by an immune response to infection (e.g., Guillain-Barré syndrome following *Campylobacter jejuni* infection[24]). It has also been suggested that neonatal exposure to infection might predispose a person to chronic illness in later life.[25] Some infectious organisms also have adverse impacts on reproductive health. Infertility is associated with sexually transmitted diseases that cause pelvic inflammatory disease[26] and with neglected tropical diseases, which can also cause maternal death and poor birth outcomes.[27]

Confirmation of causative relationships between viruses and specific chronic diseases led to development of the first two vaccines against cancer: hepatitis B vaccine, which prevents cancers of the liver,[28] and human papillomavirus vaccine, which prevents cervical cancer, as highlighted in the National Cancer Institute's *Cancer Moonshot Blue Ribbon Panel Report*.[28a] Researchers are evaluating many other potential linkages between chronic conditions and infectious pathogens (for examples, see references 29–36), as well as genetic factors that influence infectious disease susceptibility and disease progression (Table 14.3).

FACTORS THAT FAVOR DISEASE SPREAD

Increased air travel has facilitated the international spread of new threats and the resurgence of old ones, including measles and drug-resistant forms of tuberculosis. Other important factors include increased urbanization, globalization of the food supply, environmental changes that enlarge the habitats of disease vectors, population movements due to war or famine, and changes in human demographics and behaviors. In the United States, the complexity of interactions between microbes and humans is illustrated by a community outbreak of HIV infection[37] and an increased incidence of hepatitis C[38] associated with injection drug use and the US opioid epidemic. Disease spread can also be accelerated by breakdowns in public health infrastructures caused by natural disasters[39] or inadequate maintenance of water and sewage systems.[40]

The impact of urbanization and travel on disease spread is illustrated by the Ebola outbreak in West Africa. Previously reported Ebola outbreaks occurred in remote areas and were limited in size and geographic scope.[40a] During the West Africa outbreak, however, the Ebola virus spread from a rural village in Guinea to the densely populated capital cities of Guinea, Liberia, and Sierra Leone. Subsequent travel by ill persons led to cases of Ebola virus disease in Lagos, Nigeria (a megacity of 16 million people)[41]; Bamako, Mali[42]; Dakar, Senegal[43]; and Dallas, Texas.[44] In addition, a small number of infected health care workers from Spain, Italy, the United Kingdom, and the United States were medically evacuated from West Africa to their home countries, and a hospital nurse in Madrid contracted Ebola virus disease from an infected missionary

TABLE 14.3 Examples of Human Genetic Variants That Influence Disease Susceptibility or Severity

GENETIC VARIANT	EFFECT ON DISEASE
Globin gene variant alleles (e.g., sickle globin and α- and β-thalassemias)	Reduced susceptibility to malaria
Duffy null blood group	Complete resistance to *Plasmodium vivax* malaria
Blood group O	Reduced severity of *Plasmodium falciparum* malaria Increased susceptibility to cholera
Chemokine receptor CCR5 delta-32 allele	Reduced susceptibility to HIV-1 infection Reduced progression of HIV-1 infection Increased severity of West Nile virus infection
HLA B*5701 allele	Reduced progression of HIV-1 infection Hypersensitivity to abacavir
Interferon lambda-4 variant alleles	Reduced clearance of hepatitis C virus
Toll-like receptor 4 variant alleles	Increased susceptibility to gram-negative bacterial infections and septic shock
SLC11A1 (NRAMP1) variant alleles	Increased progression of tuberculosis
Interleukin 10 variant alleles	Increased susceptibility to leprosy
Apolipoprotein L-1 variant alleles	Reduced susceptibility to African sleeping sickness caused by *Trypanosoma brucei rhodesiense*

[a]A discussion of "how the history of past pandemics is written in our genomes" may be found at Pittman KJ, Glover LC, Wang L, Ko DC. The legacy of past pandemics: common human mutations that protect against infectious disease. *PLoS Pathog.* 2016;12:e1005680.
HIV, Human immunodeficiency virus.

evacuated from Sierra Leone.[45] Intensive public health efforts contained disease spread in each country, and additional cross-border spread was prevented due to enhanced disease surveillance by neighboring countries[46] and at airports.[47] The West African Ebola outbreak highlighted the need for sustained vigilance and international collaboration to ensure early detection and response to new threats.

ANTIMICROBIAL RESISTANCE

While the introduction of antibiotics in the early to mid-20th century remains one of the most significant health achievements to date, antimicrobial resistance is currently regarded as one of the greatest threats to global health and well-being.[48–54] The widespread availability and indiscriminate use of antimicrobial agents among humans and animals, along with a globalized society in which emerging resistant strains can quickly spread worldwide, has created an environment of increased antibiotic exposure that has intensified selective pressure, boosting the inherent capacity of microbes to develop and acquire resistance genes. As a result, treatment of drug-resistant bacterial infections often requires the use of less effective, more expensive, and often more toxic drugs. Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die as a direct result of these infections.[55] Moreover, the problem of antimicrobial resistance—which extends beyond bacteria and includes viral (e.g., HIV, influenza), fungal (e.g., *Candida, Aspergillus*), and parasitic (e.g., malaria) infections—threatens our economic future.[54] A 2017 analysis by the World Bank states that, by 2050, drug-resistant infections could cause global economic damage on par with the 2008 financial crisis.[54]

The evolving nature of microbes defines antimicrobial resistance. Resistance genes have been found in humans and animals in areas with limited to no antibiotic exposure, in bacterial DNA frozen in the Arctic for 30,000 years, and in bacteria from underground caves isolated for 4 million years, some of which were resistant to synthetic antibiotics developed in the 20th century.[56–58] After introduction of each new class of antibiotics has come the emergence of resistant strains—due to natural selection of bacteria with mutations that allow them to survive and live on to reproduce—followed by their rapid global spread, facilitated by international travel and trade. As early as the 1930s, sulfonamide-resistant strains of *Streptococcus pyogenes* were noted in military hospitals, and numerous bacterial strains, including *Staphylococcus aureus,* were found to be resistant to penicillin shortly after its introduction and use in the 1940s.[59,60] In 1961, just 2 years after the introduction of methicillin, methicillin-resistant *S. aureus* (MRSA) strains emerged in British hospitals,[61,62] and strains exhibiting intermediate- or high-level resistance to vancomycin have been identified since 1996.[63–66] Today, MRSA is associated with significant morbidity and mortality, spurring research on alternative treatment strategies.[67,68]

In the United States the most urgent antimicrobial resistance threats, as identified by the Centers for Disease Control and Prevention (CDC) in a 2013 report,[55] include infections with *Clostridioides difficile* (formerly *Clostridium difficile*), drug-resistant *Neisseria gonorrhoeae,* and strains of gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae (CRE), which have become resistant to all available antibiotics. Severe, life-threatening *C. difficile* infections are usually associated with recent antibiotic use and often occur among currently or recently hospitalized patients (see "Enteric Diseases" later). Strains of *N. gonorrhoeae* with decreased or no susceptibility to third-generation cephalosporins have threatened a return to potentially untreatable gonorrhea and prompted changes in US treatment guidelines that aim to prolong the effectiveness of these drugs.[69–71] CRE, which are primarily transmitted in health care settings, can cause severe disease and high mortality rates.[72,73] Patients who require prolonged hospitalization and critically ill patients exposed to invasive medical devices (e.g., ventilators and central venous catheters) are at particular risk.

Resistance to carbapenem antibiotics is mediated through the production of carbapenemases (enzymes that inactivate carbapenems), including *Klebsiella pneumoniae* carbapenemase (KPC), first identified in 2001 in the United States,[74] and New Delhi metallo-β-lactamase (NDM), first identified in 2008 in India.[75] The genes encoding KPC and NDM (carried on plasmids) have spread from *K. pneumoniae* to other gram-negative bacteria, including *Escherichia coli, Klebsiella* species, and *Enterobacter* species. More recently, emergence of the mobilized colistin resistance gene *mcr-1* and its *mcr-2* and *mcr-3* variants—genes that cause resistance to colistin, a last-resort antibiotic used to treat resistant infections—has been reported.[76–78] The *mcr* gene is particularly worrisome because it is also found on plasmids and so can transfer to other bacteria, including CRE.[78a]

Other serious threats (both domestically and globally) include multidrug-resistant tuberculosis; MRSA infections; drug-resistant forms of enteric pathogens such as *Salmonella* and *Campylobacter* (see "Enteric Diseases" later); and a newly emerged multidrug-resistant fungus, *Candida auris,* that causes health care–associated infections (HAIs) and is difficult to identify with standard laboratory methods.[79] Over the past 2 decades, highly drug-resistant strains of *Mycobacterium tuberculosis* have emerged worldwide,[80–83] including multidrug-resistant tuberculosis (defined as tuberculosis that is resistant to isoniazid and rifampin, the two most effective first-line tuberculosis drugs) and extensively drug-resistant tuberculosis (defined as multidrug-resistant tuberculosis that is also resistant to any fluoroquinolone drug and at least one of three second-line injectable drugs: amikacin, kanamycin, or capreomycin). An estimated one-third of the world's population is infected with *M. tuberculosis,* and each year approximately 9 million people develop tubercular disease and there are 2 million tuberculosis-related deaths.[83a,83b] These infections present serious public health challenges and raise the specter of virtually untreatable endemic and epidemic tuberculosis.[83c,83d]

The increasing use of antibiotics in both humans and animals, the spread of HAIs between health care settings and into communities, and a virtual standstill in antibiotic development have escalated bacterial antibiotic resistance to crisis levels and garnered the attention of public health, clinical, and policy leaders worldwide.[54] In 2015, the World Health Assembly endorsed a global action plan to address antibiotic resistance,[83e] and in September 2016 global leaders at the United Nations General Assembly in New York committed to a common effort to fight antimicrobial resistance.[83f] This was only the fourth time in the history of the United Nations that a health topic was discussed by the General

Assembly, the earlier ones being HIV, noncommunicable diseases, and the Ebola virus.

