and at least 20% prevalence of "clue cells" (vaginal epithelial cells coated with bacteria). These clinical criteria are often discussed in a modified form as Nugent scores, with a score of 7 to 10 being standardized as the diagnosis of BV and indicative of the absence of lactobacilli and a relative predominance of *G. vaginalis* and *Mobiluncus* spp. The Nugent score is rarely used by clinicians because reading the slides takes time and requires trained microscopists. Although there is much controversy regarding causation versus association, women with BV are at higher risk for pregnancy complications such as preterm birth, preterm premature rupture of the membranes, and postpartum endometritis.[194] Outside of pregnancy, BV is associated with an increased risk of acquisition of HIV, and women with BV and HIV have a greater risk of lifelong morbidity.[195–197] Although it is outside the scope of this chapter, it bears mentioning that treatment of asymptomatic BV is associated with an increased risk of preterm birth.[191,194,198] Although the pathophysiology mediating these observations is unclear, the microbiome-minded physician might choose to minimize clinical interventions that disrupt the complex vaginal ecosystem.

GROUP B STREPTOCOCCUS

Streptococcus agalactiae (colloquially referred to as GBS) is a gram-positive α-hemolytic bacterium that can cause invasive GBS disease in the early newborn (<6 days of age), characterized primarily by neonatal sepsis and pneumonia, or less frequently meningitis. In contrast to the early neonate, GBS rarely causes morbid disease in the pregnant women who carry it, but may occasionally be associated with urinary tract infections, amnionitis, endometritis, or sepsis or meningitis either during pregnancy or in the postpartum interval.[199] Therefore GBS may be considered to be a pathobiont member of the commensal gut and vaginal microbiomes, with GBS colonization of the vagina or rectum occurring in an estimated 10% to 30% of pregnant women. In an effort to eliminate neonatal mortality due to early invasive GBS disease, the current US standard for maternal GBS detection during pregnancy is universal screening by vaginal and rectal culture at 35 to 37 weeks gestation, or with preterm labor or preterm premature rupture of membranes.[200,201] Since 2011, US guidelines have provided a permissive statement for a limited role of nucleic acid amplification tests for intrapartum testing for GBS. The current US recommendation for a positive GBS culture test result (or with history of previous infant with GBS septicemia, positive maternal GBS bacteriuria during pregnancy) is intrapartum antibiotic prophylaxis, resulting in as many as 1 million U.S. women annually receiving multiple doses of penicillin or ampicillin, cefazolin, vancomycin, or clindamycin or erythromycin in labor.[199] However, other developed countries, including in the United Kingdom, with similar rates of asymptomatic maternal GBS colonization during pregnancy instead take a risk-based approach to GBS screening and treatment.[202] Irrespective of the method used to determine who receives GBS prophylaxis for the prevention of perinatal group B streptococcal disease, given a current case prevalence of invasive early newborn GBS disease of less than 0.4 cases per 1000 live births (and a prenational guidelines prevalence of 1.7 cases per 1000 live births[199]), thousands of women will be exposed to multiple antibiotic courses in order to prevent a single neonatal case. As expected, the current guidelines on either continent have had no effect on late-onset GBS disease (defined as occurring in neonates older than 6 days).

Although GBS colonization is not a risk for preterm birth per se, vertical transmission of GBS has long been independently associated with neonatal bacteremia and sepsis with worsened prognosis in the preterm (<37 weeks) neonate.[203] For several decades the standard of care in the United States was to treat women with at-risk pregnancies. As discussed previously, this approach has shifted in the last 2 decades. As a result, neonatal colonization has been dramatically altered. Ironically, the reduction of neonatal deaths from GBS septicemia has been partially offset by a proportionate increase in neonatal deaths due to infection by β-lactam-resistant *E. coli* in very-low-birth-weight (VLBW) and premature infants.[204] As a result, the overall rate of early-onset sepsis has not significantly changed, but the prevalence of resistant organisms has significantly risen.[204,205] This epidemiologic shift serves as a poignant reminder of the possible effects of broad population-based screening and treatment, and unintended consequences in affected cohorts.

THE HUMAN MICROBIOME DURING PREGNANCY

Despite several lines of evidence from many laboratories supporting the presence of a placental microbiome (as measured by metagenomic and other means) in both humans and other mammalian models,[a] there has been limited concern raised regarding the possibility of environmental contamination skewing these findings.[82,207] Ongoing studies in multiple laboratories should provide a clearer picture of the presence and functional role of placental microbes in fetal and neonatal colonization. At present, the relative contributory role of the placenta and maternal oral, vaginal, skin, and gut communities in the fetal, neonatal, and early infant microbiome remains imprecisely defined. What is evident is that neonates harbor colonizing bacteria at or very near the time of their birth. As discussed at the start of this chapter, it remains unknown whether this is due to true fetal colonization or fetal immune tolerance and ex utero colonization.

SUMMARY AND FUTURE DIRECTIONS

The human microbiome is composed of distinct microbial communities at different body sites, and these different body habitats provide niches for diverse bacterial species. Cellular elements of the microbiome may enhance immunity or prevent infections by canonical pathogens. Other microbes may serve as opportunists that typically colonize the human host without causing disease, but some of these organisms may cause infections in immunocompromised hosts. Bacterial strains, including but not limited to currently accepted probiotics, can stimulate immune responses and modulate inflammation, and antibiotic-mediated depletion of human-associated bacteria may result in immune dysregulation or infection-susceptible microbiomes. In the upcoming era of metagenomic medicine, infectious diseases must be considered in the context of the human microbiome and protective or pathogenic microbial communities. Human-associated microbes may serve as symbionts promoting interactions that are mutually beneficial or as benign commensals that simply colonize humans without bestowing any obvious benefits to the host. Future diagnostic tests may include components of the microbiome in disease evaluation, and decisions about antimicrobial therapy may rely on "typing" or profiling of the human microbiome in individual patients. The nature of the infection may dictate which body site is evaluated in terms of microbiome composition or function. Advances in understanding and management of infectious diseases will necessitate a deeper understanding of the microbiome context. The contributions or effects of microbial communities and metagenomes may have a large impact on infection susceptibility and disease pathogenesis.

[a]References 15, 41, 42, 52, 60, 62–64, 67, 68, 76, 78, 206.

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3 Prebiotics, Probiotics, and Synbiotics

Lea Ann Chen and Cynthia L. Sears

SHORT VIEW SUMMARY

Definitions
- Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."
- Prebiotics are nonviable substrates that are selectively used by host microorganisms and confer a health benefit to the host.
- Synbiotics are combinations of prebiotics and probiotics that are designed to have synergistic and/or additive effects benefiting the host. Probiotics are also marketed as food ingredients, dietary supplements, or "medical food."

Epidemiology
- Probiotics are used globally by millions of individuals.
- It is important to know that, for most marketed probiotic products, rigorous clinical trials to ascertain health benefits have not been done.

Microbiology
- Both bacterial (usually species of *Lactobacillus* or *Bifidobacterium*) and fungal (usually *Saccharomyces boulardii*) probiotics are available.
- Probiotics can be single organisms or contain several organisms, and numerous products are marketed.
- Studies have identified that at least 30% of probiotic products differ from their product labeling, with discrepancies between the stated and actual number of viable organisms, the concentration of the organisms, and the types of organisms in the product, among other concerns regarding quality and validity.
- Probiotics are not subject to minimal manufacturing standards with regulatory oversight.

Therapy and Prevention
- There are no US Food and Drug Administration–approved probiotics for disease prevention or therapeutic use.

Probiotics have been studied most extensively as therapy for acute infectious diarrhea and antibiotic-associated diarrhea and as prevention for *Clostridioides difficile* (formerly *Clostridium difficile*)–associated diarrhea and necrotizing enterocolitis in preterm infants. However, probiotics are not standard-of-care for any condition.

Although certain probiotics are thought to be generally safe, current available data are considered insufficient to address the safety of probiotics with confidence. This is particularly true in vulnerable hosts, such as those at the extremes of age, critically ill, immunocompromised, or with existing hardware or catheters.

Probiotics are defined by the International Scientific Association for Probiotics and Prebiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[1] Neither fecal microbiota transplant (FMT) nor dead organisms or bioactive molecules produced by microbes are defined as probiotics. There are no US Food and Drug Administration (FDA)–approved probiotics for therapeutic use. Prebiotics are nonviable substrates that are selectively used by host microorganisms and confer a health benefit to the host.[1] This updated 2017 definition of prebiotics maintains that prebiotics are substances that benefit the host indirectly by influencing the growth of specific microbe or microbial communities, while also expressing the further considerations that prebiotics could be noncarbohydrates and can act outside the intestinal environment, such as in the skin or vagina.[1] Synbiotics are combinations of prebiotics and probiotics that are designed to have synergistic and/or additive effects benefiting the host.[2] Most investigations continue to focus on the evaluation of potential probiotics with more limited studies available for prebiotics and synbiotics. Both bacterial (usually species of *Lactobacillus* or *Bifidobacterium*) and fungal (usually *S. boulardii*) probiotics are the subjects of an extensive literature and increasing global use, by millions of users.[3,4] It is important to note that, for most of the marketed probiotic products, rigorous clinical trials to ascertain health benefits have not been done. Further, online sources appear biased toward therapeutic benefits, suggesting the importance of patient education by clinicians regarding probiotic effectiveness.[5]

In the English literature (www.ncbi.nlm.nih.gov/pubmed/) there have been more than 19,000 publications on probiotics dating to the 1950s. Approximately half of this literature is based on human studies. Probiotics, such as yogurts or fermented milks, are ancient, dating at least as far back as documented by Egyptian hieroglyphs and now have been aggressively marketed as food ingredients, dietary supplements, or "medical food"; probiotic infant formulas also are widely available.[6,7] All yogurt contains live cultures of lactase-producing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; those yogurt products further supplemented with additional live bacteria (e.g., *Bifidobacterium* spp., other *Lactobacillus* spp.) are considered probiotics.[8] In contrast, it has been debated whether yogurt in fact represents probiotics, although some data, for example, suggest yogurt, due to containing lactase-producing bacteria, improves tolerance to lactose with potential health benefit.[4] In contrast, kefir is a fermented milk drink dating to the late 1800s, now commercialized as Kefir, which is complex, containing multiple bacterial and yeast strains.[6,9]

ISSUES REGARDING THE COMPLEXITY AND VARIABILITY OF PROBIOTICS

Marketed probiotics are highly variable, with some products labeled to contain single microbes and others composed of multiple distinct microbes, such as VSL#3, a commercial product with eight strains of bacteria from the genera *Bifidobacterium*, *Lactobacillus,* and *Streptococcus*, or products containing multiple species of a single, usually bacterial, genus (e.g., *Lactobacillus casei*, *Lactobacillus rhamnosus*). Studies to verify the composition of marketed probiotic formulations, however, have found that there are commonly discrepancies (involving at least 30% of products) between the stated and actual number of viable organisms, the concentration of the organisms, and/or the types of organisms in the product compared with the product labeling.[3] In addition, some marketed probiotics are labeled with taxonomically incorrect or fictitious microbial names.[10] Thus uncertainty exists about the composition and reliable manufacturing practices for

TABLE 3.1 Concerns About Marketed Probiotic Formulations

Marketed with taxonomically incorrect or fictitious microbial names
Standards lacking to define the number of viable organisms in available probiotics, the shelf life of the products, or appropriate storage conditions to maintain probiotic viability
Lack of clear labeling of many probiotic products on dosing or toxicity
No US Food and Drug Administration or other oversight to provide minimal manufacturing standards for probiotics
Large number of different products labeled as probiotics without adequate scientific study to define the product efficacy, the biologic basis for proposed health benefit, and/or to demonstrate product safety

TABLE 3.2 Probiotics That Have Received GRAS (Generally Recognized as Safe) Status in the United States

Bacillus coagulans GBI 30, 6086 (activated, inactivated, and spores)[b]
B. coagulans strain Unique IS2 spores preparation
B. coagulans SANK 70258 spore preparation
B. coagulans SBC37-01, spore preparation
B. coagulans SNZ1969 spore preparation
Bacteroides xylanisolvens strain DSM23964
Bifidobacterium animalis subsp. *lactis* strain Bf-6
Bifidobacterium breve M-16
Bifidobacterium lactis strain Bb12 and *Streptococcus thermophilus* strain Th4
Bifidobacterium longum BB536
B. animalis subsp. *lactis* strains Bf-6, HN019, Bi-07, B1-04, and B420
Carnobacterium maltaromaticum strain CB1 (viable and heat treated[a])
Lactobacillus acidophilus La-14
L. acidophilus, Lactobacillus lactis, and *Pediococcus acidilactici*
L. acidophilus NCFM
Lactobacillus casei subsp. *rhamnosus* strain GG
L. casei strain Shirota
Lactobacillus fermentum strain CECT5716
Lactobacillus plantarum strain 299v
Lactobacillus reuteri strain DSM 17938
L. reuteri strain NCIMB 30242
Lactobacillus rhamnosus strain HN001
L. rhamnosus strain HN001 produced in a milk-based medium
Propionibacterium freudenreichii ET-3, heat killed[a]
Saccharomyces cerevisiae strain ML01, carrying a gene encoding the malolactic enzyme from *Oenococcus oeni* and a gene encoding malate permease from *Schizosaccharomyces pombe*
Saccharomyces cerevisiae strain P1Y0, a variant of *S. cerevisiae* parent strain UCD2034
Streptococcus salivarius K12

[a]The term "probiotic" is formally limited to live organisms.
[b]US Food and Drug Administration. GRAS noticies. https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices. Accessed February 21, 2018.

a large number of the currently available probiotic preparations (Table 3.1).[1,7]

Probiotics are not subject to minimal manufacturing standards with regulatory oversight, nor are scientifically sound studies demonstrating efficacy required when marketing a probiotic product.[3,7] Hence, for most available probiotic products, studies demonstrating that the probiotic confers a demonstrable health benefit are lacking, and even less information is available to define the mechanism(s) by which particular products promote human health in different clinical illnesses. In the United States probiotics may receive "GRAS" status ("generally recognized as safe") by the FDA, even if no efficacy data exist. GRAS substances are those "for which use in food has a proven track record of safety based either on a history of use before 1958 or on published scientific evidence and that need not be approved by the FDA before being used." Probiotics that currently possess GRAS status in the United States are listed in Table 3.2 (see also www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true). International guidelines encourage the assessment of probiotics in both food and nonfood formulations,[1,11,12] and, at least in the European Union, there is increasing regulatory oversight.

CLINICAL STUDIES OF PROBIOTICS

The available probiotic studies have many limitations and biases. Of importance, potential bias is an important consideration even in the context of the available randomized controlled trials (e.g., how randomization was conducted, blinding, missing data, analytical approach). Most often, studies of probiotics lack sufficient power to detect significant differences; this is one factor in interpreting the plethora of reported meta-analyses of probiotic use. Additional concerns include the variation in probiotic formulations, trial heterogeneity (e.g., adults, children, settings of studies), as well as in variability in the status of the conditions studied, or the treatments used (e.g., particular antibiotics administered to patients) that may impact results. One area of concern for health professionals is whether probiotic administration during or following a course of antibiotics improves the recovery from the antibiotic effects. To date, there is only limited information on the effect of antibiotic spectrum of antibacterial action and/or class on microbiota alterations and the subsequent impact on use of probiotics. Similarly, our understanding of how or if probiotics alter the intestinal microbiota, mucosal or luminal, is limited; recent data indicate substantive interindividual differences and even delays by probiotic administration in microbiota reconstitution after antibiotic exposure.[13,14]

At present, prebiotics, probiotics, and synbiotics have been studied in ≈300, 1700, and 130 randomized controlled clinical trials, respectively. Overall, the reported effects often differ among studies of similar topics. This is likely, in part, attributable to variable study design and rigor. Further, verification of probiotic content and viability is not a current standard used in reporting probiotic randomized controlled studies. For example, a review of 46 clinical trials of probiotic use in inflammatory bowel disease noted that only 23 reported studies were double-blind, randomized controlled trials and that, among the 46 reviewed trials, 32 used different probiotic products, 10 used different prebiotic products, and 4 used different synbiotics.[15] An additional concern is the study criteria leading to restricted enrollment in probiotic clinical trials. For example, a highly publicized randomized, double-blind, placebo-controlled trial, evaluating a probiotic *Lactobacillus* preparation to prevent antibiotic-associated diarrhea, enrolled 135 patients. The results suggested that the probiotic yielded benefit by significantly reducing both antibiotic-associated diarrhea and the number of patients who acquired *C. difficile*–induced diarrhea. However, only 8% of potentially eligible patients were enrolled in the study, limiting the ability to generalize the study results in clinical practice.[16]

Cochrane reviews use predefined criteria to provide a structured, collaborative, and multinational approach to evaluating interventions for the prevention and treatment of disease. Given the diversity of the probiotic literature, the Cochrane reviews of probiotic studies conducted in infectious diseases conditions are summarized in Table 3.3, providing an overview of the limited number of areas that have been evaluated by this rigorous approach. Among the infectious conditions evaluated (see Table 3.3), the studied probiotics may (1) shorten the duration and stool frequency of acute infectious diarrhea, (2) decrease antibiotic-associated diarrhea in children, (3) prevent *C. difficile*–associated diarrhea in children and adults, and (4) prevent necrotizing enterocolitis (NEC) in preterm infants. Among the other infectious conditions evaluated (see Table 3.3), datasets were small and variable, and most often results were noted to be derived from low-quality evidence.

Use of probiotics for acute gastroenteritis in children is the most common use of probiotics globally. However, two prospective, randomized, placebo-controlled, double-blind, multicenter trials of *Lactobacillus*-based probiotics, conducted in Canadian and United States pediatric emergency departments, observed no differences between children who received placebo ($N = 888$) or probiotic ($N = 882$) treatment in numerous outcome measures of gastroenteritis.[17,18] These well-done trials suggest that probiotic therapy should not be recommended for children with acute gastroenteritis, at least in Canada and the United States.

Data on use of probiotics for prevention of antibiotic-associated diarrhea (AAD) for children and adults are mixed, with moderate evidence (Cochrane analysis) supporting probiotic use in children and uncertainty in adults.[19–21] Of importance, a multicenter, randomized placebo-controlled trial in the United Kingdom (PLACIDE trial) did not identify benefit in reducing AAD or *C. difficile* diarrhea. However, disease incidence was low, and thus the trial, despite its size and excellent design, may have been underpowered.[20,22]

Prevention of *C. difficile* infection (CDI) is a key area that has remained a challenge in multiple health care settings. Cochrane analyses report that moderate evidence supports use of probiotics in prevention of primary CDI, whereas insufficient data exist on whether probiotics

may contribute to prevention of secondary CDI (i.e., prevention of recurrent CDI).[23] Multiple meta-analyses, inclusive of randomized controlled trials of differing numbers and design, support, with moderate-quality evidence, that probiotics prevent CDI in adults.[24–27] These analyses do not provide clarity on which probiotic formulation is optimal, but initial analyses suggest that multispecies probiotics may be more beneficial.[26] For one probiotic combination (*L. acidophilus*, *L. casei,* and *L. rhamnosus*; known as BioK Plus), randomized controlled trials, observational clinical data, and meta-analyses support usefulness,[23,28] whereas data, for example, for single probiotics (e.g., *L. rhamnosus* GG, *Saccharomyces boulardii*) are mixed.[23] Additional evidence suggests that probiotic administration is more likely to prevent CDI when the probiotic is given close to the first antibiotic dose,[24] when two or more antibiotics are prescribed,[26] and when CDI risk is >5%.[24,26,29]

Last, although not standard-of-care, studies support the use of probiotics to prevent NEC, even though the Cochrane analysis[30] reports that nosocomial sepsis was not reduced. A subsequent well-resourced, randomized, double-blind, placebo-controlled field trial of synbiotic (*Lactobacillus plantarum* plus the prebiotic fructooligosaccharide) administration to term or late-preterm infants in rural India reported significant reduction in combined sepsis and death (primary outcome) during the first 2 months of life.[31] The Data Safety and Monitoring Board terminated the study early because interim results convincingly favored the synbiotic preparation relative to placebo.[32] These results provide a basis for additional trials, even under challenging circumstances, to define the health benefits of probiotics.