In the United States, intensified efforts to address antimicrobial resistance are described in the *National Action Plan for Combating Antibiotic-Resistant Bacteria*, issued in 2015 by the CDC.[83g] Implementation of the National Action Plan has included establishment by the CDC and the US Food and Drug Administration (FDA) of the Antimicrobial Resistance Isolate Bank, which offers panels of resistant bacteria to researchers who develop diagnostic devices and new antibiotic agents,[83h] and the Antibiotic Resistance Laboratory Network, whose seven regional laboratories help states and localities detect and track existing and emerging types of antibiotic resistance.[83i] Progress has also been made in reducing several types of HAIs—many of which are drug resistant—through improved detection, focused infection prevention and control, better antimicrobial stewardship, and coordination of prevention efforts by health care facilities located in the same city or region.[83j,83k] In the United States, HAIs are reported and tracked by the Centers for Medicare and Medicaid Services and through reporting mandates in many states, using the CDC's National Healthcare Safety Network. This secure, Internet-based surveillance system collects data from more than 12,000 facilities in all 50 states on hospital-acquired infections and related issues, including the incidence or prevalence of multidrug-resistant organisms, health care personnel safety and vaccination, and the occurrence of transfusion-related adverse events.

Reviews of antimicrobial stewardship programs in both large and small hospitals have documented their effectiveness and cost-effectiveness.[84–89] Because poor antibiotic prescribing harms patients—and because antibiotic prescribing practices vary widely and errors are common[90]—education of patients and providers on the importance of and health benefits from responsible use of antibiotics remains paramount.[90a] Critical to the improvements in HAIs and to better addressing antibiotic resistance is innovative research into better approaches and tools to detect, control, and prevent these infections.

Use of antibiotics as growth promoters in healthy food-producing animals has also been a significant concern. Globally, the amount of antibiotics used in food-producing animals surpasses the amount of antibiotics used to treat human disease.[55,91] The human health implications of this use are increasingly being recognized.[92,93] Linkages have been found between livestock-associated MRSA and multidrug-resistant *S. aureus* carriage among industry workers and between *E. coli* isolates from retail meat (chicken) and extraintestinal pathogenic *E. coli* urinary tract infections in humans.[94,95] Several nations have taken steps to address the nontherapeutic use of antibiotics in food-producing animals, beginning with Sweden in 1986 and extending to the European Union in 2006, with bans on agricultural growth promoters.[96] The FDA strategy to promote the judicious use of antibiotics important in treating humans involves working with industry to end use of antibiotics to promote growth in food-producing animals.[97] The FDA guidance recommends that medically important antibiotics be used only to address animal health needs and only under veterinary oversight.[97] Another area of concern that is being increasingly recognized and that needs better understanding is the contribution of antimicrobial resistance in the environment related to contamination from human and animal sewage, antimicrobial manufacturing, and use of antimicrobials as pesticides.[98–100]

For antimicrobial drug development, the outlook remains grim, spurring innovative investments to accelerate research and stimulate the pipeline. Despite the continued rise in antibiotic-resistant pathogens, the development of new antibiotics has dramatically slowed.[101,102] Particularly troubling, there have been no new classes of drugs to treat gram-negative bacteria in 4 decades.[85] With the discovery of new antimicrobial agents more scientifically challenging compared with earlier years, unfavorable profit margins from short-course therapies, and other disincentives, many pharmaceutical companies have abandoned the market. Steps taken to counteract these effects are imperative.[103,104] Efforts include the Food and Drug Administration Safety and Innovation Act,[105] passed into law in 2012, which seeks to increase the development of and patient access to new antimicrobial agents, and the CARB-X initiative, a partnership between the US Department of Health and Human Services' National Institutes of Health and Biomedical Advanced Research and Development Authority (BARDA), the Wellcome Trust, and Boston University, which provides competitive funds to foster research for the development of drugs to address the most urgent resistance threats.[105a]

More recent efforts have focused on the use of new technologies and host targets to better understand and reduce antimicrobial resistance.[106–108] Whole-genome sequencing (WGS) techniques can track bacterial transmission from person to person and better define and help understand transmission linkages in outbreaks of resistant HAIs.[109–113] As these technologies continue to advance, rapid, point-of-care diagnostic tests could be used to quickly identify resistant infections and target treatment. Efforts to prevent and control infectious diseases by altering the host-microbe interaction as opposed to targeting microbes (e.g., through use of fecal transplants to treat *C. difficile* or through other approaches to sustain a healthy gut microbiome) also offer tremendous promise for reducing antimicrobial resistance.

Vaccines represent the optimal solution to addressing infections and antimicrobial resistance, and research and development of new vaccines is an urgent need. Effective immunization programs have stopped emergence of resistant strains and precluded the need for new antimicrobial agents for multiple infectious diseases, allowing focus to be shifted to diseases for which drug resistance remains a major global health threat. A recent example of this impact includes the pneumococcal conjugate vaccine. Over the past several years, use of pneumococcal conjugate vaccines has significantly reduced the rate of disease.[114–119] Researchers continue to monitor evolving serotypes to detect the emergence of any resistant strains that are not targeted by the vaccine.[114,118,120]

ACUTE RESPIRATORY TRACT INFECTIONS

Infections involving the respiratory tract represent one of the most dynamic areas for emerging and reemerging diseases, producing dramatic examples such as the first recognized outbreaks of legionellosis in the 1970s and hantavirus pulmonary syndrome in the 1990s. Legionellosis has been on the rise in the United States since 2000, with 6000 cases reported in 2015; while some of this increase is attributable to increased use of diagnostic testing and better reporting, this also likely reflects a true increase in the frequency of disease. Examples in the 21st century include newly recognized pathogens such as human metapneumovirus (hMPV, first identified in 2001[121]), the coronavirus associated with SARS (identified in 2003[122]) human bocavirus (identified in 2005[123]), pandemic influenza A(H1N1) (identified in 2009[124]), and Middle Eastern novel coronavirus associated with severe respiratory illness (identified in 2012).[125] Other newly recognized respiratory viruses include two additional coronaviruses (NL63 and HKU1), novel human polyomaviruses KI and WU, rhinovirus groups C, and parechoviruses, although in some instances the role of these agents as pathogens is still being clarified.[126] More virulent strains of known respiratory pathogens have also emerged. Examples of this phenomenon include human disease associated with avian influenza viruses, especially highly pathogenic avian influenza A(H5N1) and avian influenza A(H7N9),[127,128] and extensively drug-resistant tuberculosis[129] (see "Antimicrobial Resistance" earlier).

Acute respiratory tract infection constitutes a broad category of diseases that include infections of both the upper and lower respiratory tracts, such as acute pharyngitis, epiglottitis, bronchitis, pneumonia, and influenza. Although upper respiratory tract infections are capable of causing severe illness, virtually all (98%) respiratory disease–related deaths are a consequence of infection of the lower respiratory tract, especially pneumonia. In 2015, lower respiratory tract infections caused 2.74 million deaths, making them the leading infectious cause of death and the fifth-leading cause of death globally.[3]

Among children less than 5 years of age, at least 704,000 deaths were attributed to lower respiratory tract infections, representing 12.1% of deaths in this age group.[130] The poorest countries in sub-Saharan Africa and South Asia have the largest burden, with three of every four deaths from respiratory infections occurring in these two regions. Globally, 42% of all deaths from lower respiratory tract infections (mostly due to pneumonia) occur in children younger than 5 years of age. The four most common etiologies were *Streptococcus pneumoniae*, *Haemophilus influenzae,* respiratory syncytial virus, and influenza. Between 2005 and 2015, deaths due to lower respiratory tract infections decreased

36.9% worldwide in children under 5 years old, although incidence of infection has decreased at a slower rate. In many regions, the burden has increased in people older than age 65 and because of growing and aging populations, global mortality due to lower respiratory tract infections has remained relatively stable.[130] In the United States, influenza and pneumonia are the leading cause of infectious disease–related mortality and the eighth most frequent cause of death.[131]

Human Metapneumovirus

In 2001, hMPV was first reported as a cause of acute respiratory tract infections when the virus was isolated from specimens collected over a 20-year period from hospitalized children in the Netherlands with undiagnosed upper and lower respiratory tract illnesses.[121] Subsequent serologic and virologic analyses of banked specimens have demonstrated evidence of hMPV infection as far back as the 1950s, and genetic studies suggest the virus is considerably older.[121,132] Thus this virus may have been in circulation for some time before diagnostic tools were available to detect it. A paramyxovirus, hMPV is closely related to respiratory syncytial virus, another member of the Paramyxovirinae subfamily of Paramyxoviridae.[132,133] Genetic analyses have demonstrated two major hMPV types, designated groups A and B, with two subtypes (1 and 2) within each group; subtype variants have also been reported.[132]

hMPV causes illness similar to other major viral respiratory pathogens with a winter/spring predominance, and occurs globally in both high- and low-income countries, although incidence varies by year.[133] By 5 years of age virtually all children demonstrate evidence of prior hMPV infection.[134] Clinically significant illness and severe disease most commonly occur in children younger than 2 years of age, but hMPV-related illness can occur throughout childhood.[135,136] The virus (either alone or with copathogens) has been identified in 2% to 20% of children with acute respiratory tract infections, and in 3% to 7% of children hospitalized with acute respiratory tract infections or fever.[132,135] In contrast, hMPV is rarely (<1%) identified in children or adults who are not ill and when found is usually present in much lower titers than when detected during illness.[132,135,137] A multiyear, multicenter prospective study suggested that hMPV produces 20,000 hospitalizations, 263,000 emergency department visits, and 1 million outpatient visits annually among children younger than 5 years of age in the United States.[137] Because disease is also found in older children and in adults, these numbers represent an unknown proportion of the overall burden of illness due to hMPV.[133]

hMPV is one of the leading causes of bronchiolitis in infants. In children, most severe hMPV disease manifests as pneumonia or bronchiolitis and a substantial proportion of hospitalized children have underlying medical conditions, particularly asthma.[135–138] The virus has also been associated with childhood upper respiratory tract infections (up to 10% in some series) as well as acute otitis media.[133]

Most infections with hMPV in adults are mild, but severe disease can occur, especially in the elderly and in persons with chronic pulmonary disease or congestive heart failure.[139,140] This virus has been estimated to involve 3% to 7% of acute respiratory tract infections and 4.5% of acute respiratory tract infection hospitalizations in adults.[140] Outbreaks associated with hMPV have been reported in both children and adults, especially among long-term institutionalized elderly, with case-fatality rates as high as 33%.[140–142] As with respiratory syncytial virus, hMPV can produce severe and recurrent disease in immunocompromised hosts, including transplant recipients, those with hematologic malignancies, and those with HIV infection.[133,143] Ribavirin and immune globulin have been used to treat severe disease but have not been systematically assessed; efforts are underway to develop candidate vaccines against hMPV.[132,144]

Human Coronaviruses

Although coronaviruses were once considered to be pathogens most typically associated with the common cold, their public health significance has changed considerably in recent years.[145] The 2003 outbreak of SARS is widely considered to be a major emerging infectious disease event of the early 21st century, with profound public health, economic, sociologic, and political ramifications.[146] The expanded research and monitoring of coronaviruses that resulted from the SARS episode led to the recognition of two additional human coronaviruses (NL63 and HKU1) associated with upper respiratory tract infections.[145–148] In 2012, a novel coronavirus associated with severe and fatal pulmonary disease was identified among patients in the Middle East or patients linked to the Middle East.[125] A number of studies suggest that the human coronaviruses appear to be zoonotic in origin, with bats being especially important reservoirs.[149] Because bats harbor many coronaviruses, there is the potential for future pathogenic coronaviruses to emerge from this reservoir.

SARS was recognized in February 2003 when an explosive outbreak of adult respiratory distress syndrome was carried globally by more than a dozen individuals who were all guests at a Hong Kong hotel over a single weekend while a physician from adjacent Guangdong Province with fatal respiratory illness was also present.[150–152] Before traveling to Hong Kong, the source physician had been caring for patients with a similar illness, with 305 cases of undiagnosed respiratory diseases occurring in Guangdong Province since the previous November. A global consortium of laboratories rapidly identified the causative agent as SARS coronavirus (SARS-CoV),[122] a previously unrecognized betacoronavirus. The outbreak, which involved substantial transmission in health care settings, was contained within 4 months of recognition, primarily through the employment of standard public health measures such as droplet and contact precautions in hospitals, community isolation, and quarantine.[150–152] In the interim, a total of 8096 (confirmed and probable) cases of SARS were recorded in 29 countries on five continents, with 774 (9.6%) fatalities[153]; however, 98% of the cases occurred in just five locations, with only 8 cases in the United States. No cases of SARS have been recognized anywhere in the world since 2004.

A novel human coronavirus, initially referred to as HCoV-EMC for the Erasmus Medical Center in the Netherlands and now known as MERS coronavirus (MERS-CoV), was first identified in July 2012 in a 60-year-old man from Saudi Arabia with fatal lower respiratory tract infection.[125] The earliest recognized cases were retrospectively identified in 2 persons in Jordan who were part of an 11-person hospital cluster of respiratory illnesses in April 2012.[154] Between September 2012 and March 31, 2019, a total of 2399 laboratory-confirmed cases have been reported internationally, with at least 827 deaths (a case-fatality proportion of 34.5%[155]).

The median age of MERS-CoV patients is 52 and 66% are male; most index case patients have reported at least one chronic comorbid condition.[156] Cases have been reported from 27 countries, all with a direct or indirect link to locations in and around the Arabian peninsula; the majority of cases have been reported from Saudi Arabia (82%). Illness has been characterized by severe respiratory distress and pneumonia often requiring mechanical ventilation, and some cases have been accompanied by renal insufficiency.[157,158] Diffuse alveolar damage without evidence of extrapulmonary MERS-CoV antigen detection was reported in one well-characterized analysis of autopsy tissue specimens.[159] In May 2014, the CDC confirmed two cases of MERS in the United States, one in Indiana and the other in Florida. Both cases were health care providers who had lived and worked in Saudi Arabia, where they are believed to have been infected. Both had traveled to the United States, and both were hospitalized in the United States and later discharged after fully recovering.[160] No other patients in the United States have ever tested positive for MERS-CoV infection, while more than 1020 people have tested negative, as of December 2017.[154] Person-to-person transmission of MERS-CoV has been well documented, with at least 100 spatiotemporal clusters reported in household or health care settings; however, there is no clear evidence of sustained community transmission. The largest outbreak outside of the Arabia peninsula occurred in the Republic of Korea in 2015, with 186 cases and 36 deaths; 184 of the patients were infected nosocomially.[161] Since December 2016, 31% of 199 MERS-CoV cases reported to WHO were associated with transmission in a health care facility, including 40 cases among health care workers due to insufficient compliance with infection control measures and exacerbated by delayed identification of suspected MERS patients.[156] Asymptomatic infection due to MER-CoV has been documented but the role of asymptomatic or mildly symptomatic illness in transmission is unclear. Clinical trials of MERS-CoV candidate vaccines and therapeutics are in progress.[162]

A zoonotic origin of MERS-CoV was initially suggested by genetic analyses of this novel coronavirus showing that it is the first lineage C

betacoronavirus infecting humans and has a close relationship to betacoronaviruses HKU4 and HKU5 found in bats, including insectivorous bat species present in the Middle East.[163–165] A virus with 100% sequence homology to the MERS-CoV isolated from the 2012 Saudi Arabian index case was found in an Egyptian tomb bat (*Taphozous perforatus*) trapped in the vicinity of that patient's home.[166] Transmission has also been linked to dromedary camels.[167,168] Antibodies to MERS-CoV have been detected in archived samples from camels that date back to 1983, also implicating dromedary camels as a natural reservoir.[169] Moreover, a study of 348 primary human MERS cases reported that 55% had dromedary camel exposures (e.g., direct or close contact with respiratory droplets or saliva, potentially to unpasteurized camel milk).[169a] These findings suggest that bats and dromedary camels play an important role in the ecology of MERS-CoV and could be the reservoirs for this new coronavirus. The virus targets a unique receptor and appears capable of infecting multiple mammalian cell lines, suggesting the potential for a zoonotic intermediary, and serologic evidence of infection with MERS-CoV has been reported in mammalian species.[170–172]

Human Bocaviruses

Human bocavirus, a member of the Parvoviridae virus family, is a newly described cause of lower respiratory tract and gastrointestinal infections, primarily in children. This small, nonenveloped virus with a single-stranded DNA genome is one of only two known parvovirus pathogens, the other being parvovirus B19.[173] Human bocavirus was first detected in 2005 in Sweden when investigators used molecular virus screening methods by taking cell-free, filtered supernatants of stored respiratory specimens looking for virus-sized particles and then performing random polymerase chain reaction (PCR) amplification of the genetic material.[123] Among the sequences identified was a previously unrecognized parvovirus most closely related to animal viruses of the genus *Bocavirus*. Since the original identification of bocavirus DNA in respiratory specimens, three additional bocaviruses have been found in stool specimens; these agents are now referred to as human bocavirus (HBoV) 1 to 4. HBoV1 causes predominantly respiratory disease while HBoV2, HBoV3, and HBoV4 are associated with gastrointestinal illness.[174]

Human bocaviruses have been challenging to study because of the lack of in vitro or animal models. Although primary bocavirus infection is associated with respiratory illness, HBoV1 has also been detected in asymptomatic children, raising concerns about causality.[174] Moreover, as often as 83% of the time, bocavirus is present with other coinfecting respiratory pathogens.[173] However, in most studies HBoV1 has been found more commonly in children with acute respiratory disease than asymptomatic children; copy numbers of the virus are higher in monoinfections than coinfections and are usually low in asymptomatic children. High titers of HBoV in respiratory secretions have also been associated with clinically recognized illness. Children ages 6 to 24 months are most commonly infected; serologic studies show that almost all children have evidence of previous HBoV1 infection by 6 years of age.[174,175] Human bocavirus has been reported worldwide, although infection rates vary widely and illness is most common in winter. Infection can produce either upper or lower respiratory tract infection and in children is often accompanied by wheezing.[174,176] DNA has been detected in serum and urine of children with acute infections, suggesting systemic infections can occur.[174]

Emerging Influenza Viruses

Influenza viruses are one of the most challenging infectious diseases for clinicians, researchers, and public health and agricultural officials. The viruses are constantly changing, spread rapidly in populations, and can cause significant illness. Of the four main types of influenza viruses—A, B, C, and D—only influenza A viruses circulate in multiple animal and human reservoirs, sometimes spilling over to cause illness in other species and adapting and exchanging genes to generate new influenza viruses with potential to cause pandemics.