PROPOSED MECHANISMS OF ACTION OF PROBIOTICS

Investigations are being conducted to define the mechanistic and biologic basis for the health benefit(s) of probiotics.[33] Although the mechanism(s) of action of most probiotics remain unexplored, it is generally presumed that the molecular mechanisms of probiotic activities are triggered by microbe–epithelial cell interactions at the site of probiotic application (e.g., gut, skin, vagina). Major mechanisms by which probiotics are thought to act include inhibiting bacterial growth (e.g., by bacteriocin secretion or by short-chain fatty-acid production that inhibits bacterial growth by lowering pH), suppressing expression of bacterial virulence factors, preventing colonization with pathogenic bacteria (i.e., colonization resistance), modulation of one or more of the mucosal and/or systemic immune responses, and/or improving gastrointestinal (GI) barrier integrity.[34,35] Experimental studies in vitro and in vivo are beginning to provide clues to how probiotics may act. Some data suggest that certain probiotics dampen nuclear factor kappa B activation and hence proinflammatory mucosal and/or systemic immune responses.[33,36–38] Other studies provide evidence that some probiotics augment antibody responses to immunization and/or infecting pathogens.[39–42] In some instances cell-free supernatants of studied probiotics similarly dampen inflammatory responses, suggesting that such probiotics may release cell-free antiinflammatory molecules.[36,43,44] Consistent with the idea that live organisms may not be required for probiotic-like activity, particular heat-killed probiotics or probiotic lysate formulations have yielded clinical improvement when used topically for atopic dermatitis or orally for diarrheal illnesses.[45–47] Such studies may open the way for development of specific health-promoting microbial proteins or metabolites. Although it has widely been presumed that probiotics, through mucosal adherence, displace pathogens and prevent their ability to colonize and initiate disease, experimental studies, in fact, have reported conflicting results on the ability of probiotics to displace pathogens from epithelial cells or the mucosa.[33,36,48] In human studies distinct strains of probiotics have shown differing capacities for colonization, as assessed by fecal studies.[49] Consistent with these

TABLE 3.3 Cochrane Database of Systematic Reviews: Efficacy of Probiotics in Infectious Diseases

GOAL OF PREVENTION AND TREATMENT	AUTHOR AND YEAR	NO. OF STUDIES INCLUDED IN ANALYSIS OF PROBIOTIC EFFICACY	MICROBES IN PROBIOTIC	CONCLUSION
Gastrointestinal Diseases				
Treatment of acute infectious diarrhea	Allen et al., 2011[79]	63 (7 adult, older children, or unclear age studies; 56 infant and young children studies)	Mostly *Lactobacillus casei* strain GG (13 studies), *Saccharomyces boulardii* (10 studies), and *Enterococcus* lactic acid bacteria (LAB) SF68 (5 studies)	Probiotics useful in shortening the duration of acute infectious diarrhea and decreasing stool frequency; more research needed to identify specific probiotic regimens in specific patient groups
Prevention of pediatric antibiotic-associated diarrhea	Goldenberg et al., 2015[21]	23 pediatric studies, age 0–18 yr (3938 participants)	*Bacillus* spp., *Bifidobacterium* spp., *Clostridioides butyricum*, *Lactobacilli* spp., *Leuconostoc cremoris*, *Saccharomyces* spp., *Streptococcus* spp., alone or in combination. 11 studies, single-strain probiotic; 12 studies used ≥2 probiotic strains	Moderate-quality evidence suggested a protective effect of probiotics in preventing antibiotic-associated diarrhea (number needed to treat = 10). Adverse events appeared to be rare.
Prevention of *C. difficile*–associated diarrhea in adults and children	Goldenberg et al., 2017[29]	39 studies (9955 participants)	Any probiotic strain or dose	Moderate-quality evidence suggests probiotics are effective for reducing risk of *C. difficile*–associated disease if baseline disease risk of disease exceeds 5%. However, fecal detection of *C. difficile* was not diminished.
Treatment of *C. difficile*–associated colitis in adults	Goldenberg et al., 2008[80]	4 (adult studies)	*Lactobacilli* spp., *S. boulardii*	Insufficient evidence; no evidence for probiotics by themselves; one out of the four studies showed benefit for adjunct usage
Prevention of necrotizing enterocolitis (NEC) in preterm infants	AlFaleh et al., 2014[30]	24 highly variable studies	*Lactobacilli* spp. alone or in combination with *Bifidobacterium* spp.	Probiotics reduced the risk of severe NEC and mortality in preterm infants without evidence of reduction in nosocomial sepsis
Methods of decreasing infection to improve outcomes after liver resection	Gurusamy et al., 2011[81]	2 (adult studies)	Synbiotic (containing 1×10^8 *B. breve* strain Yakult, 1×10^8 *L. casei* strain Shirota, and galactooligosaccharides [GOS])[a]; synbiotic (containing $\geq 4 \times 10^{10}$ *L. casei* strain Shirota, $\geq 1 \times 10^{10}$ *B. breve* strain Yakult, and GOS)[a]	Insufficient data in support of or against prebiotic or probiotic use to decrease postresection infections

TABLE 3.3 Cochrane Database of Systematic Reviews: Efficacy of Probiotics in Infectious Diseases—cont'd

GOAL OF PREVENTION AND TREATMENT	AUTHOR AND YEAR	NO. OF STUDIES INCLUDED IN ANALYSIS OF PROBIOTIC EFFICACY	MICROBES IN PROBIOTIC	CONCLUSION
Urinary Tract and Reproductive Health				
Prevention of urinary tract infections (UTIs) in adults and children	Schwenger et al., 2015[84]	9 studies (735 individuals, healthy or prior UTI)	Any probiotic strain, formulation, dose or frequency.	No significant benefit demonstrated but studies were small with poorly reported methods and high risk of bias
Prevention of urinary tract infections in people with neuropathic bladder	Toh et al., 2017[85]	3 studies (110 participants)	No study with oral probiotic; all studies used intravesical instillation of *E. coli* strains	No certain outcomes due to small studies with high risk of bias
Antimicrobials in bacterial vaginosis of nonpregnant women	Oduyebo et al., 2009[86]	3 (adult studies)	*Lactobacillus* spp.	Oral *Lactobacillus* augments the effects of metronidazole and is more effective than metronidazole when given intravaginally; need further studies of adverse events in *Lactobacillus* trials
Treatment of bacterial vaginosis	Senok et al., 2009[87]	4 (adult studies)	Any probiotic strain or dose	Insufficient evidence for or against the use of probiotics in treatment of bacterial vaginosis. Promising data for use of probiotics combined with metronidazole or estriol, although larger, standardized studies are needed
Prevention and treatment of vulvovaginal candidiasis in women with HIV infection	Ray et al., 2011[88]	1 (adult study)	*L. acidophilus*	No definitive evidence for or against the use of probiotics to *prevent* vulvovaginal candidiasis in HIV-infected women. No studies matching the predetermined inclusion criteria found for *treatment* of candidiasis with probiotics
Treatment of vulvovaginal candidiasis in nonpregnant women	Xie et al., 2017[89]	10 studies (1656 participants)	Any single- or multiple-species probiotic in any preparation type/dosage/route of administration. All studies used probiotics as adjuvants to antifungal drugs	Probiotics may increase rate of short-term clinical and mycologic cure but without evidence for longer-term clinical impact (low- and very low–quality evidence)
Respiratory Diseases				
Prevention of acute upper respiratory tract infections (URTI)	Hao et al., 2015[90]	12 studies (3720 participants, children, adults, all ages)	Any probiotic strain or dose	Probiotics were better than placebo in reducing the number of acute URTIs, mean duration of an episode of URTI, and cold-related school absence. However, the quality of the evidence was low or very low.
Prevention of ventilator-associated pneumonia	Bo et al., 2014[91]	8 studies (1083 participants)	*L. casei rhamnosus*; *L. plantarum*; synbiotic 2000FORTE; *Ergyphilus*; combination *Bifidobacterium longus*, *L. bulgaricus*, *S. thermophilus*	Decreased the incidence of VAP (low-quality evidence). Results were uncertain for mortality. Overall, data did not provide sufficient evidence to draw conclusions on the efficacy and safety of probiotics for the prevention of VAP in ICU patients.

HIV, human immunodeficiency virus; *ICU,* intensive care unit; *VAP,* ventilator-associated pneumonia.

observations, although *L. rhamnosus* GG (LGG) administration to 12 subjects (age 65–80 years) did not result in a detectable compositional microbiome change, functional transcriptional analyses suggested that LGG ingestion fostered an ecosystem change with new interactions among bacteria commonly regarded as health promoting.[50] Overall, debate exists over whether probiotics act via common or strain-specific mechanisms, with possibly both being important.[4] Data are clearly needed to understand the mechanisms by which specific probiotics act in specific diseases that will permit informed decisions by clinicians about the appropriate probiotic choice for use in differing clinical conditions.[51]

POTENTIAL ADVERSE EFFECTS OF PROBIOTIC THERAPY

Certain probiotics, particularly lactobacilli, lactococci, and *Bifidobacterium*, have long been proposed as generally safe based on their extensive use with likely daily ingestion by millions of people and limited reports of toxicity.[52–54] In fact, ingestion of *L. rhamnosis* GG is reported to have increased in Finland from 1 L to 6 L per person per year from 1990–2000, respectively. But fortunately, there was not an observed increase in *Lactobacillus* bacteremia.[54] Nevertheless, in general there is insufficient information on most marketed probiotic preparations to provide assurances regarding safety. This has been emphasized by a report by the Agency for Healthcare Research and Quality, concluding that current data are insufficient to address the safety of probiotics with confidence.[53] Further, a recent systematic review of 384 randomized trials of probiotics, prebiotics, and synbiotics conducted from 2015–18 identified that only 2% of trials adequately reported key safety components, and one-third of the trials gave no information on harms.[55]

Adverse events due to probiotics are generally considered in three categories: GI side effects, systemic infections, and deleterious metabolic activities.[54] Lateral gene transfer, especially of antibiotic resistance genes because antibiotics and probiotics are frequently taken together, and excessive immune stimulation have been raised as theoretical but, as yet, unsubstantiated concerns.[54] Mild GI side effects are most common but generally not concerning. In contrast, data raise concerns about the use of probiotics in vulnerable patient populations, particularly immunocompromised hosts; the severely ill (children and adults); premature newborns; those with serious comorbidities; patients with intravenous catheters, prosthetic material, or hardware; short bowel syndrome; abnormal cardiac valves; and/or the elderly, among others (Table 3.4).[52,54,56–60] In these hosts a variety of infectious complications have been reported, including bacteremia and fungemia, that have, for example, been occasionally fatal and/or led to endocarditis. For

TABLE 3.4 Populations Potentially at Risk of Harm From Probiotic Ingestion

Pregnant women
Premature neonates
Elderly individuals
Hospitalized children or adults
Immunocompromised patients (e.g., malnourished children; transplant recipients; treatment with immunosuppressive drugs, including corticosteroids; chemotherapy)
Structural heart disease (e.g., valve abnormality or replacement, history of endocarditis)
Potential for probiotic translocation across the bowel wall (e.g., active bowel leak, active colitis, neutropenia)
Critically ill children and adults
Critically ill adults
Liver transplant recipients
Patients with liver failure
Patients with HIV infection

HIV, Human immunodeficiency virus.
Modified from Doron S, Snydman DR. Risk and safety of probiotics. Clin Infect Dis. *2015;60(suppl 2):S129–S134.*

example, although *S. boulardii* (a subtype of *Saccharomyces cerevesiae,* or brewer's yeast) is an infrequent fungal bloodstream isolate, in one series 86% of *S. boulardii* fungemia episodes were identified in children or adults who ingested *S. boulardii* as a probiotic.[61] One clinical trial (PROPATRIA [Probiotics in Pancreatitis Trial] study), in particular, has raised concern about probiotic safety. In that randomized, double-blind, placebo-controlled trial designed to evaluate the effectiveness of a probiotic preparation (six different *Lactobacillus* or *Bifidobacterium* strains; total daily dose 10^{10} bacteria) on infectious complications of acute pancreatitis, there was increased mortality in the probiotic treatment group (16% in 152 patients treated with probiotics vs. 6% in the 144 patients treated with placebo; relative risk 2.53; 95% confidence intervals, 1.22 to 5.25) without any measurable impact on infectious complications.[62] The increased mortality, attributed to bowel ischemia, was significantly increased in the patients with acute pancreatitis who were treated with the probiotic. Although the mechanism(s) accounting for this striking imbalance in adverse outcomes are unknown, this trial is considered an example of deleterious metabolic effects due to probiotic use.

FECAL MICROBIOTA TRANSPLANT FOR INFECTIOUS CONDITIONS OTHER THAN *CLOSTRIDIOIDES DIFFICILE* INFECTION

The contribution of the enteric microbiome to health and disease is an expanding area of investigation. Human and murine studies of bacterial members of the microbiome, such as *Bacteroides thetaiotaomicron, Akkermansia muciniphila,* and *Faecalibacterium prausnitzii,* among others, have begun to provide insights into how common microbiome members may promote health or avert disease.[63–65] For example, *B. thetaiotaomicron* is believed to play an important role in nutrition through glycan foraging in the colonic lumen.[64,65] Conversely, microbiota disruption (also termed "dysbiosis") is believed to contribute to a wide spectrum of mucosal and systemic diseases, including obesity,[66] cardiovascular disease,[67] kwashiorkor,[68] and colon cancer,[69] among other conditions. The clinical success of FMT for therapy of CDI has spurred the study of FMT in other infectious and noninfectious conditions (e.g., metabolic syndrome, inflammatory bowel disease). Specifically, rising rates of antimicrobial resistant infections and the reported link between colonic microbiota alterations and higher rates of hematopoietic stem cell transplant (HSCT) graft-versus-host disease, bacteremia, disease relapse, and reduced survival[70–72] underpin recent clinical trials. In these trials, whether FMT can reverse colonization and disease by multidrug-resistant (MDR) bacteria and improve outcomes of HSCT are being assessed (clinicaltrials.gov; search FMT and MDR, FMT and hematologic neoplasms). Recent preliminary data reported from a clinical trial of autologous FMT versus no intervention among allo-HSCT patients suggests that autologous FMT can restore the fecal microbial diversity and composition present before antibiotic treatment.[73,73a] An alternative and emerging approach is identification of members of the microbiota that can restore colonization resistance within the colon and thus prevent colonization and infection by MDR pathogens.[74,75] These largely little-known microbiota members (e.g., *Blautia* spp., Clostridiales, bifidobacteria, *Eubacterium limosum*) are proposed as candidates for the development of next-generation probiotics.

SUMMARY AND FUTURE DIRECTIONS

The scientific definition of probiotics, "to enhance the health of the host," implies the requirement for documentation of health benefits in well-designed controlled clinical trials. At present, no marketed probiotics unequivocally meet this standard, with *L. rhamnosus* GG being the most extensively evaluated probiotic.[52,76] Furthermore, although the standard definition of probiotics refers to live organisms, the use of the term in the general population, and even within the scientific literature, is often more lax. Other unresolved issues include the impact of probiotic dosage and single- versus multiple-species composition on clinical efficacy. To date, no probiotic is approved by the FDA for any clinical use. Clinicians should discuss with patients the quality-control issues in probiotic manufacturing, the limitations of the data available on probiotic use in disease, and the potential adverse consequences of probiotic use. Defining the place of probiotics in medical care awaits improvements in the characterization of probiotics, their modes of action, and stringent reproducible studies to demonstrate benefit and safety in disease conditions. Debate remains on whether the safety and/or purported efficacy of one species or genus of probiotic can be generalized to other similar probiotics and on whether probiotics exhibit class-based versus strain- or species-specific disease activity. Based on available data, probiotic preparations should not be used in immunocompromised or critically ill populations, and caution should be exercised in individuals at the extremes of age and those with central venous catheters, disrupted mucosal barriers, short bowel syndrome, abnormal cardiac valves, prosthetic joints or valves, and other hardware or prosthetic materials. Available experimental data provide some provocative glimpses into how probiotics may act to foster human health. Beyond development of next-generation probiotics,[74,75] designer probiotics are disease-targeted recombinant probiotics engineered, for example, to absorb bacterial toxins, such as the Shiga toxins in the GI lumen,[77] or to secrete molecules that inhibit, for example, the virulence of *Vibrio cholerae*.[78] Similar to probiotic use and development, use of FMT in non–*C. difficile* infectious diseases awaits additional research on efficacy, mechanisms of action, and safety.

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The complete reference list is available online at Expert Consult.

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B Host Defense Mechanisms

4 Innate (General or Nonspecific) Host Defense Mechanisms

Alan C. Embry, Edmund C. Tramont, and Carl W. Dieffenbach

Humans are continually exposed to microorganisms in daily life. Although it is unclear the extent to which these frequent exposures impact immune function and homeostasis, we know that most of the time our armament of defense mechanisms effectively prevents disease. Host defense mechanisms against microbial invasion are a continuum that provide physical, chemical, and immune barriers operating over different time frames to prevent, contain, and eliminate pathogens. An invading microbe first encounters physical and chemical barriers—the skin, mucous membranes, the normal microbiome, and antimicrobial peptides or proteins. If microbes overcome these more immediate defenses, the next hurdle is to avoid detection by pattern recognition receptors (PRRs). PRRs bind to conserved structural motifs or molecular patterns unique to microorganisms, referred to as pathogen-associated molecular patterns (PAMPs), even though they are also present on nonpathogenic organisms.[1] Several PRR families have been described, including Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs), and retinoic acid–inducible gene-I (RIG-I)–like receptors, that is, RLRs.[2] Once activated, signaling through these receptors initiates the appropriate immune responses to eliminate an invading pathogen. The initial host defense mechanisms that sense and engage the potential pathogens are collectively referred to as the *innate immune system*. It is an evolutionarily older defense mechanism that relies on a limited repertoire of inborn sensor proteins that can provide an immediate response to pathogens without genetic rearrangement.[3] The innate immune system must be breached for an infection to occur. Even when unable to prevent or clear infection, innate mechanisms play essential roles in limiting replication and directing the microbe-specific long-lasting immune memory of the adaptive immune system (Table 4.1). The focus of this chapter is on the earliest events in the host-pathogen interaction process, the general nonspecific interactions that mitigate invasion, and the processes for the detection and possible clearance of an invading organism by the host. If infection is established, how do the initial interactions create an environment for the adaptive immune system to respond? (See Chapters 5 to 7.)

Several areas of research are significantly changing our understanding of host-pathogen interactions. First, we now understand that skin and mucosal surfaces harbor commensal microbes that act in concert with the host to maintain a state of wellness.[4] Commensal microbiota not only play a role in preventing pathogen invasion via competition but also mediate critical aspects of the development, homeostasis, and function of both innate and adaptive immune cells.[5–7] Although many of the insights to date have come from animal models, it is clear that disruptions in the balance between host and microbiota have the potential to predispose the host to local or systemic disease (see Chapters 1 and 2).[8] Second, although we have long known that the human immune system is highly variable among individuals, we are only just beginning to understand the relative contributions that heritable and nonheritable influences play on immune responses and susceptibility to infection.[9] The susceptibility, morbidity, and mortality related to almost every pathogen and to infection in general are influenced by the host's genetic makeup. This was evident years ago when it was shown that if a child's parent died of an infectious disease, such as pneumonia, the child had an increased probability of dying from an infection (Fig. 4.1).[10] Genetic associations between increased susceptibility to diseases have been mapped to sequence polymorphisms and frank mutations in many aspects of the innate and adaptive immune pathways. Such genetic effects have been identified in TLR pathways,[11–15] complement,[16,17] cytokines and chemokines or their receptors,[18–20] human leukocyte antigen (HLA) alleles,[21,22] and cellular receptors.[22,23] Although heritable factors play a substantial role in human immune system variation, a recent study of healthy human twins revealed that nonheritable influences, such as environment and microbes, may have a much greater impact in shaping our immune system.[24] It is currently unclear the extent to which nonheritable influences shape innate immune pathways and responses, but recent studies suggest that aspects of innate immunity can be functionally reprogrammed through epigenetic mechanisms. It is also increasingly evident that the delineation between innate and adaptive immunity is more of a continuum that previously understood.

Many innate immune cells mediate important roles in the initiation and development of adaptive immunity, but there is also increasing evidence that some innate cells exhibit an increased response to secondary infections or vaccination, a phenomenon referred to as innate memory or trained immunity.[25] As opposed to the highly specific nature of adaptive immunity, these enhanced secondary responses by cells, such as monocytes, macrophages, and natural killer (NK) cells, can be against the same microorganism, but more commonly provide a level of nonspecific protection against other pathogens.[26,27] One of the most intriguing examples of heterologous protection is observed with the tuberculosis vaccine, bacillus Calmette-Guérin (BCG). Epidemiologic studies suggest that vaccination with BCG at birth significantly reduces childhood mortality caused by nonrelated infections,[28,29] but evidence for a causal link is lacking. Recently, a placebo-controlled trial showed that BCG vaccination protected against an experimental infection with an attenuated yellow fever virus vaccine strain and that epigenetic and functional changes in monocytes indicative of trained immunity correlated with protection.[30] Although not conclusive, this provides evidence that trained immunity mediates at least part of the heterologous protection observed with BCG vaccination. These and many other studies demonstrate that there is still much to be learned about the roles innate immunity plays in protecting us from invading pathogens.