Influenza viruses live on the edge of chaos. The genome of influenza is relatively small and replication of these negative-sense, single-stranded RNA viruses is error prone, leading to numerous defective viruses.[177,178] Although the high error rate can be a disadvantage, it can also be an advantage, allowing a few viable escape mutants to acquire new successful adaptations for evading host immunity and continuing the spread of infection. Influenza viruses have a segmented genome—eight separate genes coding for one or more proteins necessary for constructing a functional influenza virus. Two of the eight genes provide the instructions for building two important surface glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA). Influenza virus nomenclature categorizes influenza A viruses into subtypes on the basis of the antigenic characteristics of these two surface proteins (e.g., H1N1, H7N9, H3N2, H5N1, etc.).

The HA and NA proteins are critical for virus infection and transmission. The HA protein binds to glycan receptors on respiratory and other cells, making it possible for the virus to enter the host cell and begin the process of replication. The glycan receptors on host cells are glycan chains that are tethered to the host cell at one end and have sialic acid residues at the free end; HA proteins attach to the sialic acid.[179] After replication, as the viruses are emerging on the host cell surface, the NA protein cleaves the receptors, allowing the newly formed viruses to be released. The HA protein is constantly under selective immune pressure, from either prior influenza infection or vaccination. Under this pressure, the virus must change enough to evade host antibodies that might prevent attachment of the HA to receptors on the host cells and, at the same time, not change too much and risk losing the ability to attach to host receptors. These ongoing, incremental virus adaptations are described as *antigenic drift*. This characteristic of influenza viruses allows them to find ways to evade host immunity, returning each year to infect the same population repeatedly.

Reservoirs of Influenza A

Influenza A viruses have three main reservoirs: birds, humans, and swine; however, transmission is also maintained in dogs, horses, bats, and other mammals.[180,181] The circulation of influenza A viruses in these reservoirs is essentially separate but, under certain conditions, infection can be transmitted from one species to another. Over the last 100 years, influenza A viruses circulating among humans have been limited to H1N1, H2N2, and H3N2 subtypes. The avian reservoir, mostly in migratory waterfowl, is the most diverse, supporting multiple subtypes of influenza A viruses in various combinations of HA and NA proteins (H1–H16, and N1–N9).[182] The flyways of many of these waterfowl allow influenza A viruses to travel great, intercontinental distances, introducing new viruses to naïve populations.

Domestic poultry, such as chickens, ducks, and geese, also maintain circulation of influenza A viruses. The impact of influenza on domestic poultry can be significant due to infection with highly pathogenic avian influenza (HPAI) A viruses.[183] HPAI A viruses are avian influenza A viruses of subtypes H5 or H7 (e.g., H5N1 or H7N7), which have HA proteins containing a multibasic cleavage site at the base of the protein. This protein signature is associated with increased replication of the virus and systemic spread with multiorgan failure, presenting as higher-severity illness in domestic poultry and sometimes in infected mammals. Poultry flocks infected with HPAI A viruses can manifest disease rapidly, often with 100% fatality of the flock. Because of the impact on trade and protein security, most governments require immediate reporting of HPAI outbreaks and mandate culling of all associated poultry to prevent any further spread.[184]

The swine reservoir of influenza A viruses maintains a more limited set of subtypes, generally H1 to H3 and N1 and N2. Compared with humans and birds, swine have a much greater ability to be infected with either bird or human influenza A viruses due to the presence of a more diverse set of receptors in the swine respiratory tract.[185] Influenza viruses attach to host cell receptors through binding of the HA protein to sialic acid residues. Avian influenza A viruses have a strong affinity for receptors with an α(2,3)-linked sialic acid, which are more abundant on bird respiratory and enteric epithelial cells. Human influenza A viruses prefer receptors with an α(2,6)-linked sialic acid, which are more abundant in the upper respiratory tract of humans. Swine, however, have both type of receptors, allowing infection with either bird or human influenza A viruses. Because of this, swine have been called the "mixing vessel" for influenza A viruses.[185] Avian influenza A viruses, infecting a pig, have the opportunity to adapt to binding the more human-like α(2,6) receptors. More importantly, pigs can be coinfected with avian,

swine, and human influenza A viruses, allowing the viruses to exchange genes and form new hybrid influenza viruses referred to as *reassortants*. This significant adaptive change in influenza A viruses when a human is infected is referred to as *antigenic shift*, and represents a fitness advantage that may allow the virus to transmit efficiently in humans, potentially emerging to cause a global pandemic.

Influenza A and Pandemics

In the last 100 years, there have been four antigenic shift events leading to reassortant influenza A viruses that caused pandemics in humans.[186] Each of these viruses had genes of either avian or swine origin, or both. The most devastating of these pandemics was the 1918 H1N1 "Spanish" pandemic, which caused at least 50 million deaths globally. The pandemic emerged during World War I and impacted young adults disproportionately, ultimately killing more soldiers than died in combat.[187] Subsequent pandemics in 1957 ("Asian" H2N2 pandemic) and 1968 ("Hong Kong" H3N2 pandemic) had less severe impacts, each causing approximately 1 million deaths globally.[186] The fourth pandemic in the last 100 years emerged in 2009 and was caused by an H1N1 influenza A virus with HA genes derived from the 1918 H1N1 pandemic virus.[188] Sometime in the 1930s, the H1N1 virus circulating in humans entered the swine population and underwent multiple gene reassortments, including incorporation of avian, human, and other swine influenza A virus genes. Most likely, this virus moved back and forth between humans and swine, becoming increasingly adapted to humans[189,190] and eventually achieved sustained and efficient human-to-human transmission, thus igniting the pandemic in 2009.[188]

It is very difficult to know when and where the next influenza pandemic virus will emerge; however, it is certain that one will occur. Early detection of these viruses is a critical component of pandemic preparedness and response. Addressing this need, global laboratory surveillance for influenza viruses circulating in humans is in place through the WHO's Global Influenza Surveillance and Response System (GISRS).[191] One hundred and forty-four National Influenza Center (NIC) laboratories in GISRS have access to standardized molecular testing reagents provided by the CDC to test respiratory specimens year-round. Influenza-positive specimens are sent to one of six WHO Collaborating Centers (Atlanta, London, Beijing, Tokyo, Melbourne, and Memphis) responsible for characterizing influenza viruses and selecting vaccine viruses. NICs can detect known circulating human influenza viruses, but are also able to detect "novel" influenza A virus infections (i.e., infections in humans caused by influenza A viruses circulating in birds, swine, or other animals). Since 2005, human influenza A virus infections caused by a new subtype is a condition for which WHO requires notification under the International Health Regulations (IHR)[192] (see "Conclusion: Controlling the Threats" later).

Novel Influenza A Viruses

The number of reported novel influenza A virus infections has been increasing significantly since the first reported case in 1959 (Fig. 14.1).[180,193,194] Increased surveillance and improved diagnostic tests have clearly added to the increase; however, other factors are contributing. The expanding number of pig and poultry operations, including live animal markets, that are located in or near densely populated areas provides new opportunities for human exposure to avian influenza A viruses. In addition, international air travel now makes it possible to travel to almost any place on the globe within the incubation period of influenza virus infection. Fig. 14.2 shows the considerable increase in the populations of pigs, poultry, people, and passengers since 1960. This convergence of humans, animals, and transportation may be an important reason for the significant rise in human cases due to two novel influenza A viruses of recent concern: H5N1 and H7N9.

Influenza A(H5N1)

In May 1997, a young child in Hong Kong developed acute febrile respiratory illness and died of multiorgan failure. HPAI A(H5N1) virus was isolated from a tracheal aspirate specimen.[195] This was the first known fatal case of avian influenza A virus infection. Overall, 18 virologically confirmed A(H5N1) virus infections with 6 deaths were reported in Hong Kong during 1997.[196] Seroepidemiologic studies suggested that poultry-to-human A(H5N1) virus transmission occurred among exposed poultry workers,[197] and limited human-to-human transmission may have occurred in a household[198] and through nosocomial transmission.[199] Temporary closure of live poultry markets, depopulation of 1.5 million chickens, and stopping importation of live poultry from mainland China ended the outbreak.[196] In early 2003, severe and fatal A(H5N1) virus infections were identified in family members from Hong Kong who visited southern China.[200] This was the beginning of the reemergence of human infections associated with the spread of HPAI A(H5N1) viruses among poultry to more than 60 countries. Each year, sporadic human infections have been reported in some countries, typically during colder temperature months. Although human infections have generally declined since 2006, a surge in A(H5N1) virus infections occurred in Egypt during 2014 and 2015.[201] The clinical spectrum ranges from asymptomatic infection to fulminant pneumonia, acute respiratory distress syndrome (ARDS), and multiorgan failure.[128] Close or direct exposure to sick or dead poultry or visiting a live poultry market are the main risk factors for A(H5N1) virus infection,[202,203] but limited, nonsustained human-to-human transmission has been reported among blood-related family members in multiple countries.[204,205] Prevention and control measures in poultry include surveillance for outbreaks, depopulation, quarantine, and use of H5 vaccines, but HPAI A(H5N1) viruses continue to circulate and evolve among poultry in some countries. From November 2003 to March 2018, 860 human infections with HPAI A(H5N1) virus, with greater than 50% mortality, in 16 countries were reported to WHO.[206]

Influenza A(H5N6)

Human infections with HPAI A(H5N6) virus resulting in severe pneumonia, respiratory failure, and death were first reported in China in 2014.[207,208] Although one case of mild clinical illness with A(H5N6) virus infection was reported, almost all hospitalized patients have required