PHYSICAL AND CHEMICAL BARRIERS TO THE ENTRY OF MICROORGANISMS INTO THE BODY

The skin and linings of the respiratory, urogenital, and gastrointestinal (GI) tracts each comprise thin layers of epithelial cells held together by tight junctions that form barriers against the external environment. The morphologic integrity of these surfaces is an effective first line of defense against invading pathogens, but epithelia function is more than just physical barriers. As mentioned earlier, epithelial surfaces contain communities of commensal microbes that compete with invading pathogens, make antimicrobial substances, and interact with host cells to influence immune responses. Epithelia are also specialized to defend against microbes they commonly encounter by producing a wide variety

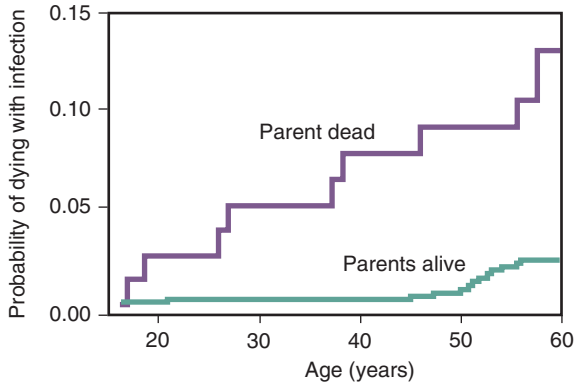

FIG. 4.1 Probability of dying from an infection before a given age for adoptees with at least one biologic parent who died before age 50 years (parent dead) of an infection versus adoptees whose biologic parents were alive at that age (parents alive). *(From Sorensen TIA, Neilson GG, Anderson PK, et al. Genetic and environmental influences on premature death in adult adoptees.* N Engl J Med. *1988;318:727–732.)*

TABLE 4.1 Features of Innate and Adaptive Immunity

	INNATE	ADAPTIVE
Components	Physical and chemical barriers Mucus AMPs Complement Innate cells (macrophages, DCs, granulocytes, NK cells)	Humoral immunity (B cells, antibodies) Cell-mediated immunity (T cells)
Receptors	Fixed in genome Rearrangement not necessary	Encoded in gene segments Rearrangement required
Recognition	Nonspecific or broadly specific molecular patterns	Antigen specific to a given pathogen
Response	Cytokines and chemokines Phagocytosis Complement action Activation of effector cells	Activation, proliferation, differentiation of antigen-specific B and T cells Secretion of antibodies Effector activity of T cells Generation of memory cells
Initiation	Many features constitutively present Immediate activation of effectors (minutes to hours)	Primary response delayed (days) Rapid recall on subsequent exposure
Duration	Can persist for days	Contraction after elimination of pathogen (weeks) Long-term memory (months/years)

AMPs, Antimicrobial proteins and peptides; *DCs,* dendritic cells; *NK,* natural killer. *Modified from Janeway CA, Medzhitov R. Innate immune recognition.* Annu Rev Immunol. *2002;20:197–216.*

of substances that inhibit microbial invasion or growth. Internal "mucosal" epithelial cells secrete mucus that can prevent microbes from adhering to their surface and can be expelled through mechanical flow. Epithelial cells also produce a diverse array of antimicrobial proteins and peptides (AMPs) and other immune mediators that can potently kill or inactivate invading microbes, often by targeting cell wall or cell membrane structures.[31]

The immune response to an invading microbe is largely dictated by the nature of the pathogen and the route of the infection. Efficient eradication of pathogens requires complex coordination between epithelial and innate immune cells at barrier surfaces, together with responding immune cells, locally and systemically.[32]

Skin

Human skin forms a complex physical and immunologic barrier to invasion by microbes. The epidermis is composed mostly of keratinocytes, which form a tight physical barrier and have a key role in detecting pathogens via a number of innate pathways. The epidermis also contains $CD8^+$ T cells and specialized dendritic cells (DCs), called Langerhans cells, that can sample microbes, migrate to draining lymph nodes, and prime the appropriate immune responses. The outermost stratum corneum layer of the epidermis supports a complex ecosystem of commensal microorganisms that are implicated in aspects of protection against pathogens, wound healing, and normal development of the immune system. The underlying dermis contains blood vessels, capillary beds, and draining lymphatics that eventually access lymph nodes. The dermis also harbors many cell types, including innate immune cells, such as macrophages, DCs, mast cells, innate lymphoid cells (ILCs), and resident $CD4^+$ and $CD8^+$ T cells.[33]

As noted, the skin possesses an array of antimicrobial properties that form a protective shield, including a battery of broad-spectrum defensive chemicals (principally peptides) able to kill or inactivate bacteria, fungi, viruses, and parasites. These AMPs respond to epithelial disruption to prevent the invasion of pathogenic organisms but also play important roles in maintaining the appropriate balance of the commensal microbiota.[34] Several AMPs in the skin, including β-defensins, the cathelicidin LL-37, and hexadecenoic acid, are depressed in atopic skin, a condition in which microbial suprainfection is common.[31]

The relative dryness, salinity, and mild acidity (the so-called acid mantle, pH ≈4.5–6) of skin, combined with normal skin microbiota, help make it an inhospitable environment for invading pathogens. Inflamed skin is more permeable and hence more hospitable to colonization. It has been speculated that oily skin may retard evaporation of water, resulting in increased numbers of colonizing microorganisms. Sebum, a lipid-rich coating that protects and lubricates hair and skin, has antimicrobial properties. However, bacterial species, including *Cutibacterium acnes*, hydrolyze triglycerides in sebum, releasing free fatty acids that ultimately promote colonization in sebaceous glands.[35] The continual desquamation of skin also aids in the elimination of microorganisms. Because few organisms can penetrate the skin, they usually gain access by some physical means, such as an arthropod vector, trauma, surgical incision, or intravenously placed catheter.

Mucous Membranes

Most pathogens enter the body through the mucosal surfaces of the respiratory, GI, and urogenital tracts. Mucus, formed by highly glycosylated proteins called mucins, which are often specific to the mucosal site, carries immune cells, antimicrobial factors, bacteria, nutrients, and waste. The continual secretion and elimination of mucus creates a flow that requires pathogens to move upstream and across epithelial surfaces. Together the physical and chemical properties of mucus create a robust barrier that is effective at immobilizing and inactivating pathogens before they contact epithelial surfaces, while also supporting commensal microbiota.[36] To subvert its function, many pathogens have developed strategies to penetrate, evade, degrade, or disrupt the production of mucus.[37]

Most mucosal epithelial cells possess the same peptide shield as those of skin.[31] However, body secretions, including saliva, cervical mucus, prostatic fluid, and tears, are endowed with unique antimicrobial properties. For example, lysozyme, one of the more potent antimicrobial substances produced in the intestine, mouth, and eye, is particularly effective against gram-positive bacteria because it enzymatically degrades peptidoglycan, a major component of bacterial cell walls.[38] However, many gram-positive and gram-negative bacteria evolved mechanisms to evade killing by lysozyme,[39] which in some cases (e.g., *Staphylococcus aureus*) may impact the balance of commensal bacteria at mucosal surfaces.[40]

Mucosal secretions contain significant levels of immunoglobulins that play important roles in the defense of these surfaces. Secretory immunoglobulin A (SIgA) is the most abundant antibody class in secretions, other than those in the genitourinary tract and lower respiratory tract, which have slightly higher levels of IgG.[41,42] Through binding to potential pathogens and toxins, SIgA can prevent their contact with the apical surface of epithelial cells and entrap them in mucus for subsequent clearance. SIgA is also capable of neutralizing pathogens or toxins that are internalized into epithelial cells.[43] In addition to

promoting the clearance of pathogens, SIgA coats the majority of commensal bacteria in the gut. This interaction facilitates targeting and sensing of commensals by tolerogenic DCs in Peyer patches of the small intestine,[44] shapes the composition of the microbiota, and contributes significantly to intestinal homeostasis.[45]

Respiratory Tract

Each breath we take brings with it the potential to inhale microorganisms that can cause respiratory disease if they are not eliminated quickly and reach the appropriate niche. To defend against this constant threat, the respiratory tract has a formidable array of antimicrobial defense mechanisms that must be compatible with the crucial function of gas exchange. Immune defenses are coordinated in relation to airway size, as effective defenses of the large airways, such as a thick mucus layer, can be harmful in smaller airways. Inhaled microbes must first penetrate and survive the aerodynamic filtration system of the upper airway and tracheobronchial tree. The airflow in these areas is turbulent, bringing large particles in contact with mucosal surfaces armed with antimicrobial defenses before they penetrate more deeply in the respiratory tract.[46]

The human trachea, bronchi, and bronchioles are lined by a highly ciliated epithelial surface that continuously propels mucus upward, leading to the mechanical clearance of any trapped pathogens that are subsequently ingested. This process (aided by coughing) is extremely efficient, leading to 90% of deposited material being cleared in less than 1 hour.[47] The airway epithelium contributes significantly to the resistance to infection through detection of PAMPs via PRRs and expression of inflammatory cytokines after infection. In addition, bronchial secretions contain antimicrobial substances able to kill or inactivate pathogens, including lysozyme, lactoferrin, β-defensins, and surfactant collectins.[48]

Once a microbe reaches the alveolus, physical expulsion becomes much less effective because, unlike the respiratory epithelium, the alveolar surface is not ciliated and lacks mucus, so it can facilitate gas exchange. As a result, alveolar epithelial cells rely on their intracellular defenses and stimulate the production of type I interferons (IFNs) to prevent the further spread of infection. Phagocytic cells such as alveolar macrophages (AMs), DCs, and neutrophils also play a more important protective role. Under homeostatic conditions, AMs phagocytose inhaled particles and degrade them without triggering inflammation or adaptive immune responses. During infection, AMs rapidly respond by activating complement (see Chapter 9), phagocytosis, producing inflammatory mediators, and recruiting neutrophils. These phagocytic cells are assisted in their defense by the collectin surfactants SP-A and SP-D, which bind to and opsonize diverse organisms, including gram-negative bacteria, viruses, and fungi.[41,49]

Like all immune defenses, these nonspecific mechanisms can be overcome by the introduction of large numbers of invading organisms (e.g., a contaminated respirator), particularly if the host is exposed for an extended period. Their effectiveness can also be decreased by environmental factors, air pollutants (e.g., cigarette smoke), mechanical respirators, concomitant infection, allergenic agents, and, in some cases, genetic defects (e.g., cystic fibrosis) and inhibitory factors of some pathogens.[46]

Gastrointestinal Tract

The GI tract must be able to sort through enormous antigenic exposure without harming the host. Although the oral mucosa has similarities with other mucosal sites, some of its immune mechanisms are unique. As a first line of defense, the oral epithelium, together with the underlying connective tissue of the lamina propria, provides resistance to the strong shear forces of mastication and helps prevent the penetration of microorganisms. Like the epidermis, the oral epithelium maintains its structural integrity and limits colonization by a process of continuous cell renewal to replace cells shed from the surface. Secretion and digestion of saliva limits the accumulation of unwanted microbes. Saliva also carries a range of host defense molecules, including mucins, lysozyme, AMPs, and SIgA and other immunoglobulins able to inactivate pathogens. Unique to the oral mucosa, the periodontal epithelium around teeth allows access of serum proteins and immune cells, providing a link between systemic and mucosal immunity.[50]

The acid pH of the stomach and the antibacterial effect of the pancreatic enzymes, bile, and intestinal secretions are effective, nonspecific, antimicrobial defense factors. The GI tract is also coated in mucus that has different properties in the stomach, small intestine, and colon. In the small intestine mucus limits the number of bacteria that can reach the epithelium and Peyer patches. In the large intestine the inner mucus layer remains relatively free of bacteria, whereas the outer mucus layer supports a subset of the commensal microbiota.[51] Paneth cells of the small intestine, located in the crypts of Lieberkühn, secrete AMPs such as β-defensins, lysozyme, REGIIIγ, and type II phospholipase A.[4,52,53] Most of these AMPs are localized in the mucus layer; thus, in addition to functioning as a physical barrier, mucus limits bacterial penetration by concentrating AMPs near the epithelial surface.[31] Mucus is continuously secreted from goblet cells and Paneth cells and moves distally with peristaltic waves, expelling potential pathogens and requiring bacteria to travel against mucus flow to reach the tissue surface.[54] Alteration of these physical and chemical parameters can lead to increased susceptibility of the host to infection and chronic disease. For example, evidence suggests that hypochlorhydric patients are more susceptible to pathogens such as *Vibrio cholerae* and *Salmonella,*[55,56] and mice deficient for the most abundant mucin, MUC2, spontaneously develop colitis and are predisposed to inflammation-induced colorectal cancers.[57,58] In addition, a number of disease states have been shown to be accompanied by the movement or translocation of gram-negative bacterial products across the epithelial barrier and into the circulation.[59] This is often accompanied by a loss of tight junctions between enterocytes or the loss of enterocytes altogether and, in some cases, may be tied to loss or impairment of Th17 cells (a subset of T cells), which stimulate mucin and AMP production, improve tight junction function, and increase IgA transport into the lumen.[60,61]

Specialized epithelial cells called microfold (M cells) and goblet cells sample luminal antigens and microorganisms for presentation to DCs and resident macrophages. Lymphocytes and other immune cells are found throughout the intestinal tract epithelium, but the majority are in the submucosal Peyer patches and lymphoid follicles (see Chapter 7).[62] The GI tract expresses PRRs on intestinal epithelial cells (IECs) and other cell types, but unlike other sites in the body where recognition of foreign microbes initiates highly inflammatory cascades, the abundance of commensals in the intestine requires an altered state of responsiveness.[63] Multiple studies demonstrate beneficial roles of commensal microbial TLR signals in gut function and homeostasis.[64,65] The ability to distinguish between commensal and pathogenic microbes is in part mediated by differential TLR signaling that occurs at apical versus basolateral surfaces of IECs. Although stimulation of basolateral TLR ligands leads to canonical activation of the transcription factor nuclear factor kappa B, apical exposure results in a net inhibitory effect through stabilization of its inhibitor, IκB. Of importance, the apical signal induces a state of tolerance to subsequent TLR stimulation, thus providing a means to differentially respond to commensal and pathogenic microbes.[66] It should be noted that although commensal microbial signals can be protective in the context of tissue damage or infection, they are implicated in a range of other diseases when homeostatic responses become dysregulated (see Chapters 1 and 2).[35,67]

Genitourinary Tract

Many immune mechanisms in the genital tract of both men and women bear similarity with other mucosal sites. Uniquely, the female reproductive tract (FRT) must be able to accept a semiallogeneic fetus while also conferring protection against pathogens. To achieve this balance, sex hormones coordinate aspects of epithelial, stromal, and immune cell number and function in each compartment throughout the menstrual cycle. Multilayered squamous epithelial cells cover the vagina and ectocervix, whereas single-layer columnar epithelial cells cover the endocervix, uterus, and fallopian tubes. The underlying stroma contains dynamic populations of immune cells; T cells are more abundant in the lower FRT, whereas granulocytes and NK cells are higher in the upper FRT. The lumen of the FRT is bathed in fluid that differs between the upper and lower tract and across the menstrual cycle. Mucus, which also varies with menstrual status, serves an important role in protecting epithelial cells from direct contact with pathogens. Cervicovaginal fluid

contains AMPs, including secretory leukocyte protease inhibitor (SLPI), human beta defensin 2 (HBD2), human neutrophil peptide 1 to 3 (HNP1–3), lysozyme, and lactoferrin. Despite the complex defense mechanisms of the FRT, accumulating evidence suggests that a window of vulnerability to infection may exist during the secretory phase of the menstrual cycle, which has programmed functions to facilitate fertilization.[68]

The human vaginal microbiota is dominated by four *Lactobacillus* spp. in most women of reproductive age, although studies show that there can be significant differences in vaginal community composition among women.[69] Under hormonal influence the vaginal epithelium contains increased amounts of glycogen that is depolymerized into simpler sugars. *Lactobacillus* spp. metabolize these sugars into lactic acid, lowering the pH of the vagina to create an environment that restricts growth of most invading organisms. The vaginal microbiota is implicated in preventing a number of urogenital diseases, including bacterial vaginosis, yeast infections, urinary tract infections (UTIs), and human immunodeficiency virus (HIV) infection.[69,70]

Urine, once considered sterile, is now known to contain commensal bacteria, although the contribution of these organisms to immune function remains to be determined.[71] The lower urinary tract is rinsed with urine four to eight times each day, eliminating potential pathogenic organisms, unless they are capable of firmly attaching to epithelial cells of the urinary tract (e.g., *Neisseria gonorrhoeae,* certain strains of *Escherichia coli*). Urinary retention or lack of complete bladder emptying impedes this flushing process. Urine is bactericidal for some strains of bacteria, mostly because of its pH, although factors such as hypertonicity, urea, and the presence of AMPs play a role. Uromodulin (also known as Tamm-Horsfall protein), a glycoprotein produced by the kidneys, is the most abundant protein in normal urine. In addition to protecting against kidney stones, it avidly binds uropathogenic *E. coli*, preventing these bacteria from gaining a foothold on the cellular lining of the urinary tract.[72]

The length of the male urethra (20 cm in an adult) also provides passive protection, and bacteria seldom gain access to the bladder in men unless introduced by instrumentation. The female urethra is much shorter (5 cm in an adult) and is traversed more readily by microorganisms, which is one reason why UTIs are much more common in women than in men.

The external surface of the nonerect penis is covered by keratinized squamous epithelium that is relatively resistant to infection unless the skin is broken or inflamed. In circumcised men, most or all of the foreskin epithelium is removed, leaving a similar dry keratinized surface also resistant to infection. In uncircumcised men the subpreputial epithelia covering the inner foreskin and glans/corona are mucosal surfaces that are more susceptible to HIV and possibly other viruses, such as herpes simplex virus (HSV) and human papillomavirus (HPV).[73]

Eye

Constant bathing of the eye by tears is an effective means of protection. Foreign substances are diluted continually and washed away via the tear ducts into the nasal cavity. Tears also contain large amounts of lysozyme, SIgA, lactoferrin, and lipocalin.[74]

INNATE IMMUNE SENSING OF INVADING PATHOGENS

PRRs are strategically located in many cell types and are localized within specific subcellular compartments to recognize and respond to invading infectious agents that breach immediate microbial defenses and physical barriers.[75] PRR families detect the full spectrum of molecules (proteins, carbohydrates, nucleic acids, and lipids in the form of PAMPs) associated with all types of pathogens, including viruses, bacteria, fungi, and protozoa. Bacterial and fungal PAMPs are often components of the cell wall, whereas because all viral components are synthesized in host cells, the main target of innate immune recognition is viral nucleic acids.[76]

Within this rapidly evolving field, several PRR families have been described. TLRs, the best-characterized PRR gene family, are expressed in both innate immune cells, such as macrophages and DCs, as well as in nonimmune cells, such as epithelial cells and fibroblasts.[77–79] There are 13 known mammalian TLRs, 10 of which are found in humans. TLRs are generally classified into two categories, depending on their cellular localization and PAMPs they recognize. Human plasma membrane-bound TLRs 1, 2, 4, 5, and 6 interact with microbial membrane components, whereas TLRs 3, 7, 8, and 9 are localized in intracellular vesicles, where they recognize viral and microbial nucleic acids.[75] TLR10 is the only human TLR member without known ligand specificity and function, although recent studies suggest that distinct from other TLRs, it functions to suppress inflammation.[80,81] PRR members of the CLR family, expressed as transmembrane or soluble proteins, bind a complex array of carbohydrates, and are essential for antifungal immunity.[82,83] Transmembrane CLRs, including dectin-1, dectin-2, mannose receptor, DC-SIGN (*d*endritic *c*ell *s*pecific *i*ntracellular adhesion molecule 3 *g*rabbing *n*on-integrin), and Mincle (macrophage inducible C-type lectin) induce intracellular signaling upon fungal recognition, whereas soluble CLRs, such as surfactant protein SP-A, SP-D, and mannose-binding lectin opsonize fungi and facilitate their recognition.[84] There are several known PRR families that detect viruses or bacteria within a cell. The NLR family, of which there are more than 20 members in humans, detects a range of microbial PAMPs in the cytosol.[85] Upon activation, NLRs recruit large signaling complexes that mediate inflammation, autophagy, or cell death.[86] The RLR family, including RIG-I, MDA5 (melanoma differentiation-associated protein 5), and LGP2 (laboratory of genetics and physiology 2), detects intracellular viral RNA.[87] In addition, an expanding repertoire of cytosolic sensors, including cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS), AIM2 (absent in melanoma 2), DAI (DNA-dependent activator of IFN-regulatory factors), DNA-PK (DNA-dependent protein kinase), IFI16 (IFN-inducible protein 16), and LRRFIP1 (leucine-rich repeat flightless-interacting protein 1) recognize and respond to cytosolic DNA and induce the production of type I IFNs.[88]

Some PRRs are also able to detect intracellular host-derived factors generated by cellular injury or tissue damage. Referred to as damage-associated molecular patterns (DAMPs), these factors can either be present before cell death or actively produced during cell death.[89] Although the original assertion that host molecules could alert the immune system and promote adaptive immune responses was controversial, specific DAMPs have now been shown to mediate inflammation via PRRs. For example, HMGB1 (high mobility group box 1) and heat shock proteins can stimulate TLR2 and TLR4.[90] Although accumulating evidence suggests that DAMP recognition can mediate aspects of innate and adaptive immunity, further research is required to better understand the extent to which DAMPs can fully substitute for PAMPs in initiating immune responses, or whether they more often operate in combination with PAMPS to elicit a specific immune response in the context of infection.

In addition to pattern recognition, the immune system has several strategies to recognize the absence of normal self-molecules that can result upon infection. The most studied example of missing self-recognition involves NK cells, the prototypical member of the ILC family (see Chapter 7). NK cells are cytotoxic innate lymphocytes that circulate through blood and tissues to defend against microbial infection and tumor progression. Patients and animals with altered NK function are more susceptible to recurrent HSV, HPV, and varicella virus infections.[91–93] The specificity and functions of NK cells are tightly regulated by a complex balance of activating and inhibitory receptors on the cell surface.[94] A major role of inhibitory receptors on NK cells is to detect class I major histocompatibility complex (MHC I) molecules, which are expressed on all nucleated cells but often downregulated during viral infection or cellular stress.[95,96] When NK cells encounter cells that have downregulated expression of MHC I inhibitory receptors (which normally prevent killing of healthy cells) they are released from inhibition, allowing the selective elimination of infected or stressed cells through the release of perforin and granzymes, as well as through engagement of target cell death receptors such as Fas.[97,98] Activated NK cells also produce proinflammatory cytokines that further stimulate innate and adaptive immune responses.[99]

Phagocytosis

Critical to the innate response are the cell types that seek and engage invading pathogens. As described earlier, both immune and nonimmune

cells at barrier surfaces express PRRs that sense and then trigger inflammatory responses. Next, specialized innate immune cells with another essential function, phagocytosis, are engaged. Phagocytosis is a complex mechanism by which relatively large particles (>0.5 μm), including altered self-particles (e.g., necrotic or apoptotic cells) or invading microbes, are engulfed within a plasma membrane envelope and then internalized into a large endocytic vesicle called a phagosome. The phagosome can then fuse with a lysosome(s) to form a phagolysosome that becomes acidified, and together with antimicrobial proteins and reactive superoxide and nitric oxide radicals usually kills the microbe.[100]

Although many cell types are capable of phagocytosis, several, including macrophages, monocytes, neutrophils, eosinophils, and DCs, are highly specialized for the process and hence termed "professional phagocytes."[101] Macrophages are the major phagocyte population in body tissues and have a diverse range of phenotypes and functions that in some cases are specialized for the location in which they reside.[102,103] Monocytes comprise ≈10% of nucleated cells circulating in blood, although significant pools exist in the spleen and lungs and can be mobilized when needed.[104,105] Neutrophils, a type of granulocyte that is the most abundant white blood cell in circulation (40%–80%), provide critical rapid defense against invading microbes. In response to inflammatory stimuli, neutrophils migrate into infected tissues, where they bind, engulf, and kill microbes, as well as release factors that inactivate extracellular microbes and prime immune responses (see Chapter 8).[106,107] There are two main functional types of DCs, both of which migrate into tissues and mediate important roles in shaping adaptive immune responses. Conventional DCs have an enhanced ability to sense inflammation, capture extracellular and cell-associated microbes, and then process them into peptides that can be presented on surface MHC molecules to induce an adaptive immune response.[108] Plasmacytoid DCs (pDCs), which circulate in blood and peripheral tissues, are best characterized for their ability to rapidly produce large amounts of type I IFNs in response to viral infection.[109,110] In addition to enhanced phagocytic activity, each of these cell types is armed with a multilayered array of antimicrobial mechanisms that inactivate pathogens, secrete cytokines, recruit immune cells, and prime adaptive immune responses.

Professional phagocytes variably express receptor systems that, together with PRRs, recognize microbes, particulate matter, and damaged host cells. Receptors that facilitate phagocytosis are classified as either opsonic or nonopsonic receptors. Opsonic receptors include Fc receptors that bind the conserved domain of IgG antibodies and complement receptors for complement-coated targets. Nonopsonic receptors involve the direct recognition of ligands on microbial surfaces. Examples include dectin-1, which recognizes β-glucans on fungal surfaces and members of the scavenger receptor family (SR-A, SR-A2, and MARCO [macrophage receptor with collagenous structure]) that bind to components of bacterial cell walls.[100] The initial contact of a target with a phagocytic cell results not only in the chemical sampling but also physical assessment of the target through the extension of pseudopodia, membrane ruffling, and the engagement of specific receptors. The physical and chemical properties of the target help instruct the nature of the subsequent response, which is also highly dependent on the state and function of the ingesting phagocyte.[111]

Many pathogens evolved mechanisms to interfere with different steps of the phagocytic process, but some, including *Mycobacterium tuberculosis* (Mtb), *Listeria monocytogenes,* and *Legionella pneumophila,* survive and replicate in phagocytes. These intracellular bacteria evolved unique strategies to perturb or evade phagosomal maturation and to withstand cellular antimicrobial factors.[112]

Autophagy

Autophagy ("self-eating") refers to a collection of processes that allow cells to digest long-lived cytosolic proteins, lipids, and organelles in autophagosomes that fuse with lysosomes. Although autophagy primarily serves as a survival mechanism in response to stress or starvation, it is now known to mediate important roles in homeostasis, development, and immunity. In concert with PRRs and other signaling pathways, autophagy can directly eliminate pathogens, regulate inflammation, secrete immune mediators, and promote adaptive immune responses through antigen presentation on MHC molecules.[113]

The importance of autophagy in immunity is demonstrated by the range of mechanisms used by microorganisms to prevent, counteract, or even exploit aspects of the process to promote their survival. For example, studies suggest that Mtb survival in macrophages is in part due to its ability to (1) inhibit the induction of autophagy, (2) disrupt autophagosome fusion with the lysosome, and (3) suppress autophagy-linked class II MHC antigen presentation.[114]

Much remains to be learned about how autophagy mediates innate and adaptive immune mechanisms that are also intrinsically linked to homeostasis. Polymorphisms in autophagy-associated genes are associated with increased risk of Crohn disease. Emerging data also implicates autophagy in other inflammatory disorders, metabolic conditions, neurodegenerative diseases, and cancers.[115] Given its many important roles, there is great interest in developing approaches to target the autophagy pathway for potential new therapies and antiinfectives.

The Inflammatory Response

The innate immune response is the assembly of signals arising from the range of PRR pathways triggered by the invading pathogen (Fig. 4.2). The nature and magnitude of the response depends not only on the class of the invading microbe but also on factors such as anatomic location, level of replication, virulence, and host-specific variables. Of importance, immune defenses are usually balanced to effectively eliminate pathogens while also minimizing damage produced by the immune system itself.[116]

When the physical and chemical barriers are breached and infectious agents enter tissues, a range of host factors and cell types are mobilized.[117] Although many of the initial response proteins are always present, it is their rapid quantitative increase that constitutes the inflammatory response. Upon sensing invading microbes or tissue damage, sentinel cells become activated and begin releasing small proteins called cytokines and chemokines (discussed later) and other mediators, such as vasoactive amines, prostaglandins, and products of complement activation. Together, these mediators induce a state of acute inflammation that increases blood flow and enables plasma proteins, and leukocytes (mostly neutrophils) to leave the circulation and accumulate at the site of infection. Once in the tissue, leukocytes become activated to destroy and remove invading microbes. If the infection is successfully eliminated, inflammation is resolved and the process of tissue repair proceeds. If the invading pathogen is not eliminated by the acute process, inflammatory signals shift to more actively engage macrophages and promote adaptive immune responses. If the combination of these responses is unable to clear a persistent pathogen, a state of chronic inflammation is established (e.g., HIV).

Cytokines and Chemotaxis

Cytokines are potent signaling proteins that orchestrate immune and inflammatory responses through communication between both immune and nonimmune cells. In response to stimuli, secreted cytokines alter the function of target cells through binding to specific cell surface receptors. Cytokines can act in an autocrine manner if the action is on the cell that secretes it, a paracrine manner if the action is on nearby cells, or an endocrine manner if the cytokine acts on distant cells.[118] Although many have both pleiotropic and overlapping functions, cytokines are known to mediate many important roles during the innate immune response and are crucial in the transition to adaptive immunity.

Detection of viral components leads to the rapid secretion of type I IFNs (IFN-α and IFN-β) and other proinflammatory cytokines. Type I IFNs can be secreted by most cell types, although pDCs, which preferentially express TLR7 and TLR9 and constitutively express IFN regulatory factor 7 (IRF7), produce the largest amounts of IFN-α/β during viral infection. Although best known for their potent ability to induce an antiviral state in both virally-infected and bystander cells, type I IFNs also have roles in defending against bacteria and other pathogens. The outcome of IFN-α/β signaling is highly context dependent. Engagement with the type I IFN receptor on target cells influences the transcription of IFN-stimulated genes dedicated to viral restriction and other cytokines, antibacterial effectors, proapoptotic and antiapoptotic molecules, and proteins involved in metabolic processes. Of importance, type I IFNs also significantly promote antigen presentation

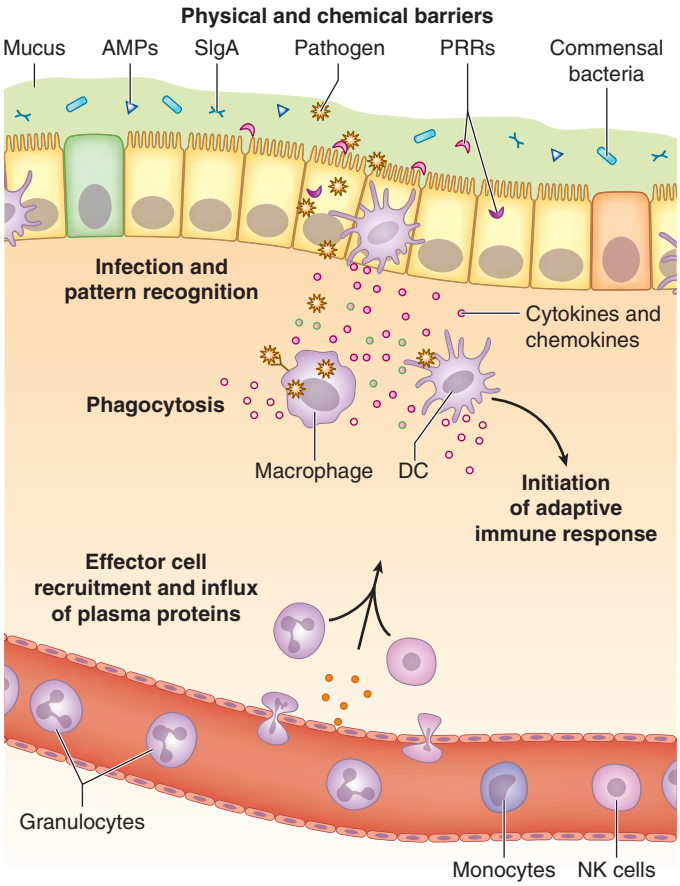

FIG. 4.2 Overview of innate immune defenses. Mucosal surfaces comprise epithelial cells interspersed with mucus-secreting cells and other cell types that produce antimicrobial factors and sample luminal antigens. Mucus carries antimicrobial peptides and proteins *(AMPs),* immunoglobulins such as secretory IgA *(SIgA),* immune cells, and other factors that can trap or inactivate pathogens before infection occurs. Commensal bacteria compete with invading pathogens, and stimulate innate immune responses. Pattern recognition receptors *(PRRs)* on the surface and within cells recognize and rapidly respond to invading pathogens by initiating signaling cascades that lead to the production of antimicrobial factors, cytokines, and chemokines. Granulocytes and macrophages ingest and kill pathogens and induce inflammation. Dendritic cells *(DCs)* produce cytokines that activate other immune cells and signal tissues to resist infection. The inflammatory response enables plasma proteins (e.g., complement) and leukocytes to leave circulation and accumulate at the site of infection. Once in tissues, they become activated to eliminate invading microbes and infected cells. If the pathogen is not eliminated, antigen-presenting cells, particularly DCs, transport antigen to lymphoid organs to promote adaptive immune responses. *NK,* Natural killer. *(Modified from Owen, JA, Punt J, Stranford S, Jones PP, Kuby J. Innate immunity. In: Owen JA, Punt J, Stranford SA, eds.* Kuby Immunology. *7th ed. New York: W.H. Freeman; 2013:142.)*

and NK-cell function, while modulating proinflammatory pathways and cytokine signaling. The net effect is the generation of adaptive responses and clearance of infection.[119]

Other proinflammatory cytokines important in the early immune response include tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6. Together, these powerful cytokines mediate central roles in stimulating natural immunity and the recruitment and activation of inflammatory cells. Cytokines, especially TNF and IL-1, increase the expression of adhesion molecules on endothelial cells and leukocytes that aid in the binding and transmigration of leukocytes into sites of inflammation. IL-1α, TNF, and IL-6 act on both the liver and central nervous system and are responsible for producing the fever and malaise that often comes with infection. In the liver these cytokines induce acute-phase response proteins, including C-reactive protein and other mediators, to promote elimination through complement activation and phagocytosis.[118]

Chemokines are a special family of cytokines that attract leukocytes (chemotaxis) to sites of pathogen invasion, although some also have direct antimicrobial activity and mediate roles in additional processes.[120] Defined by the number and arrangement of conserved cysteine residues, the majority of chemokines fall into two groups. Members of the CXC family, whose first two cysteines are separated by a single amino acid, stimulate the chemotaxis of neutrophils, monocytes, DCs, NK cells, B cells, and T cells. Members of the CC family, whose first two cysteines are adjacent, function primarily on monocytes, macrophages, and lymphocytes, with some activity on other cells types. Chemokines are produced by macrophages and other cells after recognition of invading microbes or in response to proinflammatory cytokines. The interactions of chemokines on target cell populations are extraordinarily complex. Although there appears to be a level of redundancy, with multiple chemokines signaling through the same receptor, differential expression of chemokine receptors and adhesion molecules provides the appropriate instructions for leukocyte trafficking.[121] It is then the coordinated action of chemokines, other proinflammatory cytokines, and vasoactive mediators that regulate the migration and activation of leukocytes in a stepwise process to appropriately respond to an infection.

PATHOGEN INTERFERENCE WITH INNATE IMMUNE RESPONSES

Essentially all successful pathogenic bacteria, viruses, fungi, and protozoa have evolved ways to evade or suppress innate immune responses to facilitate their establishment and replication in a host. Major points of pathogen interference parallel three major steps in innate immunity—PAMP recognition, intracellular signaling, and chemokine/cytokine expression triggered by the activated transcription factors.

Altering PAMP structure to avoid activation of PRRs is an important strategy in the establishment of infection for many pathogens and commensals. For example, *Helicobacter,*[122] *Coxiella,*[123] *Legionella,* and *Rhizobium* all have altered lipid A structures that are poorly recognized by TLR4.[124] Several fungal species, including *Candida* and *Pneumocystis*, alter their surface glycans during different stages of life to prevent recognition by CLRs, the predominant receptor family for sensing fungi.[125] Viruses such as the influenza A virus (IAV) and dengue virus replicate in membranous cellular compartments to avoid detection by intracellular RLRs, although others modify their viral genome (e.g., Crimean-Congo hemorrhagic fever virus) or shield viral RNA through binding of viral or host proteins (e.g., Ebolavirus and IAV).[126] In addition to avoiding activation of PRRs, bacteria have also developed multiple mechanisms to evade complement activation (see Chapter 9), including mimicry of complement regulatory proteins and expression of proteases or small proteins that can cleave or inhibit complement components.[127]

Many pathogens express PAMPs that can be sensed by PRRs but evolved strategies to evade intracellular signaling pathways. The most studied are viruses, some of which directly inhibit the activation of PRRs or target downstream signaling molecules to interfere with the expression of type I IFNs or the function of antiviral effector proteins. Inhibition can be mediated by virally encoded proteins that intervene in posttranslational modifications (e.g., phosphorylation) or by degrading key signaling components. The first example of viral inference with innate immunity was the blockage of the proteins responsible for the antiviral effects of IFNs.[128,129] It is now known that viruses such as human herpesvirus family members also encode microRNAs, which decrease the expression levels of specific mediators of TLR signaling. The result is avoidance of innate pathways, giving these viruses the upper hand.[130] Bacteria can also have profound effects on innate signaling pathways. For example, the anthrax toxin subunit lethal factor targets the mitogen-activated protein kinase (MAPK) kinase (MKK) for proteolytic degradation, which induces apoptosis of macrophages and DCs exposed to this toxin.[131]

A third major point of pathogen interference is on the initial inflammatory response. For example, poxviruses have devised two ways of interfering with the activation of IL-1β through the NLR pathway. First, infected cells secrete a caspase inhibitor that prevents the maturation of pro–IL-1β.[132] Second, the virus-infected cells also secrete a soluble IL-1β receptor, effectively reducing the serum concentration of this important cytokine.[133] Taken together, these adaptations demonstrate the diverse approaches that microbes take to successfully establish infection.

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5 Adaptive Immunity: Antibodies and Immunodeficiencies

Holly H. Birdsall and Arturo Casadevall

SHORT VIEW SUMMARY

- Antibodies are a principal mode of host defense against bacteria, fungi, parasites, viruses, and exotoxins prior to entry into host cells. Knowing the types and kinetics of antibody-mediated effector functions enables the clinician to better judge whether and when patients will recover from infections.
- Understanding the factors that initiate and perpetuate antibody production explains how vaccines can be optimized for protection against infectious agents and why it is particularly difficult to generate protection against pathogens whose surfaces are dominated by polysaccharide antigens.
- Defects in antibody production are the most common forms of inherited immunodeficiencies and are the only types that can be readily and effectively treated without resorting to bone marrow transplantation.
- Detection of a patient's antibody response to a pathogen is often the only means of diagnosing an infection. By further identifying the particular classes of specific antibody, the clinician can often ascertain whether the infection is ongoing or resolved.
- Specific antibodies, often in the form of monoclonal antibodies, are used as reagents to identify antigens, including infectious agents, in tissues. Some monoclonal antibodies are used for prevention and therapy of infectious diseases. An appreciation of the available assays, their strengths, and their potential pitfalls helps the clinician interpret test results.
- Infections can generate copious quantities of antigens that become incorporated into immune complexes with antibodies. Deposition of these immune complexes in blood vessel walls, renal glomeruli, or other vascularized beds causes inflammation that exacerbates the tissue injury caused by the infection.
- Depending on the structure of their Fc piece, antibodies can engage with either activating or inhibitory Fc receptors on cells and thereby enhance or downmodulate immune and inflammatory responses.
- Infections can promote the production of autoreactive antibodies, which, in turn, lead to autoimmune disease.

Antibodies are serum proteins that aid in the neutralization and clearance of pathogens or antigens. Antibodies are produced by B lymphocytes, or B cells. As a pre-B cell matures, it rearranges and selectively mutates the portion of its DNA that encodes the antigen-binding site in the antibody molecule. Each B-cell clone does this in a different way, leading to millions of B-cell clones, each producing antibodies with a slightly different configuration at the antigen-binding site. The opposite end of the antibody molecule remains constant, allowing it to interact with fixed (or invariant) elements of the immune system, such as neutrophils and monocytes that ingest and kill antibody-coated pathogens.

Antibodies were initially discovered in the 1890s through their ability to neutralize toxins. In the late 1800s, it was observed that animals immunized with bacterial toxins produced a circulating substance that could neutralize the toxin's activity. First called an *antitoxin,* this substance was later given the more general name *antibody.* In electrophoretic analyses of serum proteins, antibodies migrate in the third, or "gamma," globulin peak, which led to the alternative name *gamma globulins* or, finally, *immunoglobulins.*

IMMUNOGLOBULIN STRUCTURE

Basic Antibody Structure

Antibodies look a bit like lobsters, with the two claws serving as antigen-binding clefts (Fig. 5.1). The tail of the lobster/antibody interacts with receptors on cells of the immune system: neutrophils, monocytes, macrophages, B lymphocytes, dendritic cells, and, in certain cases, mast cells. The tail or carboxyl-terminal end of certain immunoglobulins also binds to specialized transport receptors that carry the antibody across epithelial barriers into secretions or across the placenta into the fetus. Near the insertion point of the claws into the lobster's body is a binding site for C1q, the first protein of the complement cascade, which helps to kill and clear pathogens and other antigens.

The basic antibody unit is composed of two identical light chains and two identical heavy chains. Each of the four polypeptide chains is made up of loops or domains of about 110 amino acids bridged by disulfide bonds. This structural motif is characteristic of the immunoglobulin superfamily that also includes some cell adhesion molecules, CD4, CD8, CD28, and members of the B7 family of costimulatory molecules. Light chains have two domains, and heavy chains have four or five domains. The loop at the amino ("lobster claw") end of the heavy and light chains is called the *V domain* because of its highly variable amino acid sequence. The other domains have relatively constant sequences and are called *C domains.* Each light chain is attached to its heavy-chain partner by disulfide bonds bringing together their V domains to form the antigen-binding site. The variation in amino acid sequence of the V domains is actually focused in three *hypervariable regions;* when the protein folds, the six hypervariable regions (three from the light chain and three from the heavy chain) form the walls of the antigen-binding cleft. The hypervariable regions are also called *complementarity-determining regions* (CDRs).

The two heavy chains are linked to each other by disulfide bonds to form the immunoglobulin molecule's lobster tail. There are five variations in heavy-chain constant domains, termed mu (μ), gamma (γ), alpha (α), epsilon (ε), and delta (δ). The *antibody classes* that they form are called immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin E (IgE), and immunoglobulin D (IgD), respectively. Antibody classes are also called *isotypes* because they were defined by the use of antibodies generated by immunizing phylogenetically distant animal species with human immunoglobulins. *Allotypes* are minor variations within an isotype that are found in some, but not all, humans and were discovered using antibodies generated by immunizing humans (or nonhuman primates) with immunoglobulin from other humans. *Idiotypes* are variations between antibodies that are otherwise of identical isotype and allotype. Idiotypic variations tend to be located in or near the antigen-binding site. For example, an IgG antibody specific for measles would have a different idiotype from an IgG antibody specific for mumps.