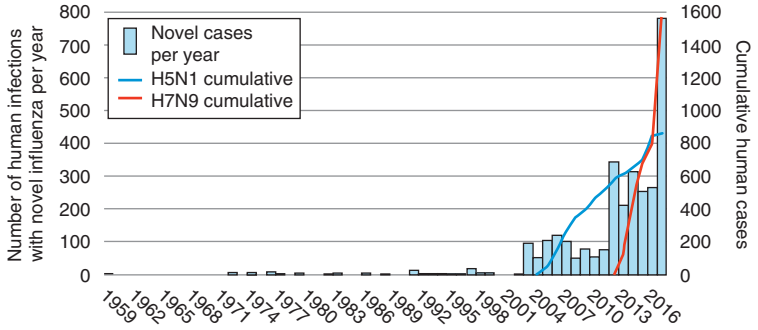

FIG. 14.1 Increasing number of human cases of novel influenza A virus infection, 1959 to 2017. Cumulative cases of H5N1 and H7N9, as reported by the World Health Organization. Graph includes avian H4, H5, H6, H7, H9, and H10; and swine H1 and H3 (not pH1N1). *(Data from Freidl GS, Meijer A, de Bruin E, et al. Influenza at the animal-human interface: a review of the literature for virological evidence of human infection with swine or avian influenza viruses other than A(H5N1).* Euro Surveill. *2014;19:pii-20793.)*

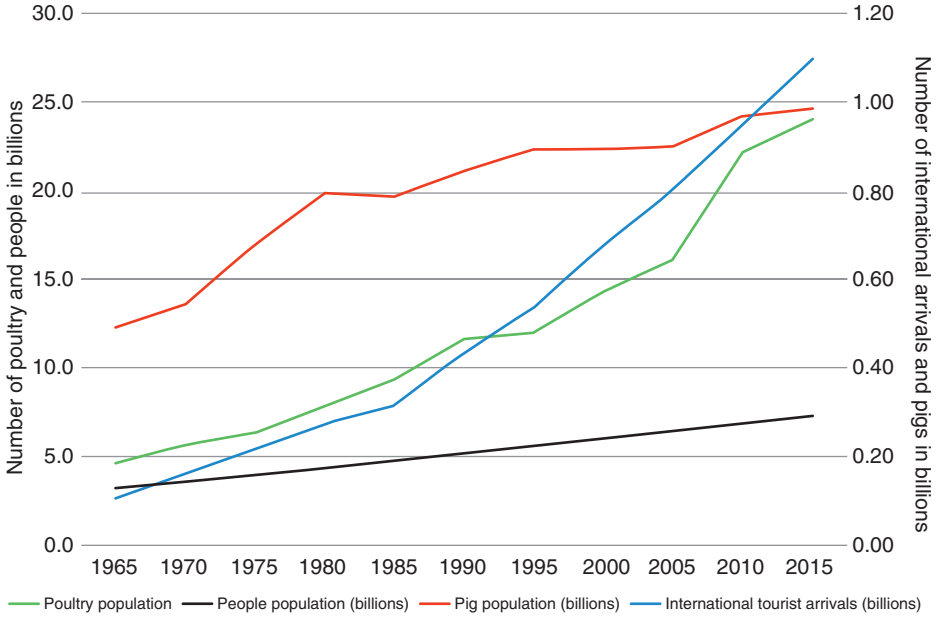

FIG. 14.2 Poultry, pigs, people, and travel trends. *(Data from Areppim AG. International tourist arrivals.* http://stats.areppim.com/stats/stats_ita.htm*; United Nations Department of Economic and Social Affairs. World population prospects: the 2017 revision.* https://www.un.org/development/desa/publications/world-population-prospects-the-2017-revision.html*; and United Nations Food and Agriculture Organization. Data, agriculture.* http://www.fao.org/faostat/en/#data.*)*

invasive mechanical ventilation, with high mortality.[209] As of November 2018, 22 sporadic human cases of A(H5N6) virus infection with high mortality were reported in China.[210] HPAI A(H5N6) viruses that have infected humans are reassortant viruses that are related genetically to HPAI A(H5N1) viruses circulating among poultry in China. HPAI A(H5N6) virus caused severe pneumonia in experimentally infected ferrets, but did not transmit via droplets.[211] HPAI A(H5N6) viruses, including genetically related as well as unrelated reassortant viruses, have been identified in wild birds and in poultry in multiple countries and regions of the world.[212–216] Other related HPAI viruses of H5 subtypes, such as H5N2, H5N5, and H5N8 (often referred to as "H5NX") viruses, have emerged through genetic reassortment and have been detected in wild birds or have caused poultry outbreaks in North America, Europe, and Asia.[217–219] As of April 2019, no human infections with HPAI A(H5) viruses other than H5N1 and H5N6 viruses have been reported.

Influenza A(H7N9)

In the early spring of 2013, the first three human infections with A(H7N9) virus were identified in eastern China.[127] All three infected persons died; this was the first time that a low-pathogenicity avian influenza (LPAI) A virus had caused fatal outcomes. This Asian-lineage A(H7N9) virus was a reassortant virus with the H7 and N9 genes derived from other avian influenza A viruses and six genes derived from A(H9N2) viruses circulating among poultry. During the spring of 2013, most A(H7N9) virus infections were sporadic and occurred in older men with a recent history of exposure to poultry in urban areas of eastern China.[220] The main risk factor identified for A(H7N9) virus infection was visiting a live poultry market.[221] Most reported A(H7N9) virus infections have resulted in severe pneumonia, and infected persons were admitted to intensive care units with complications such as respiratory failure, ARDS, and multiorgan failure.[222] Clinical management has included antiviral treatment with an NA inhibitor and supportive care. NA inhibitor resistance has emerged during antiviral treatment.[223,224] Some cases of asymptomatic or mild illness with A(H7N9) virus infection were identified, and some studies estimated that many mild cases occurred but were not detected, suggesting a wide clinical spectrum of infection.[225–227] Closure of live poultry markets in large urban areas was effective in reducing human A(H7N9) virus infections.[228,229] However, as A(H7N9) viruses spread among poultry into rural areas, exposure to backyard poultry was identified as a risk factor for human infection.[230]

Annual epidemics of sporadic human A(H7N9) virus infections have continued to occur in China, with a surge in infections during 2016 and 2017, prompting pandemic concerns.[231–233] This huge increase in human infections was attributed to geographic spread of A(H7N9) viruses among poultry throughout China and ongoing viral evolution into multiple lineages.[234] During 2016, HPAI A(H7N9) viruses emerged to cause poultry outbreaks in several areas of China with associated severe and fatal human infections in 2017.[234] Limited, nonsustained human-to-human A(H7N9) virus transmission has occurred in small clusters of cases among both blood relatives and unrelated persons, but no increase in human-to-human transmission was identified during 2013 to 2017.[235] Human infections with A(H7N9) virus have been exported from China to Malaysia,[236] Taiwan,[237] and Canada.[238] During 2017 and 2018, very few human A(H7N9) virus infections were reported. The reasons for the dramatic reduction in human cases are unknown, although multiple factors may have contributed, including widespread H5/H7 vaccination of commercial sector poultry in China. As of April 2018, 1567 human infections with A(H7N9) virus and 615 deaths (39%), all infected in China, had been reported to WHO.[239]

Other Influenza A Virus Subtypes

Sporadic human infections with HPAI A(H7) viruses other than A(H7N9) have resulted in a wide range of disease severity. Multiorgan failure with fatal outcome occurred in one case of HPAI A(H7N7) virus infection in the Netherlands during widespread poultry outbreaks in 2003.[240] However, most human infections with HPAI A(H7N7) virus have resulted in mild upper respiratory tract illness or conjunctivitis.[241] Conjunctivitis has been reported with HPAI A(H7N3) virus infection in Mexico.[242] A wide range of illness has also been reported for LPAI A virus infections. Conjunctivitis has been reported from infections with LPAI viruses A(H7N2) in the United Kingdom,[243] A(H7N3) in Canada and the United Kingdom,[244,245] A(H7N7) in the United Kingdom,[246] and A(H10N7) in Australia.[247] Upper respiratory tract illness has occurred from infections with LPAI viruses A(H6N1) in Taiwan,[248] A(H7N2) in the United States and the United Kingdom,[243,249] A(H7N3) in Canada and the United Kingdom,[244,245] A(H9N2) in China, Hong Kong, Bangladesh, and Egypt,[250–252] and A(H10N7) in Australia.[247] Lower respiratory tract illness has been reported from infections with LPAI viruses A(H7N2) in the United States, Hong Kong, and China,[249–253] A(H7N4) in China,[254] A(H9N2) in Hong Kong and China,[253,255] and A(H10N8) in China.[256]

In addition to LPAI A(H7N9), fatal outcomes have been reported in China with A(H9N2)[257] and A(H10N8)[256] virus infections. Cat-to-human transmission of an LPAI A (H7N2) virus resulting in upper respiratory tract illness was reported in the United States.[258] Experimental infection of a seal with a closely related LPAI A(H7N7) virus resulted in seal-to-human transmission with resultant conjunctivitis in the United States.[259]