There are only two classes of light chains, kappa (κ) and lambda (λ), and they appear in all five immunoglobulin classes. On average,

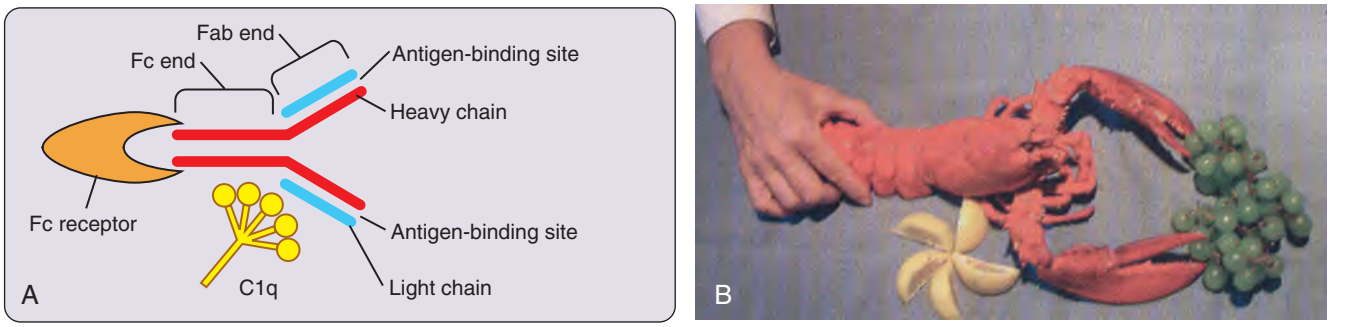

FIG. 5.1 Structure of antibodies. (A) Antibody molecules are composed of two heavy chains *(red lines)* and two light chains *(blue lines)* held together by disulfide bonds. The two heavy chains join to form a tail (Fc end), which can interact with Fc receptors on a variety of cells. The heavy and light chains each contribute to the Fab end. At the 5′ or amino-terminal end, these chains form two identical antigen-binding sites, much like two lobster claws (B). Near the hinge region of the antibody, there is a binding site for C1q, the first component of the complement cascade.

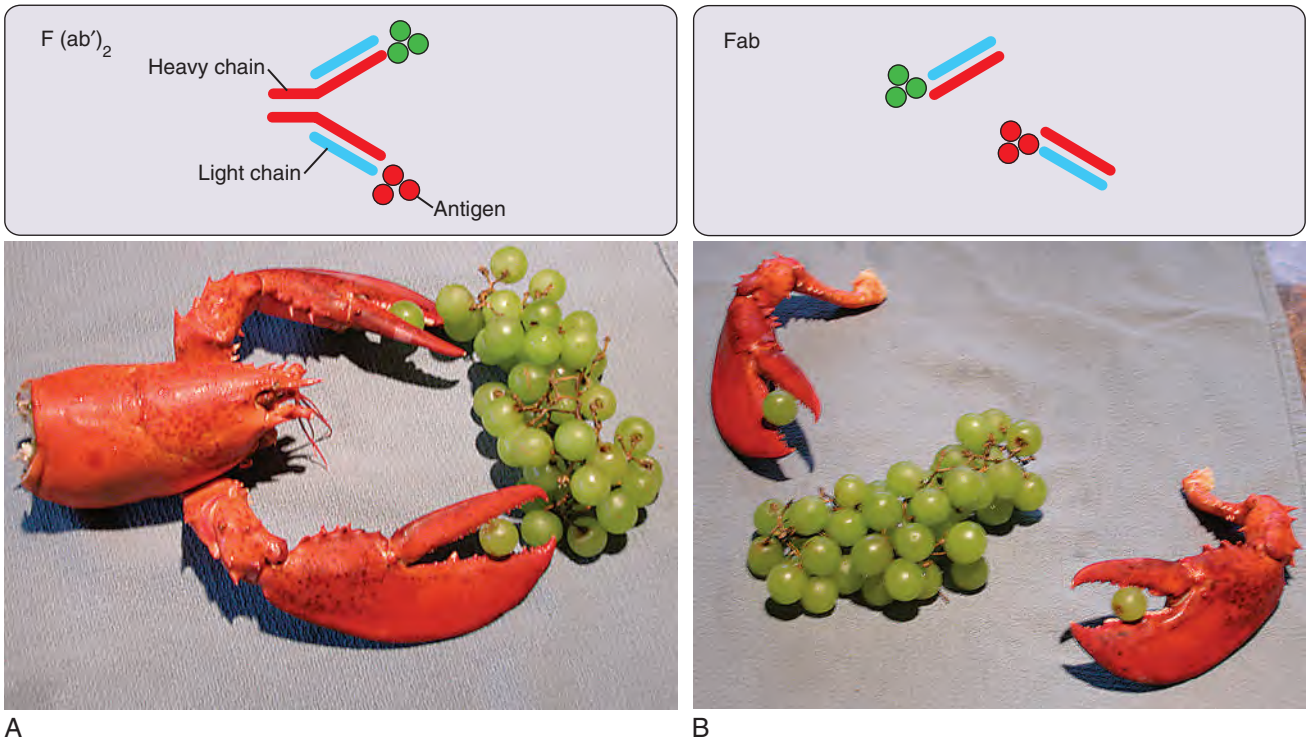

FIG. 5.2 Cleavage fragments of antibodies. (A) Papain digests the immunoglobulin molecule into an F(ab′)$_2$ fragment. This fragment is still dimeric, but it can no longer interact with Fc receptors on cells. Under reducing conditions, the disulfide bonds holding the two heavy chains together can be broken, leaving two monomeric Fab′ fragments (not shown). (B) Pepsin digests away all of the Fc piece, leaving two monomeric Fab pieces.

60% of antibody molecules use κ chains and 40% use λ chains. This information can be useful in the diagnosis of lymphomas. If virtually all of the B cells use the same light-chain class (i.e., all κ or all λ), it is likely that they arose by clonal expansion from a single malignant precursor.

F(ab′)$_2$, Fab, and Fc Pieces

Several regions of the immunoglobulin molecule have more specific names. The *Fab fragment* is the antigen-binding end, and the other end is the *Fc piece*. In the lobster analogy, the Fab region is the head and claws and the Fc region is the tail (see Fig. 5.1). All antibodies of a given isotype have the same Fc regions such that when Fc fragments were first generated by enzymatic cleavage, the identical molecules often crystallized—hence, the "c" designation. Cell receptors for the Fc pieces of antibodies are called FcRs. A Greek letter further indicates their isotype specificity; for example, an FcγR binds IgG and an FcεR binds IgE.

Papain cleaves our imaginary antibody-lobster at approximately midthorax, resulting in an Fc tail piece attached to a dimeric F(ab′)$_2$ piece (Fig. 5.2). Under reducing conditions, the disulfide linkage between the two heavy chains is broken, splitting the lobster/antibody in a sagittal direction to generate two monomeric Fab′ molecules. By way of contrast, pepsin digests the tail into tiny fragments, leaving just the two Fab monomers—claws with no lobster head (see Fig. 5.2).

Proteolytic fragments of antibodies are useful experimental reagents. Antibodies used to stain cells in immunohistochemical or immunofluorescent assays are often predigested into F(ab′)$_2$ fragments to eliminate antigen-nonspecific binding by FcRs found on many types of cells. Antibodies can be used as surrogate ligands to interact with cell surface receptors. To determine whether cross-linking of the cell surface receptor is required for signaling, the experimenter can compare the effect of dimeric intact or F(ab′)$_2$ fragments with the effect of Fab or Fab′ fragments that are monomeric and unable to cross link.

Antigen Binding, Affinity, and Avidity

Affinity refers to the strength of the interaction, which reflects the goodness-of-fit and energetics between the antigen-binding site and the antigen. Affinity is influenced by electrostatic, hydrogen-binding,

van der Waals, and hydrophobic interactions. *Avidity* measures the interaction of the intact antibody molecule and involves binding site affinity plus the additive effect of multiple antigen-binding sites. IgG, IgE, and IgD have two antigen-binding sites per antibody molecule. IgA can dimerize to generate four antigen-binding sites, and IgM is a pentamer with 10 antigen-binding sites. Because it is unlikely that all 10 antigen-binding sites will disengage simultaneously, IgM molecules can have relatively high avidity for a multivalent antigen even when the affinity of their antigen-binding site is relatively low.

The *epitope* is that portion of the antigen that fits into the antigen-binding cleft. The antigen-binding cleft can accommodate as many as 6 to 12 amino acids. *Linear epitopes* are composed of contiguous amino acids or oligosaccharides, whereas *conformational epitopes* are formed by protein and polysaccharide segments that are brought into close apposition by molecular folding. Peptide vaccines generate antibodies to linear epitopes. Denaturation or degradation can abolish native conformational epitopes while also generating novel conformational epitopes. A large antigen may have many epitopes and can react with multiple antibody molecules at the same time.

Immunoglobulin Classes

The concentrations of the five isotypes in serum vary widely, reflecting both different numbers of B cells producing each isotype and different intrinsic half-lives of the immunoglobulin classes. The isotype of an antibody dictates where in the body it is likely to be found and what types of effector functions it can mediate (Table 5.1).

Immunoglobulin M

IgM has the largest molecular weight of all of the isotypes, 900 kDa, which keeps it largely restricted to the intravascular compartment. It is composed of five immunoglobulin monomers whose μ chains are either covalently linked through disulfide bridges or held noncovalently by a joining, or "J," piece produced by the B cell. Steric hindrance typically allows only 5 of IgM's 10 antigen-binding sites to engage antigen simultaneously. Still, this multivalent binding ability allows IgM to provide effective defense despite its characteristic low affinity for antigen. IgM antibodies defend the host by blocking binding of pathogens to cells and by aggregating infectious agents to facilitate their clearance. IgM antibodies fix (activate) complement more efficiently than any other isotype. Monomeric IgM is displayed on the surface of B cells. This membrane IgM allows the B cell to detect encounters with cognate antigen and triggers its subsequent activation and proliferation.

Immunoglobulin G

IgG is the most plentiful isotype in the serum because of its high production rate (25 mg/kg/day) and its half-life of 23 days, which is 4 to 10 times longer than that of the other isotypes. As a 150-kDa monomer, IgG can move into the extracellular fluid so that less than half of the body content of IgG is in the circulation at any one time. IgG is the only isotype that is carried across the human placenta to the fetus. Beginning at 20 or 21 weeks of gestation,[1] maternal IgG crosses into the fetal circulation by means of a special placental transport receptor, FcRn.

There are four subclasses of the γ heavy chain: IgG1, IgG2, IgG3, and IgG4. They vary in amino acid composition and degree of glycosylation. IgG1 and IgG3 can fix complement, but IgG2 and IgG4 do not. Antibodies to proteins are largely IgG1 and IgG3. Antibodies to polysaccharides tend to be of the IgG2 subclass,[2] and individuals deficient in IgG2 may show an increased susceptibility to infections with encapsulated organisms.[3] Responses to helminths tend to be of the IgG4 class,[4] but there is no evidence that individuals deficient in IgG4 have a higher susceptibility to these organisms.[2]

TABLE 5.1 Characteristics of the Immunoglobulin Classes

	ISOTYPE			
CHARACTERISTIC	**IgM**	**IgG**	**IgA**	**IgE**
Half-life in serum (days)	10	21	6	2
Normal serum level in adults (mg/mL)	0.6–3.5	6.4–13.5	0.7–3.1	0.00004
Transported into secretions	±		+	
Crosses placenta to fetus		+		
Blocks binding of pathogens or toxins	+	+	+	
Opsonizes for phagocytosis via FcR		+		
Fixes complement via C1q	+	+		
Mediates ADCC		+		
Binds mast cells				+

ADCC, Antibody-dependent cellular cytotoxicity; *Ig,* immunoglobulin.
Modified from Stites DP, Stobo JD, Wells JV, eds. Basic and Clinical Immunology. *7th ed. Los Altos, CA: Appleton & Lange; 1991, with permission.*

Immunoglobulin A

Each day, humans produce about 66 mg of IgA per kilogram of body weight, which is about twice the quantity of IgG produced.[5] However, serum levels of IgA are relatively low, because most of the IgA is produced by submucosal plasma cells and immediately transported into secretions. There are two subclasses of IgA. IgA_1 is monomeric and found primarily in the serum. IgA2 can polymerize into multimers linked by the J piece and is transported into the secretions. Dimeric IgA, produced by submucosal B cells, binds to secretory component (SC) produced by epithelial cells. The IgA is endocytosed and carried through the cytoplasm of the epithelial cells. On the apical side, SC is cleaved, releasing the IgA into mucosal secretions. A fragment of the SC remains associated with the IgA molecule and protects it from cleavage by proteases in the secretions. IgM can also be carried across epithelium by this process.[6] Some pathogens express ligands that allow them to co-opt this transport system and use it to cross in the reverse direction into the subepithelium.[7] IgA-coated Epstein-Barr virus may gain entry into nasopharyngeal cells by this route.[8]

IgA defends mucosal surfaces against invading pathogens. IgA blocks the binding of bacteria, viruses, and toxins to cell receptors. IgA cross links pathogens and facilitates their clearance by ciliated epithelium. In the intestinal tract, IgA binds food antigens and prevents triggering of proinflammatory responses. IgA does not activate complement or bind to phagocytes. The relative inability of IgA to initiate inflammatory responses allows food antigens to be sequestered without deleterious consequences.[9] Failure of this process in IgA-deficient individuals may account for their increased frequency of allergic diseases.[10]

Immunoglobulin D

IgD is produced by all B cells during early stages of differentiation and is expressed on the cell membrane, where it has a key role in cell signaling. However, very little IgD is found in the serum, and IgD currently has no known effector role in host defense.[11]

Immunoglobulin E

High-affinity FcεRs scavenge IgE so quickly that its half-life in the circulation is only about 2 days, and very little is found in the serum. Once bound to mast cells, IgE persists for a long time, perhaps for the life span of the mast cell. Infused IgE can be detected on murine mast cells for up to 7 weeks.[12] IgE displayed on the mast cell surfaces mediates immediate hypersensitivity or allergic reactions. IgE appears to have a role in defense against parasitic infections. Mast cells are needed to clear intestinal helminthic infections,[13] and IgE-deficient mice, when infected, have higher burdens of *Schistosoma mansoni.*[14]

EFFECTOR FUNCTIONS MEDIATED BY ANTIBODIES

Historically, antibodies were considered as linker molecules or "transducers" to tag the pathogen and become a physical link between the pathogen and the killing mechanism, typically a leukocyte. As such, they were viewed as having no intrinsic antimicrobial function. However, in recent years several antibodies have been shown to have direct antimicrobial effects on bacteria and fungi.

Blocking or Neutralization

Invasion of host cells is a critical step in infectious processes, and a major protective function of antibodies is preventing the binding of viruses, toxins, or bacteria. The challenge for vaccine developers is to identify which microbial epitopes are integral to the pathogenic process and then devise vaccines that generate specific antibodies to block those interactions. Generation of protective immunity can be extremely difficult if, for instance, the key epitope is located deep within a cleft in the native protein and is inaccessible to antibodies. Steric hindrance is not the only way that antibodies can prevent infection. For example, picornavirus has multiple binding sites, but infection can be blocked by a single antibody. This suggests that antibody binding affects the charge characteristics or conformation[15] in the pathogen. Verifying that an antibody has "blocking" or "neutralizing" activity is key. Antibodies that bind to a pathogen but fail to effectively neutralize or block infection can paradoxically facilitate infection by allowing the pathogen to be taken into the cytoplasm through FcR or other receptors.[16] Such antibodies can also exacerbate the morbidity of an infection by triggering inflammatory processes without actually controlling the infection.[17]

Blocking adherence of bacteria and viruses to mucous membranes is probably the major defensive role of IgA in secretions. Viruses can also be neutralized by IgA within the cytoplasm of epithelial cells in the course of transepithelial transport.[18] Blocking is independent of the Fc piece; it can be accomplished by antibodies of any isotype and even antibodies from other species. This accounts for the efficacy of equine antitoxin in the early treatment of diseases such as tetanus.[19] However, nonhuman immunoglobulin is perceived as foreign by the immune system and triggers an antibody response. The complexes that form between horse immunoglobulin and human antibodies to horse immunoglobulin caused serum sickness, a condition with considerable morbidity and some mortality. Antisera produced in other animals are rarely used in the United States today except in cases such as snake antivenin, when it is not feasible to generate human hyperimmune sera. However, immune animal sera continue to be used in less resource-rich areas of the world for such purposes as prophylaxis against rabies.

Complement Activation

Complement is a series of serum proteins that augment or "complement" the action of antibodies by facilitating phagocytosis, attracting leukocytes, and directly lysing microbes (see Chapter 9). Antibodies interacting with and initiating the complement cascade are said to activate or "fix" complement.

IgG and IgM, but not IgG4, IgA, or IgE, have binding sites for C1q, the first protein in the classical complement cascade. To be activated, C1q must interact with at least two C1q binding sites. As a pentamer, IgM has five C1q binding sites, so only a single IgM molecule is needed to activate the cascade. For IgG, with only one C1q binding site per molecule, activation requires that C1q straddle at least two IgG molecules that are sufficiently close together. Because the C1q binding site is not accessible until the antibody binds to antigen, complement is not activated by soluble immunoglobulin in the circulation. Binding to antigen leads to a conformational change in the IgG molecule that increases the affinity of the C1q binding site 10,000-fold.[20]

When C1q is engaged by antibodies, it undergoes a conformational change of its own and activates the next member of the cascade. Eventually, C1r, C1s, C4, C2, C3, C5, C6, C7, C8, and C9 are activated, in that order. Complement provides immune defense by enhancing the uptake of C3b-coated pathogens, directly lysing target cells, and promoting the influx of immune effector cells. Antibodies augment the defensive efficacy of complement by greatly accelerating the rate at which complement is activated and by focusing the effect of complement onto the surface of the antibody-coated particle.

Opsonization

Neutrophils, monocytes, and macrophages are collectively referred to as *phagocytes* on the basis of their ability to ingest antigens. Phagocytes pull pathogens into phagosomes, where the organisms are killed with toxic agents, such as reactive oxygen species, nitric oxide, and enzymes. Phagocytes have pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) endogenous to many microbes (discussed later). Phagocytes also have receptors for the Fc end of IgG molecules (FcγRs) and for the C3b fragment of complement, and they use these to recognize and ingest IgG- or C3b-coated targets. Facilitation of phagocytosis is called *opsonization,* and IgG and C3b, in this role, are called *opsonins.* As an opsonin, IgG expands the repertoire of the immune system by enabling phagocytes to recognize pathogens, such as viruses, that do not express any PAMPs. C3b can bind spontaneously to the surface of microbes through the alternate pathway of complement activation. However, the accumulation of C3b on the surface of the pathogen is greatly accelerated when antibodies bind to the microbe first, fix C1q, and activate the complement cascade through the classical pathway.

Phagocytes can attach to C3b-coated targets by means of their C3b receptors. However, to complete the phagocytic process, the leukocyte needs to receive a second stimulus. This signal can come from the interaction of its FcR with the Fc of IgG bound to the pathogen, or it can come from C5a fragments generated by activated complement. Signaling through the FcγR also triggers an oxidative burst that increases the ability of the phagocyte to kill the organism it has just ingested. There are no FcμRs on phagocytes, so IgM cannot opsonize in this manner. However, a single molecule of IgM can activate complement through the classical pathway, leading to the deposition of many C3b molecules that can act as opsonins. IgA does not activate complement by the classical C1q pathway but can provide a site for deposition of C3b and thereby activate complement through the alternate pathway.[21]

There are two major families of receptors for IgG, type I and type II.[22] Type I Fcγ receptors include FcγRI, FcγRIIA, FcγRIIB, FcγRIIC, FcγRIIIA, and FcγRIIIB, and largely mediate effector functions of IgG such as opsonization and antibody-dependent cellular cytotoxicity (ADCC). Type II Fcγ receptors include DC-SIGN (*d*endritic *c*ell–specific *i*ntercellular adhesion molecule-3-grabbing *n*onintegrin) and CD23, and largely mediate antiinflammatory functions through induction of interleukin (IL)-33, suppressor monocytes, and helper T cell 2 (Th2) and T-regulatory (Treg) cells. Conformation of the Fc region of IgG is dictated by the composition of the Fc-associated glycan located in a cleft between the C_H2 regions of the two heavy chains.[22] Branching fucose moieties in the cleft induce an "open" configuration that promotes binding to type I FcR. Sialylization of the central N-linked glycans in the cleft promotes binding to type II FcγR. Fc glycoengineering can be used to modify the N-linked glycans on therapeutic antibody preparations to increase their desired efficacy in vivo. IgG1 and IgG3 interact effectively with FcγRs, but IgG2 and IgG4 do not.[22]

FcγRI has a high affinity for IgG, and it is the only FcγR that can bind monomeric IgG. FcγRI is constitutively expressed on monocytes and macrophages and can be induced on neutrophils, eosinophils, and basophils. FcγRII and FcγRIII have much lower affinity for IgG and only bind IgG in immune complexes or in aggregates. When engaged in immune complexes, IgG undergoes a conformational change that increases its affinity for the FcR. Furthermore, the additive effect of multiple FcRs interacting with the IgG molecules in the immune complex increases the overall avidity of the interaction. The restricted ability of FcγRII and FcγRIII to only bind to IgG in immune complexes ensures that they are only engaged during an ongoing immune response to antigen and not to monomeric IgG during steady state.