Variant Influenza A Infections From Swine

Sporadic human infections with multiple subtypes of influenza A viruses circulating among pigs have been reported worldwide for many years. Swine influenza A viruses that have infected humans are referred to as "variant viruses" and denoted with "v" after the subtype. Most reported variant virus infections have occurred in children. Most infections with variant viruses (H1N1v, H1N2v, H3N2v) have resulted in mild upper respiratory tract illness (H1N1v, H1N2v, H3N2v),[260–263] conjunctivitis (H1N1v, H3N2v),[262,263] or gastrointestinal symptoms (H3N2v).[263] However, hospitalizations, including one death, were reported for 5.2% of H3N2v virus infections during a multistate outbreak associated with exposures to pigs at agricultural fairs in 2012 in the United States.[263] Critical illness, including respiratory failure and ARDS, with H1N1v virus infection have been reported in an adult man in Italy[264] and a child in the Netherlands.[265] Fatal outcomes of other variant virus infections have been reported.[261] Most variant virus infections likely result from sporadic swine-to-human transmission, but limited, nonsustained human-to-human transmission has been reported.[261,263,266]

Influenza Risk Assessment

Given the diversity and significant increase in human cases due to avian- and swine-origin influenza A viruses, public health officials have developed tools to assess the pandemic potential of these viruses.[267,268] One of these tools, the CDC's Influenza Risk Assessment Tool (IRAT),[267] uses 10 scientific criteria covering an influenza A virus' characteristics (e.g., receptor binding tropism, genetic variation, antiviral drug resistance markers, transmissibility and pathogenesis in animal models), epidemiology, ecology, and the disease it causes in humans. These data are used to assess potential pandemic risk based on two different scenarios: (1) the likelihood that the virus will emerge to transmit efficiently in humans and cause a pandemic, and (2) the disease severity impact the virus would cause in humans if it were to emerge. Fig. 14.3 provides examples of IRAT scores for selected novel influenza A viruses, indicating that A(H7N9) viruses currently have the highest risk scores among those viruses evaluated. The IRAT and other risk assessment tools provide frameworks for assisting public health officials in determining the appropriate response to an emerging novel influenza A virus.

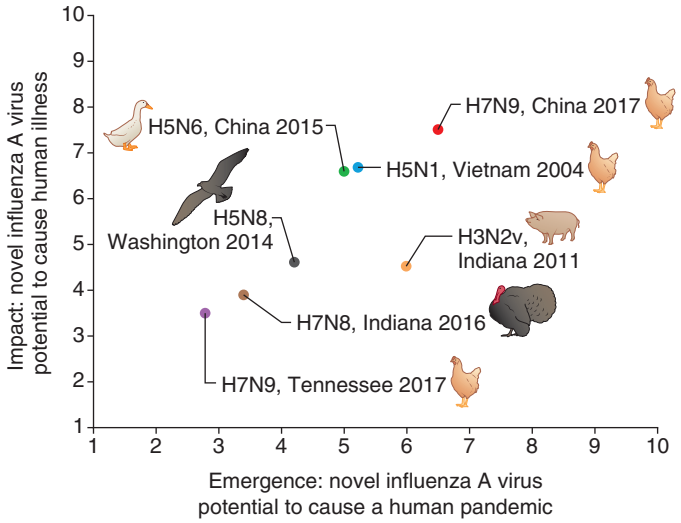

FIG. 14.3 Influenza Risk Assessment Tool. Average risk scores with selected viruses.

In conclusion, influenza A viruses maintain reservoirs of circulation in humans, birds, swine, and other animals. The high error rate of virus replication, coupled with the ability to exchange gene segments among avian, swine, and human viruses, allows the virus to continue evading host immunity, to spill over and cause disease across species, and to periodically introduce pandemic viruses into the human population. Maintaining surveillance in humans, birds, and swine is critical to identifying, preventing, and controlling emerging novel influenza A viruses with pandemic potential.

ENTERIC DISEASES

Enteric diseases encompass all the attributes of emerging and reemerging infectious diseases, and their emergence and spread is influenced by microbial, human, and environmental factors, as discussed previously. Enteric pathogens are constantly changing in their ability to survive in their reservoir environments and in their ability to infect and harm the human host. New variants are discovered with increasing frequency, due in part to advances in technologies such as WGS that are applied to large numbers of pathogen isolates and that can identify changes in genetic virulence factors. In addition to increased international travel and mass production (including huge farms and dairies), increased international trade in food is expanding the number and types of foods that may serve as vehicles for foodborne diseases. For example, in the United States, imports of fruit and nuts more than doubled and imports of fish and seafood increased over 60% during 1998 to 2007.[269] Moreover, individual food items may contain ingredients from multiple countries.

Other factors that facilitate the spread of enteric diseases include wars, social disruption, and associated population migrations, which often result in the breakdown of basic public health and health care services, leading to reemergence of ancient scourges such as cholera, with devastating consequences. Weather events can also increase human exposures to enteric pathogens, especially with the dramatic destruction and flooding from hurricanes and typhoons associated with waterborne diseases. Less obvious are the gradual environmental effects of changes in climate. These effects were evident in an outbreak of *Vibrio parahaemoliticus* infections due to contaminated oysters in 2004.[270] Typically, *V. parahaemoliticus* contamination of shellfish is associated with warm water harvest areas, but in this case the oysters were harvested from Prince William Sound in Alaska. Investigators determined that the water temperatures in the sound reached record high temperatures above 15°C at the time of harvest, the threshold above which *Vibrio* sp. tend to proliferate. This outbreak extended the northernmost documented source of vibriosis by more than1000 km. An enteric pathogen has also been associated with intent to harm, another factor contributing to reemergence of infectious diseases. Prior to the anthrax-laden letters responsible for the bioterrorism event in 2001, the only bioterrorism event in the United States was due to *Salmonella* contamination of salad bars in restaurants in Oregon in 1984.[271] Members of a Rajneesh cult had intentionally contaminated the salad with *Salmonella,* resulting in over 750 cases of salmonellosis in the community, in an attempted to disrupt local elections.

The WHO Global Burden of Disease Study for 2015 ranked diarrheal diseases as the second leading cause of acute incident diseases (2.4 billion)[272] and communicable disease mortality (1.3 million deaths) worldwide, behind lower respiratory infections.[130] This study noted a number of important characteristics of the global diarrheal disease burden. As a sign of progress, the death rate from diarrheal disease decreased 36% between 2005 and 2015. Although diarrheal disease affects persons of all age groups and in all geographic locations, the greatest burden of death falls on children younger than 5 years, accounting for 499,000 deaths. In 2015, rotavirus enteritis was the leading cause of death due to diarrheal diseases (15.2%) among all ages, followed by shigellosis (12.5%) and salmonellosis (6.9%). Rotavirus enteritis was also the leading cause of death due to diarrheal diseases (29.3%) among children younger than 5 years, followed by cryptosporidiosis (12.1%) and shigellosis (11%). Among all ages and for children younger than 5 years, the population attributable fractions for deaths due to diarrheal diseases decreased most for rotavirus enteritis from 2005 to 2015 (40.7% and 43.6% decreases, respectively), likely due to the increasing use of rotavirus vaccines. *C. difficile* infection was a major cause of mortality

due to diarrheal diseases in high-income countries and was the only cause for which the attributable fraction increased from 2005 to 2015 (40.1%).

Routes of Transmission for Enteric Pathogens

The pathogens that cause enteric diseases are transmitted primarily by three routes—foodborne, waterborne, and person-to-person—though other environmental and zoonotic exposures also play a role in transmission. For example, *Clostridium perfringens* and *Bacillus cereus* are transmitted almost exclusively through food, *Shigella* species and norovirus are transmitted primarily from person to person, and *Giardia* species are principally waterborne. Many enteric pathogens can be transmitted by more than one route, including simultaneously. The relative importance of these transmission patterns varies by pathogen and setting; however, systematic studies to determine the population attributable fractions for different transmission routes are difficult to perform. Foodborne transmission has been studied most rigorously. Estimates in the United States suggest that 48 million episodes of foodborne illness occur annually, resulting in approximately 128,000 hospitalizations and approximately 3000 fatalities,[273,274] and that endemic waterborne disease results in 4.3 to 32.8 million cases of acute gastrointestinal illness annually.[275]

The interaction among foodborne pathogens, their environments, and human behaviors are especially complex and in constant flux. Some foodborne pathogens, such as *Campylobacter,* Shiga toxin–producing *E. coli* (STEC), and nontyphoidal *Salmonella,* have animal or environmental reservoirs, and humans are often incidental hosts, acquiring the pathogen after eating foods or ingredients contaminated from those reservoirs somewhere along the chain of production, slaughter, and processing. Other pathogens, such as norovirus or *Shigella,* have a primary human reservoir and cause foodborne illness when an infected human contaminates foods. In addition, our food supply is changing as more food is imported from distant lands, food processing becomes more centralized and industrialized, and consumer tastes and cooking practices evolve. Food animals are raised in close quarters and are slaughtered and processed with ever-greater efficiency. Fresh fruits and vegetables are available year round, often shipped from distant countries. Processed foods such as peanut butter[276] and flour[277] have caused large outbreaks when food safety measures were insufficient to prevent microbial contamination. In the kitchen, microwaving is replacing traditional cooking, which means that the heating that kills microbes is less thorough and more difficult to monitor. Consumers may desire local foods and foods eaten with minimal cooking as well as convenience. In the United States, many states permit the sale of raw unpasteurized milk, despite the raw milk–associated outbreaks that occur more frequently in those states.[278]

These changes in food production and human behavior result in regular emergence of new food vehicles for foodborne diseases (Table 14.4). For example, the increased popularity of packaged fresh leafy vegetables has been associated with numerous outbreaks due to STEC and *Salmonella.* In 1996 a large outbreak of *E. coli* O157:H7 infections linked to packaged fresh spinach shook the produce industry in the United States. Investigators identified 225 cases in 27 states; 39 (14%) case-patients developed hemolytic-uremic syndrome and 5 died.[279] An environmental investigation of source spinach-growing fields in California identified multiple potential sources of *E. coli* O157:H7 contamination from surrounding water sources and animals.[280] Two different STEC serotypes were isolated from spinach during the investigation, including Orough:NM and O146:H21, which further illustrated the multiple sources of environmental contamination. Also in 1996, thousands of cases of cyclosporiasis due to *Cyclospora cayetanensis* occurred in the United States and Canada among persons who consumed fresh raspberries imported from Guatemala.[281] Before this episode, only small numbers of cases, primarily associated with travel to developing countries,[282] had been reported in North America.

Among other modes of transmission, the United States has documented a significant increase in outbreaks due to *Cryptosporidium* contamination of recreational water venues.[283] Different types of water venues have become popular and are associated with multiple outbreaks, including splash pads with water spouts and recirculating water. Since first identified in 1988, recreational water–associated outbreaks due to *Cryptosporidium,* a choline-tolerant parasite, increased from one outbreak in 1988 to 37 outbreaks with 890 cases in 2011 and 2012.

Enteric diseases due to zoonotic transmission have been associated with a wide variety of pathogens and animals. In the United States, the proportion of illnesses from seven pathogens due to animal contact was estimated to be 14%, representing approximately 450,000 illnesses annually.[284] *Campylobacter* had the largest proportion of illnesses attributed to animal contact (17%), followed by *Cryptosporidium* (16%) and *Salmonella* (11%). In a change in human behavior that may be specific to the United States, raising poultry in urban backyard poultry flocks has become more popular.[285] Poultry sold for backyard flocks are produced by approximately 20 hatcheries in the United States, which sell more than 50 million chicks annually. Live poultry–associated *Salmonella* outbreaks increased substantially from 17 outbreaks from 1990 to 2005 to 36 outbreaks during 2006 to 2014.

TABLE 14.4 New Food Vehicles Identified in US Multistate Infectious Disease Outbreaks Since 2006

1. Bagged spinach
2. Carrot juice
3. Peanut butter
4. Broccoli powder on snack food
5. Dog food
6. Pot pies/frozen meals
7. Canned hot dog chili sauce
8. Fresh hot chili peppers
9. Black pepper
10. Tahini sesame paste
11. Raw cookie dough
12. Aquatic water frogs
13. Fresh papaya
14. Frozen mamay fruit pulp
15. Bologna
16. In-shell hazelnuts
17. Pine nuts
18. Par-cooked, broiled chicken livers
19. Scraped tuna
20. Cashew cheese
21. Sugar cane juice
22. Sprouted chia seeds
23. Almond butter
24. Caramel apples
25. Sprouted nut butters
26. Dried mushrooms (in truffle oil puree)
27. Pistachios
28. Wheat flour
29. Powdered meal supplements
30. Soy nut butter

International Spread: Cholera in the Western Hemisphere

The movement of pathogens has had major repercussions in the reemergence of enteric diseases. The introduction of *Vibrio cholerae* O1 into the Western Hemisphere has caused two major epidemics in the past 30 years. Cholera had not been recognized in South America during the 20th century, but in January 1991 cases were identified in coastal areas of Peru.[286] Within weeks, thousands of cases were occurring, and cholera quickly became the most commonly diagnosed cause of diarrheal illness in many parts of Peru.[287,288] During the next 3 years, the disease spread throughout mainland South and Central America, significantly altering the distribution patterns of etiologic agents of diarrheal disease. Epidemic cholera in Latin America has since been essentially eliminated with major improvements in safe water sources.

In October 2010, 9 months after a severe earthquake, a cholera epidemic was identified in Haiti that spread within 2 months to the entire country and across the border to the Dominican Republic.[289,290] Molecular epidemiologic studies have indicated that the *V. cholerae* strain is very similar to South Asian strains,[291] and epidemiologic investigations revealed that initial cases occurred downstream from a

camp where United Nations peacekeepers from Nepal were stationed.[292,293] The source of introduction of the epidemic strain was a politically sensitive topic.[294] Although the incidence has been reduced, cholera transmission persists. Cases in Haiti and the Dominican Republic accounted for greater than 30% of the global cases reported to WHO in 2016.[295]

Evolution of Enteric Pathogens

Changes in pathogens have been recognized as a factor of emergence in a number of events in recent years. A dramatic example occurred in 2011 when a large outbreak of STEC O104:H4 stuck Germany, associated with fenugreek sprouts.[296,297] This pathogen had a newly recognized combination of virulence factors. In addition to producing a virulent subtype of toxin (Shiga toxin 2) encoded on a prophage, it contained genes associated with enteroaggregative *E. coli* and a plasmid-encoded extended-spectrum β-lactamase gene.[298] The combination of efficient attachment to intestinal epithelial cells and a toxin particularly toxic to endovascular cells in one *E. coli* strain resulted in a devastating outbreak. The contaminated seeds used for sprouting originated in Egypt[299] and were distributed widely, resulting in over 4000 illnesses, 800 cases of hemolytic-uremic syndrome, and 50 deaths in Germany and 15 other countries.[297]

Multiple drug resistance in bacterial enteric pathogens has emerged in the United States and internationally. The problem is most severe in foodborne pathogens of animal origin, primarily in *Campylobacter* and *Salmonella* strains.[300] A multidrug-resistant strain of *Salmonella typhimurium* known as definitive type 104 (DT104) emerged in the United States and Europe in the 1990s.[301,302] Patients infected with multiply-resistant strains are more likely to be hospitalized.[303] Fluoroquinolone-resistant strains of *C. jejuni* infections have also emerged; some infections are associated with foreign travel while others are acquired domestically and have been associated with poultry consumption.[304] In a study of resistance in over 1100 *Shigella* isolates in the CDC's Foodborne Diseases Active Surveillance Network (FoodNet) sites from 2008 to 2010, 74% were resistant to ampicillin, 36% to trimethoprim-sulfamethoxazole, 28% to tetracycline, and 0.5% to ciprofloxacin; 5% of isolates were resistant to five or more antimicrobials.[305] Resistance was more common in persons with a history of recent foreign travel. A foodborne outbreak in Los Angeles in 2012 was caused by *Shigella sonnei* with decreased susceptibility to azithromycin, the first such outbreak identified in the United States.[306]

Another example is the emergence of a new strain of multidrug-resistant *S. typhimurium* (multilocus sequence type ST313) in sub-Saharan Africa. The strain causes invasive nontyphoidal disease, sometimes accompanied by diarrhea, primarily in patients with HIV infection, malaria, and malnutrition.[307,308] Application of WGS-based phylogenetic methods has identified two closely related, highly clustered lineages estimated to have evolved independently approximately 52 and 35 years ago. Lineage II strains have replaced lineage I strains, perhaps because of their acquisition of chloramphenicol resistance.[305]

The incidence and severity of *C. difficile*–associated disease has increased dramatically in adults and children in the United States, Canada, Europe, and Australia.[309–312] Moreover, while *C. difficile*–associated disease used to be primarily health care associated, community-associated cases are on the rise. In the United States during 2011 in Emerging Infections Program sites the overall adjusted incidence per 100,000 persons was 147 (95% confidence interval [CI], 129–165).[313] Sixty-six percent of *C. difficile* infections were health care associated, resulting in an incidence of 95 (95% CI, 86–105); 24% had hospital onset of illness. Community-associated *C. difficile* infections accounted for 46% of cases, resulting in an incidence of 52 (95% CI, 43–61). Persons over 65 years old had by far the greatest incidence at 628 (95% CI, 567–687). The death rate was higher for health care–associated disease (8.9; 95% CI, 5–13) than for community-associated disease (0.7; 95% CI, 0.4–0.9). Disease due to a strain of restriction endonuclease analysis group BI, pulsed-field gel electrophoresis type NAP1, and PCR ribotype 027 (BI/NAP1/027) is responsible for much of this increase, including many nosocomial outbreaks.[314–316] Two distinct epidemic lineages, both of which are resistant to fluoroquinolone antibiotics, have recently been identified.[311] The FQR1 lineage, first seen in 2001, is associated with North American outbreaks and sporadic infections in South Korea and Switzerland. The FQR2 lineage originated in 2003 in North America but subsequently spread more widely, causing hospital outbreaks in the United Kingdom, continental Europe, and Australia.[317] Since 2005, another *C. difficile* strain (PCR ribotype 078) has emerged in the Netherlands and in the United Kingdom.[318] This strain also appears to be hypervirulent and is more likely to affect younger persons and to be associated with community-onset disease. Isolates of *C. difficile* also have been obtained from retail ground meat samples,[319] prompting the need for evaluation of the potential role of foodborne transmission in community-associated cases. Recent experiences with fecal microbiota transplantation have suggested an important role for this approach in the management of patients with severe *C. difficile* infections.[320,321]

In addition to changes in toxin production in emerging *C. difficile* strains, investigators have observed changes in other clostridial toxins. A purported new *Clostridium botulinum* toxin type was found in 2013, designated toxin type H at the time.[322] *C. botulinum* has been known to produce seven botulinum toxin types (A–G), and noninfant botulism is treated with Botulism Antitoxin Heptavalent (Emergent BioSolutions Canada), an equine-based product of polyclonal antibodies that treats all known serotypes of botulinum toxin. *C. botulinum* and its associated toxins are considered select agents due to their potential use as biological terrorism agents,[323] thus a new toxin type that could escape the action of available antitoxins was a security concern. The identification of a new botulinum toxin type stirred controversy concerning information that should or should not be shared about the new toxin based on the potential dangerous misuse of the information for nefarious purposes.[324] Additional investigation found the new toxin to have a hybrid structure similar to the structures of toxins A and F. Furthermore, available type A antitoxin was able to neutralize the toxin, deescalating the security concern.[325]