FcγRs can also be divided into activating versus inhibitory.[22] The activating FcRs are FcγRI, FcγRIIA, FcγRIIC, and FcγRIIIA, which have immunoreceptor tyrosine-based activation motifs (ITAMs) that provide docking sites for the Syk family of kinases, with subsequent activation of downstream pathways. Signaling through FcγRIIIA on natural killer (NK) cells and monocytes triggers ADCC and production of interferon-γ.[23] Antibodies with no neutralizing effect in vitro may still have protective activity in vivo through their ability to activate cells through their FcγRs.[24] There are also clinical correlations between expression of low-affinity variants of FcγRIIA and increased susceptibility to bacterial infections and sepsis. For example, homozygosity of the R131 variant of FcγRIIA, which has lower affinity for IgG2, is found more frequently in patients who develop sepsis as opposed to infected but nonseptic patients, and the H131 low-affinity variant is twice as prevalent in children who

develop sepsis with *Streptococcus pneumoniae,* as opposed to healthy blood donor controls.[25,26]

FcγRIIIB has no intracellular domain and can signal only through interaction with other receptors. The only FcγR that can antagonize the immunostimulatory signals of the other activating FcγRs is FcγRIIB with its immunoreceptor tyrosine-based inhibition motifs (ITIMs). For example, FcγRIIB is present on B cells, where it exerts a negative signal to downregulate antibody production when the formation of immune complexes indicates a relative excess of antibody.[27]

The ability of antibodies to engage activating and inhibitory receptors means that the antibody response in infection has the capacity for modulating the immune and inflammatory response. In this regard, the ability of an antibody response to be pro- or antiinflammatory will reflect a complex formula that includes the type of isotype made, the ability of the antibody to activate complement, the Fc receptor(s) engaged, and the type of antigen-antibody complexes that are made.

In addition to its role in transporting IgG across the placenta, FcRn has a pivotal role in extending the half-life of IgG in the circulation.[28] FcRn is found on the surface of macrophages and endothelial cells that pinocytose IgG. FcRn binds to IgG in the endosome, directs it away from the lysosomes, and instead recycles the IgG to the cell surface and back into the circulation.

There is an FcR for IgA (CD89) on monocytes, neutrophils, macrophages, and perhaps eosinophils, particularly those in the lungs.[29] There is no FcR for IgM, and antibodies of these classes cannot serve as direct opsonins for phagocytosis.

Antibody-Dependent Cellular Cytotoxicity

ADCC is carried out by monocyte-macrophages, NK cells, and neutrophils. ADCC allows these effector cells to kill targets, such as tumor cells and virally infected cells, that are too large to be ingested. Perforins, granzymes, and in some cases reactive oxygen intermediates are involved in this microbicidal activity. The role of the IgG antibody in ADCC is to bind to the FcγRIII or FcγRII on the NK cell or monocyte and identify the target cell to be killed. ADCC activity is a mechanism by which some nonneutralizing antibodies are able to protect against viral infections.

Direct Antibody-Mediated Antimicrobial Functions

Although antibodies have classically been considered as connector molecules between microbial antigens and effectors of the immune system such as phagocytes and complement, there is increasing evidence that some antibodies have direct antimicrobial activities. IgM and IgG are directly microbicidal to the outer surface protein of *Borrelia burgdorferi* in the absence of complement.[30] Antibodies to fungal mannoproteins are directly fungicidal against various fungi, including *Candida albicans.*[31] Although the mechanisms by which antibody can mediate direct microbicidal activity are not well understood, there is evidence that antibodies can induce metabolic changes in certain microbes. For example, antibody binding to the capsules of both the fungus *Cryptococcus neoformans*[32] and the bacterium *S. pneumoniae*[33] results in transcriptional changes that predispose the former to antifungal agents and activate an autolytic pathway in the latter.

Antibody-Mediated Catalysis

Many antibodies have catalytic-like motifs in their binding sites that allow them to hydrolyze antigens. Antibody-mediated catalytic activity has the potential to aid host defenses by cleaving microbial antigens that are involved in pathogenesis.[34] Consequently, some of these catalytic activities have been associated with resistance to infection. Specifically, an antibody that cleaves the urease made by *Helicobacter pylori* has been shown to reduce bacterial burden in experimental models of infection.[35] Similarly, other monoclonal antibodies have been shown to cleave viral and fungal antigens. Antibody-mediated catalysis is generally much slower than that observed with classical enzymes, but given that infectious processes take time, this activity could contribute to host defense by deactivating microbial components.

KINETICS OF ANTIBODY PRODUCTION AND DIAGNOSIS OF INFECTIONS

On first encounter with an antigen, the immune system generates a primary response that is detectable within 5 to 7 days (Fig. 5.3). This is the timing of the response in a laboratory setting with highly sensitive assays. The apparent kinetics of a patient's antibody response would be influenced by the sensitivity of the assay available to measure the antibodies such that a more sensitive assay would be able to detect a response earlier than a less sensitive assay. The primary antibody response consists of IgM antibodies with a relatively low affinity for antigen. Within several days to weeks, some antibody-producing B cells switch to making antibodies that have the same antigen specificity but of an IgG, IgA, or IgE isotype. To switch isotypes, B cells must receive signals from activated helper T cells that are typically specific for and activated by the same antigen as the B cells. Under the influence of T cells, some B cells also become memory cells.

On subsequent exposure to the same antigen, memory B cells rapidly divide and begin to produce large quantities of antibody within as little as 1 to 2 days. Faster kinetics and greater quantities of antibody are the hallmarks of a secondary antibody response. Secondary responses are also largely IgG, IgA, or IgE isotype, as opposed to IgM. The average affinity of antibodies in a secondary response is much higher than in a primary response. As the B cells divide in the lymph node germinal center, point mutations occur in the hypervariable region of the gene encoding the antigen-binding site. B-cell survival depends on continued antigen stimulation. B cells whose mutations increase their affinity have a competitive survival advantage. The net effect is to produce IgG antibodies with a progressively higher affinity for the immunizing antigen. As more and more antigen is incorporated in antigen-antibody complexes and metabolized, competition among B cells for antigen stimulation increases.

As the antigen stimulus becomes more scarce, this and other regulatory influences act to slow the production of antibody. Only a low level of residual production of IgG persists in response to antigen associated with follicular dendritic cells. However, quiescent memory B cells lie in wait for the next challenge with this same antigen. Should antigen reappear, these memory cells can rapidly activate, proliferate, and begin producing antibodies.

When the timing of antigen exposure is well defined, as with vaccines, it is easy to distinguish between the primary and secondary responses.

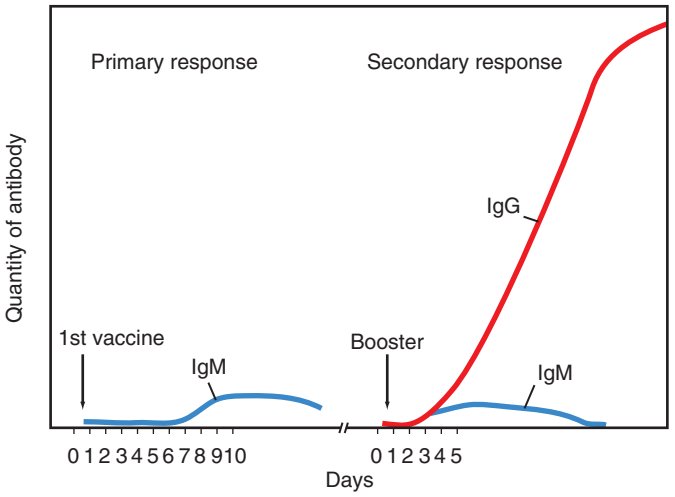

FIG. 5.3 Primary and secondary immune responses. A first exposure to antigen stimulates production of immunoglobulin M (IgM) antibodies (*blue line*), which become detectable within about a week. The antigen-specific B cells proliferate, and some become memory cells. On subsequent exposure to the same antigen, these memory cells are rapidly activated. Antibodies, mainly of the IgG class (*red line*), appear within about 2 days, and the quantity of antibody produced is much higher than that seen with the primary response. The affinity of the antibodies in the secondary response is also markedly higher than that seen in the primary response.

However, in the case of infection, antigen is released over a prolonged interval and there is no clear border between primary and secondary responses. So long as new clones of B cells emerging from the bone marrow are encountering antigen, IgM will be produced. Therefore the presence of IgM antibodies is indicative of active infection. By contrast, a response that is solely of the IgG isotype is considered to indicate a resolved infection. During the first few weeks of an infection, antigen-specific B cells proliferate and produce ever-increasing quantities of antibody. Therefore a rising titer of specific antibodies also indicates an active infection.

To compare titers, serum should be obtained at presentation (the acute serum) and again 1 or more weeks later (the convalescent sample). For maximum accuracy, the acute serum should be stored in a freezer and sent to the laboratory at the same time as the convalescent sample, so that the two samples will be assayed at the same time with identical reagents.

LABORATORY MEASUREMENT OF ANTIBODIES

Quantification of Total Immunoglobulin

Serum IgG, IgM, and IgA are readily quantified by most clinical laboratories. Age-specific reference tables must be used for children, because normal ranges for the various immunoglobulin classes are highly dependent on age. Two-thirds of IgG is of the IgG1 subclass, so a deficiency in this subclass would be apparent when total IgG is measured. However, patients who are missing IgG2, IgG3, or IgG4 could still have normal total IgG levels, and it may be appropriate to measure the subclasses individually.

By far the most common immunodeficiency is an absence of IgA, often in conjunction with a deficiency of one or more subclasses of IgG. Measurement of serum IgA is adequate to identify most IgA deficiencies. Deficiencies of the secretory piece, such that patients have serum IgA but no secretory IgA, is a very rare defect. Measurement of total IgE is not useful in the diagnosis of atopy or allergy, because serum levels may be normal or only mildly elevated in patients with clinically significant atopic disease (e.g., asthma, allergic rhinitis, urticaria). However, serum IgE may be strikingly elevated in parasitic infections.

Serum Protein Electrophoresis for Monoclonal Gammopathies

Antibody responses are typically polyclonal, meaning that many clones of B cells are stimulated, and the array of antibodies produced recognizes many different epitopes. These antibodies have diverse electrophoretic mobilities and produce a broad gamma globulin peak on protein electrophoresis. In multiple myeloma or other monoclonal gammopathies, a single B-cell clone proliferates in an unrestricted manner and produces large quantities of a single antibody type. This homogeneous product, referred to as a monoclonal antibody or M protein, appears as a spike with a single electrophoretic mobility.[36] Light chains from this clone may appear in the urine as Bence Jones proteins.

Measurement of Functional Antibody

When infectious agents cannot be cultured or otherwise specifically identified, diagnosis may depend on the ability to demonstrate that the patient is mounting a specific humoral response to the suspected pathogen. Common clinical examples would be hepatitis B, hepatitis A, Epstein-Barr virus, and cytomegalovirus, in which it is far easier to measure an ongoing antibody response than to culture the virus as a means of diagnosis. An ideal antibody test should be quantitative so that it can be used to look for rising titers indicative of active infection. If the test can also discriminate between IgG and IgM antibodies, then the presence of an IgM response can be used as indication of an active infection.

The most common format is a solid-phase immunosorbent assay. Antigen, such as an extract from the infectious agent, is immobilized on a plastic surface such as a microtiter plate or beads (Fig. 5.4A). The patient's serum is allowed to interact with the antigen, and nonspecific, unbound antibody is washed away. Then a detection reagent is added to measure the quantity of patient antibody bound to the antigen. The detection reagent is an antibody to human immunoglobulin that has been prepared by immunizing a sheep, rabbit, goat, or other animal. The greater the quantity of patient antibodies adherent to the antigen, the greater the amount of anti–human immunoglobulin that binds. Class-specific antiimmunoglobulin reagents can be prepared by immunizing the animal with a single isotype of human immunoglobulin (i.e., only IgG or only IgM) and removing antibodies that cross-react with light-chain antigens that would be present on other isotypes. Using these, it is possible to determine whether the patient's antibodies are IgM, IgG, IgA, or IgE. The anti–human immunoglobulin is typically purified and conjugated with a reagent to facilitate quantification. In an enzyme-linked immunosorbent assay (ELISA), the antiimmunoglobulin is conjugated with an enzyme, such as a peroxidase or a phosphatase, that converts a substrate to a colored or luminescent product. In a radioimmunoassay, the anti–human immunoglobulin is

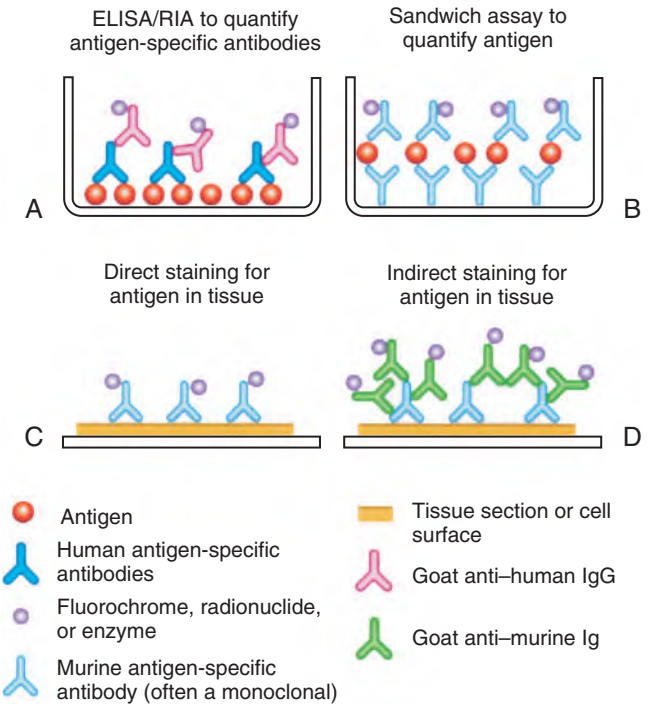

FIG. 5.4 Antibody-based assays. (A) In a solid-phase assay for antigen-specific antibodies, antigen is coated on a surface, typically a plastic well. The patient sample (e.g., serum) is incubated with the antigen, after which unbound antibodies are washed away. Patient antibodies bound to the antigen are detected with an antibody to human immunoglobulin that is produced by immunizing another species (in this case, a goat). The goat anti–human immunoglobulin *(Ig)* is conjugated with a moiety that allows it to be detected. This could be a radionuclide, a fluorochrome, or an enzyme that converts an added substrate to a colored or luminescent product. (B) A sandwich format can be used to measure antigen. A capture antibody is immobilized in the plastic well. The source of antigen is added, and then a second antigen-specific antibody is added to detect the bound antigen. This detection antibody is conjugated with a radionuclide, fluorochrome, or enzyme. The two antibody layers in the sandwich assay must recognize different epitopes on the antigen; otherwise, the first antibody would occupy all the epitopes and there would be none left for the second antibody to bind. (C) Direct staining for antigens in tissue or cells uses antigen-specific antibodies (often monoclonal antibodies) that have been purified and conjugated with a detection molecule such as a radionuclide, a fluorochrome, or an enzyme. (D) Indirect staining for antigens in tissues or cells uses an unconjugated antigen-specific antibody as the first reagent. This antibody is detected with the use of a second antibody (directed against the first antibody) that has been conjugated with a radionuclide, a fluorochrome, or an enzyme. The indirect technique often gives a higher signal than the direct technique, because multiple secondary antibodies (goat anti–mouse Ig in this case) can bind to one primary antibody (mouse monoclonal antibody in this case). However, the background staining for the indirect technique is also considerably higher than is seen with a primary or direct technique. *ELISA,* Enzyme-linked immunosorbent assay; *IgG,* immunoglobulin G; *RIA,* radioimmunoassay.

labeled with a radioisotope. In a fluorescence-linked immunosorbent assay (FLISA), the antiimmunoglobulin is conjugated with a fluorochrome. Ideally, the assay should include a standard curve prepared with purified specific antibody so that the results can be reported as actual concentrations, not optical densities or counts per minute.

A solid-phase immunosorbent assay can be isotype specific, sensitive, quantitative, and adapted for analysis of high numbers of samples. Its principal drawback is the potential for a false-positive result from two possible causes. Ideally, only antigen-specific antibodies from the patient's serum would bind to the immobilized antigen. However, nonspecific antibodies will also bind to the plastic well or bead containing the immobilized antigen (background binding). These nonspecific antibodies will be detected by the anti–human immunoglobulin. Background binding becomes a particular problem when a patient has abnormally high levels of serum immunoglobulin, as may occur in certain chronic infections such as malaria. For this reason, a background determination in which patient serum is added to wells without antigen should be included to estimate nonspecific uptake of immunoglobulin. The second cause of false-positive results involves the nature of the immobilized antigen. In many assays, the antigen is the pathogen or a relatively crude homogenate of the pathogen as a complex mixture of biologic products. A patient may coincidentally have antibodies that cross-react with an epitope present in the mixture. For this reason, positive results in solid-phase immunosorbent assays such as the human immunodeficiency virus (HIV) ELISA, which has been designed to be highly sensitive at the cost of being less specific, must be verified using the more specific Western blot.

Western Blot

A Western blot assay is a solid-phase immunosorbent assay in which the antigens are geographically distributed by molecular weight, so that it is possible to determine exactly which antigens are being recognized by the patient's antibodies. To accomplish this, the antigen mixture is first separated by molecular size using polyacrylamide gel electrophoresis and then transferred, or blotted, onto nitrocellulose paper. Patient serum is incubated with the paper strip, unbound antibodies are washed away, and bound antibodies are detected with anti–human immunoglobulin as in the ELISA. Because the antigens are distributed by molecular weight along the strip, the size—and therefore the likely identity—of the antigen recognized by the antibody can be identified.

The Western blot is useful for identifying false-positive reactions caused by cross-reactive antibodies that react with something unrelated in the antigen mixture. Recognition of just one antigen from a given pathogen could be attributed to chance cross-reactivity, but it is unlikely that a patient would have cross-reactive antibodies that recognize multiple antigens from a pathogen. It is more likely that such a patient has been exposed to or infected with the pathogen and has mounted a polyclonal antibody response. This is the basis for interpretation of the Western blot assay for HIV. Reactivity with only one HIV viral antigen is presumed to represent chance cross-reactivity or a very early HIV infection, before the patient has mounted a polyclonal response. Reactivity with two or more HIV antigens is interpreted as an immune response to an ongoing infection with HIV.

Agglutination and Complement Fixation

The ability of antibodies to cross link and aggregate antigens is the basis of the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP). The microbes or their extracted antigens are coated onto particles such as latex beads or erythrocytes and then added to serum. Agglutination by specific antibodies is apparent on visual inspection. IgM antibodies can cross link two large particles, but IgG may be too small to bridge the distance. Therefore, anti–human IgG is often added in a second step as a "developing reagent" to agglutinate particles coated with specific patient IgG.

Agglutination cannot identify the isotype of the patient antibody. However, these assays are useful because they can be read within minutes and require only minimal laboratory resources. Results are typically reported as antibody titer, which is the maximum extent to which a sample can be diluted and still give a positive result. For example, a titer of 1:160 means that 1 part serum can be mixed with 159 parts buffer and still produce a positive reaction. Sera are typically tested in serial twofold dilutions, and titer differences between samples are not considered to be statistically different until there is a fourfold or greater difference in the titers of the two samples.

Complement-mediated lysis or complement fixation assays can be sensitive but technically challenging. In these assays, red blood cells are artificially coated with the desired antigen and then mixed with patient serum (as a source of antibodies). Fresh guinea pig serum (as a source of complement) is added. Patient antibodies that have bound to antigen on the red cells activate complement, and the red cells are lysed. The advantage of this assay is the ease of reading the macroscopically visible results. Limitations include the need for a source of biologically active complement that behaves in a consistent manner. Antigen-coated erythrocytes are unstable and cannot be stored for long periods. As would be predicted from the abilities of different immunoglobulin isotypes to activate complement, this format is excellent for measuring IgM, moderately effective for measuring IgG, and not useful for measuring IgA antibodies.

Immunofluorescence and Immunohistochemistry

A patient's specific antibodies can be identified using immunofluorescence and immunohistochemical techniques. The principle is the same as for the ELISA; the only difference is that the antigen is present in a histologic tissue section or immobilized on a slide. Examples of antigens used in this format include treponemes to look for antibodies to *T. pallidum* and virus-infected cells to look for virus-specific antibodies. Patient serum is incubated with the slide, unbound antibodies are washed away, and reactive antibodies are detected using a conjugated anti–human immunoglobulin, just as in the solid-phase immunosorbent assay. Antibodies conjugated with a fluorochrome are visualized with a fluorescence microscope. Immunohistochemistry studies use antibodies conjugated with an enzyme that deposits a precipitate onto the tissue wherever antigen is expressed. Use of an anti–human immunoglobulin that is selective for a particular immunoglobulin class makes it is easy to identify the isotype of the patient's antigen-specific antibodies.

Interpretation of immunohistochemistry and immunofluorescence findings is subjective, or at best semiquantitative, with results typically reported on a scale of 0 to 4+. Although antibodies cannot be formally quantified, an experienced laboratory technician may be able to estimate the relative quantity by determining the titer or the extent to which serum can be diluted and still give a positive test. As previously mentioned, samples should not be considered to be different unless their titers differ by at least two serial dilutions. Because assays are typically carried out with twofold dilutions, a significantly higher titer should be fourfold or greater. Appropriate controls must be included. At a minimum, a negative control should be run using the conjugated anti–human immunoglobulin alone (without addition of patient serum) to evaluate the extent to which the anti–human immunoglobulin reagent binds to the tissue. Background signal can often be reduced by using $F(ab')_2$ fragments of the anti–human immunoglobulin to prevent adsorption to tissues expressing FcR. Antibodies produced in goats or sheep are preferred, because they have a lower affinity for human FcR. It may also be appropriate to run a negative control using normal human serum in place of the patient's serum to evaluate nonspecific uptake of human immunoglobulin.