Noroviruses

Noroviruses are transmitted not only from person-to-person but also by food, water, and contact with contaminated environmental surfaces.[326–328] These viruses have become an important cause of diarrheal illness outbreaks among cruise ship passengers, hospitalized patients, nursing home residents, college students, restaurant patrons, and military personnel, reflecting their high infectivity and low infectious dose (<20 viral particles).[329] A systematic literature review published in 2014 indicated that norovirus disease is associated with 18% (95% CI, 17–20) of all cases of acute gastroenteritis.[330] Prevalence was similar across age groups, but higher among cases of acute gastroenteritis in community and outpatient settings (24% and 20%, respectively) compared with inpatient settings (17%). Prevalence was also higher in developed countries (20%) compared to high-mortality developing countries (14%), suggesting a more prominent role for other pathogens in developing countries. Noroviruses are the leading cause of epidemic gastroenteritis in the United States.[331,332] Following the introduction and use of rotavirus vaccines, noroviruses have also become the leading cause of medically attended acute gastroenteritis in US children under 5 years of age; these viruses were found in 21% of children seeking medical care for acute gastroenteritis in 2009 and 2010.[333] In March 2012, a new GII.4 norovirus strain named GII.4 Sydney was identified in Australia and subsequently spread to the United States, the United Kingdom, and a number of other countries.[334] GII.4 norovirus outbreaks have been associated with higher rates of hospitalization and mortality compared with outbreaks caused by other genotypes.[335] New variants of the GII.4 genotype have emerged every 2 to 4 years over the last 2 decades, likely driven by escape from population immunity.[336] These genotype variants represent an additional mechanism of enteric pathogen change resulting in emergence of novel strains as a result of antigenic shifts.

Future Trends

The observation of changes in virulence factors and antigens such as in *E. coli, Clostridium* species, and norovirus, as described previously, provides an increased appreciation of the extent to which pathogens may change with acquisition, rearrangement, or mutation of associated genes. Indeed, the genes responsible for virulence may be encoded on plasmids or other transmissible genetic elements and thus may recombine

to produce pathogens with enhanced virulence, which may emerge rapidly in varied populations and places.

Looking to the future, we can expect to identify the emergence of more pathogens and pathogen subtypes, including pathogens with a differing and expanded repertoire of antibiotic resistance determinants. Clinical laboratories are increasingly adopting syndromic panel-based diagnostic tests that can rapidly detect a wide range of bacterial, viral, and parasitic enteric pathogens and yield more positive results than conventional testing methods, resulting in an increase in pathogen-specific diagnosis among enteric illnesses.[337] The routine and systematic application of next-generation DNA sequencing technology to the surveillance and investigation of listeriosis in the United States has resulted in the detection and successful investigation of more and smaller listeriosis clusters.[338] WGS analyses allow the high-resolution characterization of pathogens, including genetic determinants of virulence, antibiotic resistance, antigenic shifts, and a host of other factors related to changes in pathogen behavior. Large publicly available DNA sequence databases enable research for the discovery of new pathogen virulence factors and antibiotic resistance determinants.

However, these new technologies also present challenges. The adoption of syndrome panel-based diagnostic tests that can detect multiple pathogens increases the chance of identifying a noninfecting transient pathogen, a minor pathogen, or multiple pathogens, which can confuse diagnosis. In a particularly ironic twist, the use of these diagnostic panels threatens the ability to conduct DNA sequence-based pathogen surveillance and investigation.[339] This is because these PCR-based diagnostic panels detect potential pathogens directly from sample material and do not result in cultured isolates. Next-generation DNA sequence technologies require cultured isolates for analyses. The logical path to resolve this challenge is likely the convergence of diagnostic and DNA sequence technologies with metagenomics.[340] The technical ability to sequence and analyze any or all DNA contained in a clinical sample is in its infancy but shows great promise for clinical, public health, and research applications and may lead to the next paradigm shift in microbiology.

VECTOR-BORNE DISEASES

Vector-borne diseases are caused by pathogens transmitted by blood-feeding arthropods, typically insects and ticks. In all cases the pathogen is amplified in the vector and in some—*Plasmodium* and *Borrelia* are examples—it is transformed. Vector-borne pathogens include representatives from most major categories of disease-causing agents—viruses, protozoa, bacteria, and helminths—and are responsible for some of the world's most destructive epidemics. Malaria, plague, yellow fever, typhus, and dengue have been known since antiquity but recent years have brought new threats from once obscure or unknown pathogens, such as the mosquito-borne chikungunya and Zika viruses, and tick-borne agents. Vector-borne pathogens are predominately zoonoses, and vectors frequently bridge the gap between humans and animal reservoirs that rarely come into direct contact, an especially important contribution to pathogen emergence. Sometimes humans remain dead-end victims, as happens with West Nile virus and Lyme disease, but for others, such as dengue and Zika viruses, the pathogens have gained the ability to be vectored between humans. Some human *Plasmodium* species that made the transition from animals to exclusively humans eons ago still retain the capacity for infecting wild, nonhuman primates.

There are, so far, only two highly efficacious vaccines in widespread use against vector-borne pathogens, those against yellow fever and Japanese encephalitis viruses. This puts the burden of preventing and controlling the large number of other vector-borne diseases on eliminating or reducing contact with the vectors. This can be done by denying vector access to humans, as with bed nets or repellents, or by eliminating the vector, using insecticide or altering habitat ("source reduction"). An advantage of vector control is that elimination of a single vector species can often eliminate transmission of more than one pathogen. The mosquito *Aedes aegypti,* for example, transmits dengue, yellow fever, chikungunya, and Zika viruses; most malaria vectors, such as *Anopheles gambiae,* can transmit all species of human *Plasmodium.* A second advantage is that insecticides and many other control tools will work against a variety of vectors species. But despite early successes,[341] vector control, which typically requires a large, well-organized work force and access to property, has often proved to be unsustainable. The indiscriminate use of insecticides—both in public health and agricultural applications—has resulted in widespread vector resistance while few new active agents are coming to market.[342] Development of methods of biologic control, such as release of sterile male mosquitoes or replacement of wild populations of mosquitoes with those that are pathogen resistant, are being actively pursued.[343]

The *Aedes*-Transmitted Viruses: Dengue, Zika, Yellow Fever, and Chikungunya

The small black and white mosquito, *A. aegypti,* evolved to cohabit with humans. It lays its eggs in water-filled artifacts common in and around dwellings, often rests in houses, and tends to bite during daylight. Although most common in the tropics, its range increasingly extends into temperate zones; a cognate species, *Aedes albopictus,* is even more accommodating of cool climate and has been responsible for epidemics of chikungunya virus in Italy[344] and France.[345] The close proximity of *A. aegypti* to humans makes it a potent vector of four of the most consequential arthropod-borne viruses ("arboviruses"), all of which have caused major epidemics since 2013.

Dengue

Dengue is the most important mosquito-borne viral disease in the world, estimated to infect nearly 400 million people annually.[346] Epidemics are common: more than 80,000 cases were reported from Sri Lanka in 2017, a year in which there were also major outbreaks in Vietnam, Burkina Faso, and Cote d'Ivoire. Dengue fever is caused by four related but antigenically distinct flavivirus species (DEN-1, -2, -3, and -4), sometimes referred to as serotypes. They are single-stranded, positive-sense RNA viruses that possibly jumped from simians to humans during historical times; sylvatic transmission still occurs in parts of West Africa and Southeast Asia.[347] There has been a dramatic increase in reported cases of dengue disease since 2000, especially in Latin America and Africa.[348] The reasons for worldwide increase are likely a combination of increased global movement of people and goods, warming climate, insecticide resistance, and, especially, urbanization. Infection with any of the four dengue species confers lifelong immunity to only that species, and the most severe forms of disease occur in those subsequently infected by another species. While most infections are mild, and often go unreported, severe dengue (dengue hemorrhagic fever and dengue shock syndrome) is life threatening if not properly managed.[349]

The decades-long quest for an efficacious vaccine against all four dengue species has been complicated by concerns about the association of severe disease with secondary heterotrophic infection, and by the lack of an animal model that could be used to establish correlates of protection. The first vaccine to successfully complete clinical trials, Sanofi-Pasteur's Dengvaxia, appeared commercially in 2015, but its limited efficacy against DEN-2 and DEN-4 has been further complicated by a relatively high rate of postvaccination hospitalization in children who had been seronegative at the time of vaccination.[350,351] At least four other tetravalent vaccines are in clinical trials, including live-attenuated, subunit, DNA, and purified inactivated candidates.[350,351]

Zika

Zika virus is also a flavivirus of apparent simian origin. Discovered in 1947 in a sentinel macaque during yellow fever investigations in Uganda, Zika infections were rarely reported in humans until an epidemic in Micronesia in 2007. It subsequently moved south and eastward through the Pacific before reaching Brazil by 2014.[352] Its epidemic spread through the naïve populations of the tropical Americas during 2015 to 2017 dramatically illustrates the dangers inherent in the emergence of a pathogen. Before Brazil, Zika virus was characterized as causing mild, inconsequential disease in no more than about 20% of those infected. Its symptoms might not be distinguished from mild dengue: fever, rash, and myalgia, although with significantly higher occurrence of conjunctivitis. Although details of its pathogenesis are still being investigated, it is clear that Zika virus infection of pregnant women, especially during the first trimester, was responsible for an epidemic of congenital brain abnormalities, most obviously manifested as microcephaly. No other