Enumeration of Antibody-Producing B Cells: The ELISPOT Assay

The enzyme-linked immunosorbent spot (ELISPOT) assay is designed to enumerate antibody-producing B cells. It can also be modified to enumerate cytokine-producing mononuclear leukocytes. To measure antibody production, B cells are allowed to settle onto a surface that has been coated with antibodies to the isotype of interest and are cultured in place for several hours. For example, to measure IgG production, the surface is coated with anti-IgG, which captures the IgG molecules as they are secreted by the B cells. The secreted and trapped B-cell products are identified by a second antiimmunoglobulin reagent that had been conjugated with a tracer, just as in the ELISA. After development, the spots are counted. Each spot represents a cell that secreted

the product of interest. To enumerate cytokine-producing cells, the surface is coated with anticytokine antibody, and the developing reagent is a second, tagged antibody to the same cytokine. A similar approach has been modified for use in the flow cytometer. Fluorochrome-tagged antigen can used to enumerate B cells with antigen-specific membrane immunoglobulin.[37] Alternatively, antigen-specific B cells can be identified as $CD19^+/CD20^-/CD27br/CD38br$ plasmablasts in the circulation after infection or vaccination.[38]

Assays to Measure Antigen

The assays described earlier can also be modified to detect antigen using antigen-specific antisera. Monoclonal antibodies are ideal reagents for these assays, because they are a reproducible source of antibodies with a well-characterized specificity. It must be recognized that these immunoassays measure antigen on the basis of its ability to interact with specific antibodies, not by its biologic activity. Unless the antibody measures a conformational epitope, it is very likely that the assay will detect both active and inactive (e.g., denatured) antigens. Soluble inhibitors may not interfere with an immunoassay but can block activity in vivo. For example, some cytokines have soluble inhibitors that block their biologic activity. An immunosorbent assay might indicate that a particular serum contained large quantities of cytokine when, in fact, because of the presence of inhibitor, very little cytokine *activity* was present.

Solid-phase immunosorbent assays typically use a sandwich format in which purified antibody to antigen is immobilized on a surface such as a plastic well and forms the bottom layer of the sandwich (see Fig. 5.4B). The patient sample containing antigen is allowed to react with the immobilized antibody. Then more antigen-specific antibody is added as the top of the sandwich. This second detection antibody is conjugated with a radiolabel, enzyme, or fluorochrome. When monoclonal antibodies are used, the capture and detection antibodies must recognize different epitopes on the antigen. Otherwise, the capture antibody will react with all of the available epitopes, leaving none for the second antibody.

Sometimes, diagnosis requires the identification of pathogens in the patient's tissue. Immunofluorescent and immunohistochemical techniques are used to locate antigens in patient tissues. For example, to look for virus, sections of patient tissues are probed with antibodies, often monoclonal antibodies, that are prepared by immunizing animals with viral proteins. The antigen-specific monoclonal antibodies can be directly conjugated with fluorochromes or enzymes and used in a so-called direct, or one-step, detection (see Fig. 5.4C).

Alternatively, the antigen-specific antibody such as a mouse monoclonal antibody is added first, followed by a conjugated anti-IgG prepared in another animal. This is a two-step or indirect assay (see Fig. 5.4D). Indirect assays often generate a stronger signal because several conjugated anti–mouse immunoglobulin antibodies can bind to each mouse immunoglobulin. However, this technique also has a higher background, and appropriate negative controls are critical for interpretation of the assay. Cells in suspension can be stained with multiple antibody specificities, each labeled with a different fluorochrome, and analyzed by flow cytometry. The flow cytometer enumerates the cells, measures the fluorescence associated with each, and discriminates among fluorochromes with different emission spectra. Control samples must be analyzed in parallel, using fluorochrome-conjugated monoclonal antibodies that are of the same isotype but are specific for irrelevant antigens, to measure background nonspecific uptake. To improve the signal-to-noise ratio and decrease nonspecific binding, FcRs on cells can be blocked with irrelevant IgG from another species and reagent antibodies can be prepared as $F(ab')_2$ fragments or both.

Immunofluorescence and immunohistochemistry can also be used to detect autoreactive antibodies that bind to antigens in autologous tissues. A direct assay is possible if the involved tissue can be sampled. Patient's autoantibodies bound to their own tissues are detected by the addition of conjugated anti–human immunoglobulin antibodies in a direct test. However, sometimes the involved tissue is not accessible, and the only option is to look for autoantibodies in the serum. This indirect technique involves incubating the patient's serum with tissue obtained from surgical samples or autopsies and using conjugated anti–human immunoglobulin to detect the patient antibodies that bind.

Measurement of Immune Complexes

Fixed tissue phagocytes in the liver and spleen are responsible for clearing complexes of antigen and antibody. However, if their capacity is exceeded, immune complexes circulate and deposit in tissues, where they activate complement and incite phagocytes to attempt to ingest the tissues to which the antibodies have bound. Diagnosis of immune complex–mediated diseases can be made by demonstrating deposits of immune complexes in the targeted tissues using immunofluorescent or immunohistochemical techniques. A second diagnostic strategy is to measure the quantity of complement components, particularly C3 and C4, in the serum, because these levels decrease when complement is activated by immune complexes. However, interpretation of the results can be difficult, because C3 is an acute-phase reactant and its levels increase during inflammatory conditions.

A third diagnostic strategy is to look for circulating immune complexes. Several approaches can be used.[39,40] Some assays depend on the ability of IgG or IgM antibodies to fix C1q when they are engaged with antigen. Serum can be incubated with immobilized antibodies to C1q to determine how much immunoglobulin coprecipitates with the C1q. Radiolabeled C1q can be added to the serum; after all the serum proteins in the size range of immune complexes have been precipitated, the amount of coprecipitating C1q is quantified. Another type of assay is based on the fact that circulating immune complexes contain bound C3b. C3b (and the attached complexes) are extracted from the serum using immobilized antibodies to C3b or the Raji cell line, which has an avid and abundant receptor for C3b. The quantity of human immunoglobulin that coprecipitates with the C3b is used as an estimate of the quantity of immune complexes present.

The various strategies for measuring immune complexes do not always give concordant results, which suggests that complexes may vary in size, composition, and biologic behavior among patients or at different stages of a disease process.

B-CELL MATURATION AND IMMUNOGLOBULIN PRODUCTION

There is not enough DNA in the human genome to encode each of the millions of antibody specificities that a person can produce when appropriately challenged. Rather than encode each antibody specificity in the germline sequence, B cells create antibody diversity by systematically mutating the genes that encode components of the antigen-binding cleft and selecting for those with the highest binding affinity. The DNA rearrangement process uses enzymes unique to lymphocytes, such as recombination-activating genes (*RAG1* and *RAG2*), as well as the full array of ubiquitous DNA repair enzymes common to all cells. B cells begin by producing IgM antibodies. With further antigen stimulation and signaling from T cells, progeny of these B cells switch to production of IgG, IgA, or IgE antibodies with the same antigen-binding site as the initial IgM.

DNA Rearrangement and Generation of Diverse Antigen-Binding Sites

The bone marrow produces about 10^9 pro-B cells per day. To become B cells, these precursors must undergo a specific series of gene rearrangements (Fig. 5.5 and Table 5.2). The first step is to rearrange the DNA to form a functional μ heavy chain. Four gene segments must be brought together to make a μ chain: the V or variable segment, the D or diversity segment, the J or joining segment, and the μ heavy chain gene. There are 50 functional gene segment choices for V, 27 choices for D, 6 choices for J, and 1 μ gene. Through random selection of these segments, 8100 ($50 \times 27 \times 6$) different IgM specificities can be generated by combinatorial diversity. One of the D gene segments is spliced to one of the J gene segments, and the resulting DJ gene product is then spliced to one of the V gene segments. Note the overlapping nomenclature: "V" is used to designate the V gene segment and also the V domains at the 5′ ends of the heavy and light chains.

The antigen-binding cleft is formed by V domains from the heavy and light chains. Each of these V domains has three hypervariable regions or CDRs. Two of the CDRs are encoded in the V gene. The third CDR is encoded by DNA that spans the splice junction between the V and J segments. Sloppiness or wobble in the splicing of these

genes inserts more variability onto the antigen-binding site through junctional diversity. DNA splicing requires cleavage, and the cut ends of the DNA are not immediately reattached to the next gene. Instead, the cut end is sealed back on itself to form a hairpin loop. Before splicing, the loop is reopened, but not necessarily at the same place. A shift in the exact site of the opening cut introduces further variability into the gene sequence. Additional nucleotides can be introduced to (or removed from) the cut end before it is spliced to the next gene to add even more diversity to the gene sequence.

These DNA rearrangements are a risky process for the B cell. Stop codons can be inadvertently introduced. Two of three times, the reading frame is shifted out of sequence and the gene no longer encodes a functional protein. Before it proceeds, the B cell must verify that the rearranged VDJ gene from the first gene encodes a functional μ chain gene. To test for functionality, the B cell attempts to pair the μ chain with a surrogate light chain and express the product on the cell membrane. It is not known how the B cell confirms that these two proteins are successfully displayed. However, if the criteria for success are met, the heavy chain gene on the other chromosome is prevented from rearranging by a process called *allelic exclusion*. If the μ chain does not display properly, the pro-B cell attempts to rearrange the other heavy chain allele.

Once the heavy chain rearrangement is successful, the pro-B cell divides, becomes a pre-B cell, and begins to rearrange the light chain (see Table 5.2). The rearrangement process for light chains is the same as for heavy chains, with the exception that light chain genes have only V, J, and constant region segments—they do not have a D segment. However, the odds of success are twofold better for light chains than for heavy chains. The κ chains are tried first; and if neither of the κ chain rearrangements is successful, the B cell can try to rearrange the λ chains. Once the light and heavy chains have been successfully rearranged to form functional membrane IgM, the cell officially becomes an immature B cell that is ready to encounter antigen (see Table 5.2).

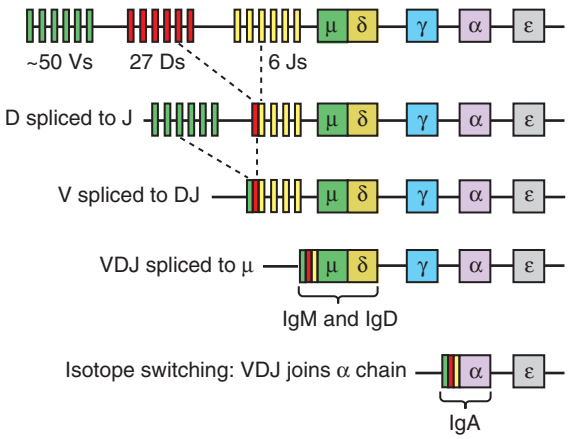

FIG. 5.5 DNA rearrangement. The scheme for rearranging heavy $CD4^+$ chain genes begins with splicing one of the D gene segments to one of the J gene segments. The DJ product is spliced to the V segment, and this is spliced to the μ chain gene. DNA encoding μ and δ chains are adjacent to each other, and the B cell can produce a single messenger RNA molecule spanning both. Through alternative splicing in posttranslational steps, the B cell produces both IgM and IgD, which are expressed on the cell's surface. The B cell also produces secreted IgM. After it receives signals from activated T cells, the B cell can switch from production of IgM to production of IgG, IgA, or IgE. The VDJ segment is spliced onto the new heavy chain genes, thereby preserving the antigen specificity of the antibodies produced.

Deletion of Autoreactive Clones

The next step is to eliminate B cells that are producing autoreactive antibodies that might injure the host. Immature B cells at this stage express only IgM on their surface and are located in the bone marrow. Antigens in this environment are likely to be self-antigens, or autoantigens. If a B cell's surface IgM binds to and is cross-linked by antigen (autoantigen), that B-cell clone is removed from the repertoire. B cells may be induced to undergo apoptosis in a process called *clonal deletion,* or they may become anergic or unresponsive to antigen. There is one potential rescue option for the autoreactive B cell. If sufficient levels of the enzyme RAG remain in the cytoplasm, the autoreactive B cells can try to rearrange a new light chain gene, which might result in a new IgM that no longer reacts with self-antigens. This process is called *receptor editing.*

Antigen Stimulation: First Signal

As the B cells leave the bone marrow, they begin to express both IgD and IgM on their membrane. To produce both isotypes concurrently, the B cell generates a long messenger RNA molecule with the rearranged VDJ region, the μ gene, and the δ gene. Through alternative splicing, the B cell can use this messenger RNA molecule to produce either IgM or IgD. This is a very different process from that used for isotype switching. The class switch from IgM to IgG, IgA, or IgE is irreversible because the B cell splices the VDJ sequence to a new heavy chain gene (γ, α, or ε) and discards the intervening DNA (see Fig. 5.5).

Production of IgD is an important threshold in the life of a B cell. Before this stage, cross-linking of surface IgM with antigen, which would have been an autoantigen from the bone marrow, led to inactivation or death. From this point forward, interaction with antigen has a stimulatory effect; moreover, the B cell's survival now depends on repeated stimulation by antigen. Naïve B cells leaving the bone marrow have about 1 week in which to locate their cognate antigen. Eighty percent of B cells fail to do so and die.[41]

Membrane IgM is the B-cell's (antigen) receptor, or BCR. When the BCR interacts with cognate antigen, membrane IgM reorganizes into lipid rafts in the B-cell membrane. The cytoplasmic tail of membrane IgM has no signaling capability on its own. Signaling is carried out by two other cytoplasmic proteins, Igα and Igβ, that associate with the IgM in the lipid raft. Like CD3 and the zeta (ζ) chain in T cells, Igα and Igβ have ITAMs. When IgM becomes cross-linked, these ITAMS are phosphorylated, which allows docking of the tyrosine kinase Syk. Syk is the B-cell equivalent of ZAP-70 in T cells. Syk and other tyrosine kinases activate the Ras–mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol-specific phospholipase C pathway, leading to increased cytosolic calcium and diacylglycerol. Ultimately, transcription factors such as nuclear factor of activated T cells (NFAT), nuclear factor kappa B (NF-κB), and activator protein 1 (AP-1) are produced.[42,43]

Coreceptors Amplify or Suppress Antigen Signaling

Antigen-mediated signaling can be greatly enhanced by the participation of several B-cell coreceptors. CD21, CD19, and CD81 form the B-cell

TABLE 5.2 Stages of B-Cell Development

CELL STAGE	PRO-B	PRE-B	IMMATURE B	MATURE B
DNA rearrangement for immunoglobulin	μ Chain being rearranged	Heavy chain complete; light chain being rearranged	Heavy and light chains complete	Isotype switch, somatic hypermutation in germinal center
Membrane immunoglobulin	None	μ Chain with surrogate light chain	IgM	IgM and IgD
Response to interaction with antigen	None	None	Receptor editing or deletion of autoreactive clones	Survival dependent on antigen stimulation

Ig, Immunoglobulin.

coreceptor complex. CD21, also known as complement receptor 2 (CR2), binds C3d. C3d is a degradation product of C3b, which accumulates on the surface of pathogens when complement is activated. While the pathogen antigens interact with membrane IgM, the C3d can cross link the CD21 on the B cells. This brings the cytoplasmic tail of CD19 into proximity with the BCR, where it can be phosphorylated by Syk and bound to Igα or Igβ. The phosphorylated CD19 ultimately activates phosphatidylinositol 3-kinase. Src family kinases linked to CD21 can also phosphorylate ITAMs in Igα and Igβ. The net effect of coreceptor involvement is to increase the concentration of signaling molecules. In a murine model, involvement of the coreceptor complex, modeled by cross-linking CD21 with antibodies, reduced the quantity of antigen needed to induce an immune response by 1000-fold.[44]

Other receptors, such as FcγRIIB, have an opposite effect and downregulate membrane IgM signaling. The cytoplasmic tail of FcγRIIB has an ITIM that inhibits the activity of ITAMs. ITIM activates a phosphatase that dephosphorylates the ITAM and interrupts the signaling pathway. As a result, when immune complexes containing antigen and IgG interact with the B cell, the enhancing signal delivered by the interaction of membrane IgM with antigen is countered by an inhibitory signal arising from the interaction of IgG with FcγRIIB.[27] This regulatory role for FcγRIIB was demonstrated by cross-linking membrane IgM with IgG anti-IgM as a surrogate for antigen. Intact IgG anti-IgM, which interacted with both membrane IgM and FcγRIIB, suppressed antibody responses. Treatment with $F(ab')_2$ fragments of the same anti-IgM, which could cross link membrane IgM but could not interact with FcR, stimulated antibody production.[45]

Second Signals and Interactions Between B Cells and T Cells

To switch to production of IgG, IgA, or IgE, B cells need to receive a signal from activated $CD4^+$ helper T cells (Fig. 5.6). Activated T cells express CD40 ligand (CD40L, or CD154), which binds to CD40 on B cells and activates the B cell. The critical role of CD40–CD40L interactions is best illustrated by the defects seen in individuals with a congenital deficiency of CD40L and a condition called *hyper-IgM syndrome*. Without CD40L, T cells cannot stimulate CD40 on B cells. B cells from affected patients produce greater than normal quantities of IgM but are unable to switch to production of other isotypes or to form germinal centers in lymph nodes. Key survival factors for B cells include B-lymphocyte stimulator (BLyS, also referred to as B-cell activating factor [BAFF]), IL-21, and APRIL (*a p*roliferation-*i*nducing *l*igand).[46]

Adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1, or CD54) and lymphocyte function–associated molecule 1 (LFA-1, or CD11a/CD18), stabilize the B cell and the T cell as a conjugate pair. The two cells may remain in contact for many hours as the T cell secretes cytokines in a polarized direction into the space between them. B cells can respond to numerous T-cell–derived cytokines, including IL-2, IL-4, IL-5, IL-6, and transforming growth factor-β (TGF-β). IL-10 and TGF-β drive the B cell to switch to the IgA isotype, whereas IL-6 induces it to become a high-rate IgA-producing plasma cell.[47] IL-4 and IL-5 promote switching to the IgE isotype.

After stimulation by the T cell, the B cell begins to divide every 6 to 7 hours and generates several thousand daughter cells. At this point, nature raises the stakes for the B cell. The B cell is doomed to die by apoptosis through a decrease in the level of BCL2 and an increase in Fas. The B cells can survive if they find antigen to interact with their surface immunoglobulin, which causes their level of $BCL\text{-}X_L$ to increase; if this does not occur, the B cell dies through Fas-mediated pathways of apoptosis. Moreover, the B cells cease expression of surface IgD and express only low quantities of IgM.

To enhance their ability to bind antigen, B cells undergo somatic hypermutation within the DNA segments coding for the antigen-binding clefts in an attempt to increase affinity for antigen. Both class switch recombination and somatic hypermutation are initiated by the same enzyme—activation-induced deaminase (AID). AID deaminates cytidine in single-stranded DNA to uracil, causing U:G mismatches that are processed as repairs, mutations, or DNA double-strand breaks.[48] B cells can introduce one mutation per 10^3 base pairs in this particular sequence,

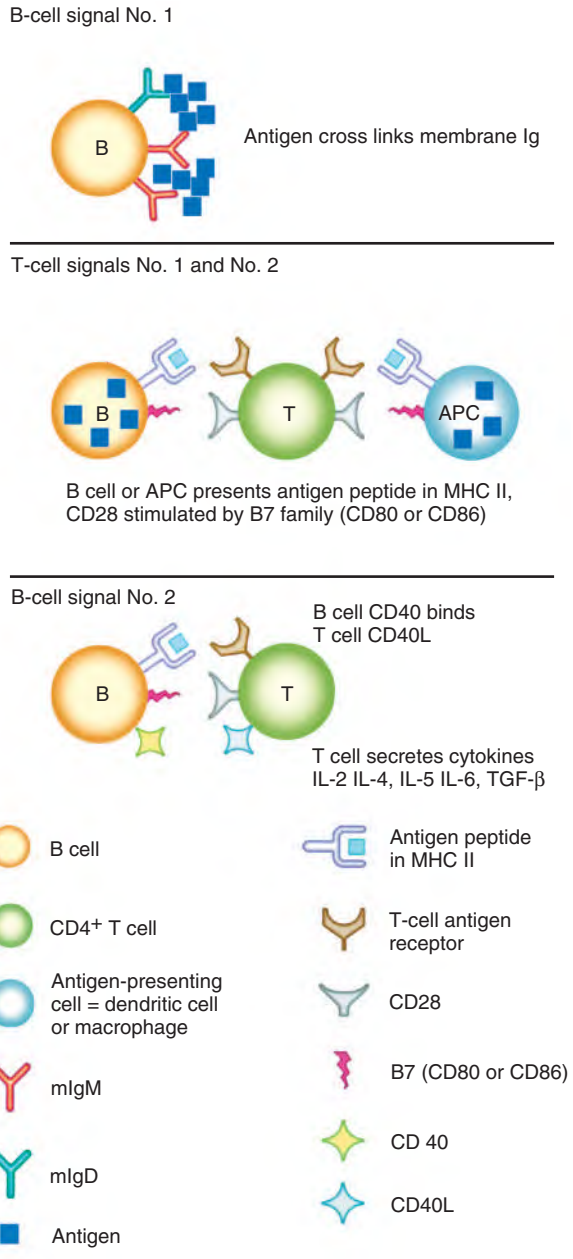

FIG. 5.6 B cells and T cells each need two signals. *Top,* The first signal for B cells comes when antigen cross links membrane immunoglobulin (Ig). If this occurs when the immature B cell is expressing only IgM, the signal is inhibitory. However, once the B cell is expressing both IgM and IgD, encounter with antigen is stimulatory. *Middle,* T cells are activated by encounter with antigen-presenting cells (APCs) such as dendritic cells. T cells need to recognize their cognate antigenic peptide expressed in a major histocompatibility complex (MHC) class II molecule, and they need a signal through their CD28 receptor. The ligand for CD28 is one of the members of the B7 family (CD80 or CD86). APCs upregulate their expression of B7 molecules when they encounter proinflammatory stimuli such as pathogen-associated molecular patterns on the antigen. This second APC signal confirms to the T cell that the antigen is "dangerous" and an immune response is needed. *Bottom,* To switch from IgM to other isotypes and to form memory cells, B cells need signals from activated T cells. These second signals include the interaction of CD40 on B cells with CD40 ligand (CD40L, or CD154) on activated T cells and various T cell–derived cytokines. *IL,* Interleukin; *MIgD,* membrane IgD; *MIgM,* membrane IgM; *TGF,* transforming growth factor.

which is 10 million–fold greater than the baseline mutation rate for somatic cells. Because continued stimulation by antigen is a prerequisite for survival, as antigen becomes scarce, B cells must compete by modifying their antigen-binding sites to generate a sequence with a higher affinity for antigen. Only those B cells making antibodies with the highest binding affinity will be able to compete for the critical survival signal provided by antigen. As a result, the average affinity of the antibody response becomes higher and higher.

Somatic hypermutation takes place within the follicular region of the lymph node or spleen in germinal centers.[49] B cells move through the basal light zone, competing for antigen expressed on follicular dendritic cells. Survivors become memory cells and plasma cell precursors.[50] Plasma cells can produce antibodies at a highly accelerated rate for a period of days, or perhaps even longer. Other B-cell progeny become memory cells and long-lived plasma cells that appear to survive for years.[51] Memory cells can sample antigen complexed with C3d and retained on the surface of follicular dendritic cells to maintain stimulation over prolonged period of time.

How B Cells Find and Activate T Cells

How does a B cell find the one-in-a-million T cell that is specific for a peptide from the same antigen? After B cells leave the marrow, they circulate through the bloodstream and pass through the lymph nodes or spleen, where they may encounter cognate antigens assembled from the periphery by dendritic cells. B cells that fail to find their matching antigen pass on to the next node. B cells that find a suitable antigen begin to express the chemokine receptor CCR7, which draws them toward chemokines produced by stromal cells in the T-cell zone.[52] Activated T follicular helper cells expressing CXCR5 are drawn, in turn, toward the chemokines produced in the B-cell zone.[53]

B cells can also increase their odds of contacting activated T cells specific for the same antigen by becoming antigen-presenting cells (APCs), which activate T cells. B cells ingest antigen and present relevant peptides to activate the very T cells they need. Unlike monocytes or dendritic cells, which pick up antigens on a random basis, B cells have an antigen-specific capture mechanism—their membrane IgM. Antigen captured by membrane IgM is brought into an endosomal compartment, degraded into peptides, and displayed at the cell surface in major histocompatibility complex (MHC) class II molecules to activate $CD4^+$ T cells. When membrane IgM is cross-linked by antigen, the B cell is activated to display even more MHC class II antigen, which further enhances its capacity to be an APC for $CD4^+$ T cells. Because the antigen was picked up with the B cell's antigen-specific membrane IgM, the B cell is presenting just the right peptides to engage T cells with the appropriate antigen specificity. The importance of B cells as antigen-specific APCs should not be underestimated. T-cell–mediated diseases can be ameliorated by the B-cell–specific monoclonal antibody rituximab, which appears to act by interfering with antigen presentation by B cells to T cells.[54] B cells also produce IL-10 and TGF-β and may be involved in the development of regulatory T cells.[55]

In addition to their ability to select the antigen-correct T cells, antigen-activated B cells can provide the necessary costimulatory molecules required to activate the T cell's CD28 receptor.[56] T cells require two signals to be activated. The first signal comes through their antigen receptor, and the second signal comes through CD28 and is typically provided by APCs: monocytes, macrophages, dendritic cells, or B cells. The two-signal requirement ensures that T cells do not mount an immune response to self-antigens. T cells have no way to discriminate pathogens from harmless antigens. If T cells were able to be activated with just one signal, they would respond to every peptide that fit their antigen receptors, and the host could be overwhelmed by inflammatory processes. It is the role of the APC to tell the T cell whether a particular antigen is "dangerous" and merits an immune response. APCs do this by expressing the B7 ligands whenever they encounter danger. There are two homologous members of the B7 family: B7-1 (CD80) and B7-2 (CD86). B7 molecules bind to CD28 and deliver the critical second signal that activates T cells and keeps them alive. T cells stimulated by a single signal (i.e., antigen without B7) undergo apoptosis.

How do the APCs identify danger? APCs identify pathogens through an assortment of receptors collectively called *pattern recognition receptors.* PRRs bind to PAMPs, such as lipopolysaccharides, peptidoglycans, lipoteichoic acids, mannans, bacterial DNA, and double-stranded RNA. When PRRs are engaged by PAMPs, the APC "recognizes" that this is a pathogen and begins to display the B7 costimulatory molecules. Cross-linking of membrane IgM, MHC class II molecules, or CD40 upregulates B7 molecules on B cells and transforms them into effective APCs.

Conjugated polysaccharide vaccines depend on the ability of B cells to serve as their own APCs to present antigen to T cells with a little bit of "bait-and-switch" (Fig. 5.7). Polysaccharides make poor vaccines, because they cannot stimulate the production of specific IgG antibodies. B cells producing antibodies to polysaccharides cannot make the switch from IgM to IgG and cannot generate memory cells, because there are no specific T cells to provide the necessary second signals. There are no polysaccharide-specific T cells because T cells are programmed to recognize peptides (i.e., protein fragments) displayed in MHC molecules. Polysaccharides do not generate peptides, so it is not possible to have polysaccharide-specific T cells. Conjugate vaccines are designed to "fool" T cells into providing help to polysaccharide-specific B cells. A conjugate vaccine links the polysaccharide antigen to a protein, such as tetanus toxoid, for which the host already has specific T cells. Polysaccharide-specific B cells internalize the conjugate using their polysaccharide-specific membrane IgM. The conjugate is degraded, and peptides from the conjugated tetanus toxoid are displayed in the B cell's MHC class II molecules. T cells specific for tetanus toxoid peptides pair up with this polysaccharide-specific B cell and provide the necessary help to drive isotype switching and formation of memory cells.

T-Cell–Independent Antigens

B cells typically require two signals to become efficient antibody-producing cells. The first signal comes from antigen, and the second signal usually comes from T cells. Some antigens can provide a type of second signal to the B cell without the participation of T cells. Examples of T-cell–independent antigens include the polysaccharides of *Haemophilus influenzae* type b, peptidoglycans, *Staphylococcus* protein A, and many viruses. T-cell–independent antigens typically have highly repetitive motifs and a flexible structure. Their repetitive structure and flexible backbone allow them to interact with multiple membrane IgM molecules that aggregate into a single focus and deliver a potent signal to the B cell. Many T-cell–independent antigens are pathogens that become coated with C3b, which degrades to C3d. C3d cross links CD21 on B cells, which shares a lipid raft with membrane IgM and further amplifies the signal to the B cell. B cells activated by T cell–independent antigens are still dependent on cytokines but may receive these from non–T-cell sources, such as macrophages. New data also suggest that neutrophils, stimulated by IL-10 from splenic sinusoidal endothelial cells, can also induce immunoglobulin class switching, somatic hypermutation, and antibody production by activating mantle zone B cells through a mechanism that involves the cytokine IL-21 as well as BAFF and APRIL, which are two Toll-like receptor–inducible B cell–stimulating factors related to the ligand for the T-cell molecule CD40.[57]

B cells in the marginal zone of secondary lymphoid tissue tend to express membrane IgM specific for T-independent antigens, particularly carbohydrate antigens. These B cells respond to bloodborne pathogens such as bacteria that are trapped by macrophages located around the marginal zone. Because they do not require the participation of T cells, marginal-zone B cells provide a pseudoinnate response to bacteremia by quickly releasing IgM antibodies.

Downregulation of Antibody Production

Once the antigen is cleared, antibody responses decline. There are several mechanisms that shut down an antibody response. Sequestration of antigen deprives B cells of the activating signal they require to survive.[58] Immune complexes formed between antigen and excess IgG antibody deliver inhibitory signals to the B cell by interacting with FcγRIIB, as described previously.[45] Anti-idiotypic antibodies spontaneously arise during an immune response and may have a role in both upregulating and downregulating immune responses.[59] Anti-idiotypic antibodies are autoantibodies that react with epitopes in or near the antigen-binding site of an antibody. Because they recognize epitopes unique to a particular

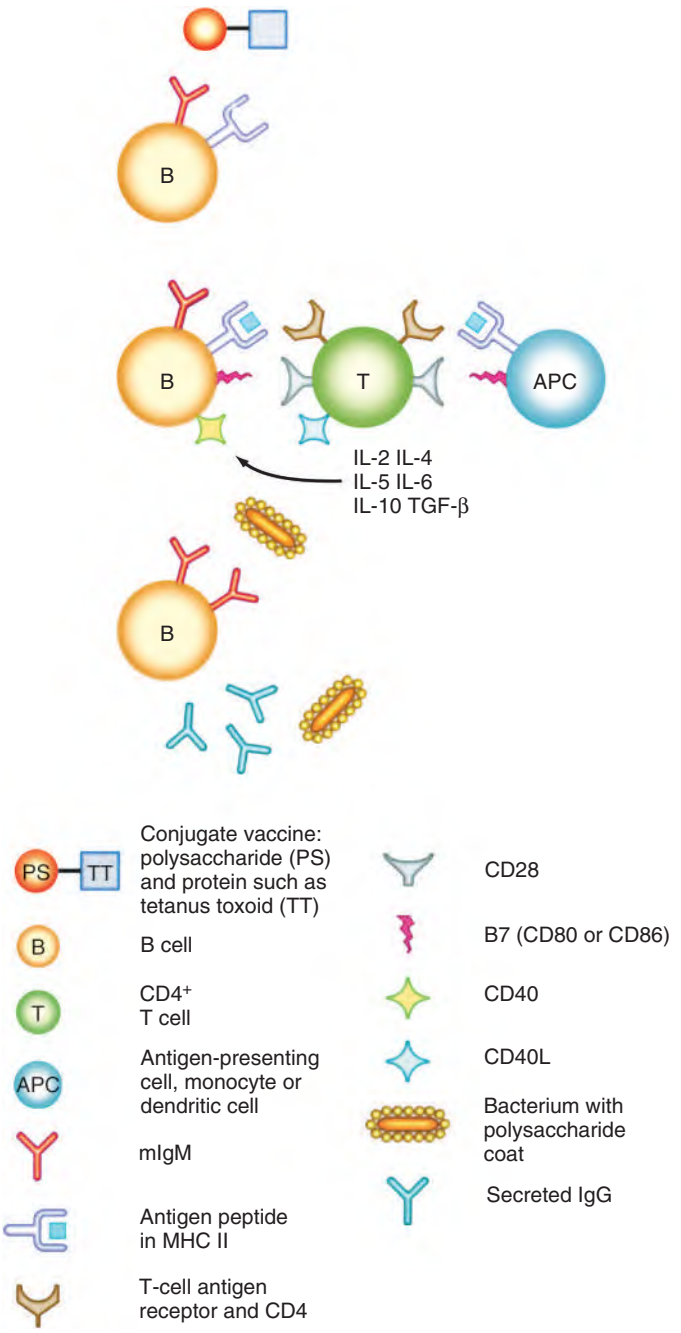

FIG. 5.7 Conjugate vaccines. The conjugate vaccine is designed to induce production of immunoglobulin G (IgG) antibodies to polysaccharide (PS) antigens, which would normally be unable to induce IgG antibodies. Polysaccharides do not induce IgG antibodies because antigen-presenting cells (APCs) cannot degrade them into the peptides that must be presented in major histocompatibility complex (MHC) class II molecules in order to stimulate antigen-specific T cells. The vaccine consists of the PS antigen linked to a protein antigen, such as tetanus toxoid (TT), to which the host has primed antigen-specific T cells. B cells that are able to make PS-specific antibodies ingest the vaccine through their PS-specific membrane IgM. The B cells degrade the vaccine molecule and present the TT peptides in their MHC class II molecules to a TT-specific activated $CD4^+$ T cell. The T cell can be activated by the B cell or by prior interaction with a dendritic cell displaying TT peptides. The activated TT-specific T cell provides PS-specific B cells (displaying peptides from TT) with the necessary help in the form of CD40–CD40L interactions and cytokines. Having received the necessary signals from a T cell, the PS-specific B cell can switch to production of IgG and can generate long-lived memory cells. *CD40L,* CD40 ligand; *IL,* interleukin; *mIgM,* membrane immunoglobulin M; *TGF,* transforming growth factor.

clone, they can modulate B cells in a highly selective manner. Anti-idiotypes interacting with membrane IgM, before the immature B cell expresses IgD, can suppress antibody production. Declining numbers of T cells also help temper the antibody response. With persistent activation, T cells begin to express both Fas and its ligand, FasL. Reciprocal interactions between activated T cells expressing both Fas and FasL lead to mutual apoptotic death.[60] Late in an immune response, activated T cells also begin to express cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 binds the B7 costimulatory molecules with a much higher affinity than does CD28.[61] Whereas CD28 sends a stimulatory signal, CTLA-4 sends an inhibitory signal to the T cell, so the net effect on the T cell is to suppress its activity. Finally, as antigen becomes scarce, fewer T cells and B cells receive the antigen signals necessary for continued activation and survival.

B1 Cells

The "usual" B cells discussed until now are called B2 cells to distinguish them from B1 cells. B1 cells are located primarily in the peritoneal and pleural spaces[62] and have a unique surface marker, CD5. B1 cells appear to regenerate continuously in the periphery rather than in the bone marrow. They produce only IgM, which suggests that they do not receive help from T cells. Their antigen-binding clefts are encoded directly by germline sequences without further modification, and the antigens they recognize tend to be microbial polysaccharides and effete or denatured host proteins. Antibodies produced by B1 cells may serve a housekeeping function by facilitating the clearance of cellular debris and denatured proteins.[63] B1 cells are thought to be the source of the many "natural" antibodies to microbial antigens, particularly those found on normal gut flora. These antibodies are present in low levels even in individuals who have not been deliberately immunized. Animals that have been raised in "germ-free" environments have low levels of circulating natural antibodies that react with a variety of commensal organisms, suggesting that B1 cells may be part of the endogenous immune system.

ANTIBODY-MEDIATED PATHOLOGY

Gell and Coombs Classification of Hypersensitivity Responses

Hypersensitivity reactions are immune responses that cause tissue injury and morbidity for the host. In some cases, an aberrant autoimmune response is specifically directed to host antigens and host cells. In many other cases, an exuberant immune response to an infectious agent injures host cells that are innocent bystanders. The Gell and Coombs classification divides hypersensitivity reactions into four types based on their underlying mechanism of action.[64] Types I, II, and III are mediated by antibodies and are discussed here. Although few diseases can be uniquely attributed to a single Gell and Coombs class, the classification scheme is a useful foundation for understanding pathogenetic mechanisms. It should also be noted that the term *hypersensitivity reaction* is often used, in a potentially paradoxical manner, to describe defense mechanisms that are actually beneficial to the host. For example, granulomas formed in response to tuberculous organisms help to contain the bacillus and protect the host, yet they are referred to as a type IV hypersensitivity mechanism.

Type I Hypersensitivity

Type I reactions involve mast cells. Mast cells contain preformed stores of histamine and heparin. They also produce leukotrienes, prostaglandins, cytokines such as tumor necrosis factor-α, and proteases. Local release of mast cell contents causes wheal-and-flare reactions, urticaria, and hives. Massive simultaneous degranulation of large numbers of mast cells throughout the body causes anaphylaxis, resulting in reduced blood pressure, a loss of fluids through permeable vessel walls, and constriction of smooth muscle. Mast cells can be degranulated by cross-linking of IgE antibodies held in FcεRs on the mast cell surface. This can be carried out by antigens with multiple repetitive epitopes or by antibodies to the IgE molecules or the FcεR. Opioids and contrast dyes can also degranulate mast cells without acting through IgE or the FcεR. As a result, the responses they trigger are referred to as *anaphylactoid* reactions. Fragments from the third and fifth complement proteins (C3a and C5a) can also degranulate mast cells, and these molecules are referred to as

anaphylatoxins. The clinician struggling to deal with IgE-mediated reactions to harmless substances such as penicillin or ragweed recalls with difficulty that type I responses have a beneficial role in the defense against intestinal parasites.

Type II Hypersensitivity

Type II reactions involve antibodies reacting with antigens on the surface of host cells. Cells decorated with antibodies may be lysed by activated complement or phagocytosed by neutrophils or monocyte-macrophages. Autoantibodies may recognize self-antigen on host cells. For example, autoimmune hemolytic anemia can be caused by antibodies to red blood cell antigens. Antibodies can also target external antigens that have bound to host cells. For example, patients with antibodies to penicillin can develop hemolytic anemia when penicillin binds to their red blood cells.

Origin of Autoreactive Antibodies

It may be helpful, at this point, to review the mechanisms that prevent the development of autoimmunity and how these processes can be circumvented. The immune system makes every effort to remove autoreactive B cells. Early in their development, B cells are induced to undergo apoptosis or to become unresponsive if they encounter cognate antigen. This reaction occurs during the window of vulnerability that persists while immature B cells express IgM and before they express IgD. Such B cells are likely to be situated in the bone marrow, or in early transition into the periphery, and any antigens they encounter are likely to be self-antigens. As B cells move into the periphery, where they are likely to encounter foreign antigen, they lose their IgD and interaction with antigen becomes an activating, life-prolonging stimulus. However, central induction of tolerance appears to be only partially effective, because virtually all people have circulating B cells that can be driven, in vitro, to produce antibodies reactive with autoantigens.

The nature of antigen signaling also appears to dictate whether a B cell will become responsive or anergic. Stimuli that tend to induce anergy include persistent stimuli, oligovalent antigens, low-affinity interactions, and immune complexes that can interact simultaneously with ITIM-containing FcγRIIB on the B cell. It is important to recognize that mature B cells in germinal centers undergo somatic hypermutation and generate new antigen-binding sites well after they are no longer vulnerable to the regulatory mechanisms that apply to pre-B cells in the bone marrow.

Control of B cells is imperfect, so much of the responsibility for preventing autoimmune reactions falls on the T cells.[65] T cells maturing in the thymus are carefully examined for their ability to bind to autologous antigens. A unique system allows thymic epithelium to express a variety of proteins normally found only in extrathymic tissues. The T cell is tested against a surprisingly wide repertoire of self-antigens while still within the thymus. Immature T cells that bind with high affinity to autologous antigens are deleted by a process called *negative selection*. After they leave the thymus, T cells cannot be activated by cognate antigen alone; they require a second signal transmitted through CD28, typically from APCs. T cells that receive a signal through their antigen receptor without an accompanying signal through CD28 are induced to undergo apoptosis. CD28 signaling is accomplished by B7 costimulatory molecules displayed on APCs. APCs upregulate the expression of B7 molecules when they recognize danger signals or PAMPs on a pathogen. APCs displaying peptides from host antigens typically do not express B7 molecules. However, in the course of an infection, an APC could display autologous peptides at a time when it has also been stimulated by proinflammatory cytokines to express B7 costimulatory molecules. Therefore during an infection or other inflammatory condition, T cells recognizing peptides from self-antigens may be activated and may, in turn, provide help to autoreactive B cells. This is one of the mechanisms postulated to lead to autoimmune responses.

Another possible mechanism leading to autoimmunity is molecular mimicry, which involves pathogen-derived antigens that closely resemble host antigens. Examples of molecular mimicry include cross-reactive epitopes found on the M protein of *Streptococcus pyogenes* and also on proteins in the myocardial sarcolemma,[66] and cross-reactive peptides found in both *Trypanosoma cruzi* and human neurons.[67] The first peptide recognized as a result of molecular mimicry may not trigger an autoimmune disease, but immune responses tend to expand to other epitopes on the same protein in a process called *epitope spreading*.[68] Epitope spreading is thought to occur when B cells producing antibodies to one epitope on a protein take up that protein through their membrane IgM and process it to antigenic peptides. The B cell displays those diverse peptides, in large numbers, along with costimulatory signals presumably induced by a coexistent inflammatory stimulus. This activated B cell, in its role as an APC, activates T cells that recognize other epitopes from the same protein. This expands the repertoire of CD4 cells that are activated and allows them to help a still wider array of B cells.[69] Eventually, this expanding repertoire can include autoreactive T cells and B cells whose specificity leads them to injure host tissues.

Type III Hypersensitivity

Type III hypersensitivity reactions are inflammatory responses triggered by soluble immune complexes that deposit in various tissues. Phagocytes try to ingest the immune complexes bound to tissues. Although tissues decorated with complexes are too large to be ingested, the phagocytes nonetheless try, and in the process they release injurious proteolytic enzymes and proinflammatory cytokines. Immune complexes can also activate complement, which deposits on the cell surface. Host cells usually protect themselves from complement attack with proteins that block formation of the membrane attack complex and accelerate the inactivation of complement components. However, when immune complexes activate complement through the classical pathway, the host may be unable to produce inactivator proteins at a sufficient rate. The site of type III hypersensitivity reactions depends entirely on where immune complexes deposit; the antigenic specificity of the antibodies is irrelevant. For example, complexes of antibodies and hepatitis antigens cause vasculitis when they deposit in blood vessel walls and glomerulonephritis when they deposit in the kidney.

Infectious diseases are commonly associated with type III hypersensitivity reactions because the infection generates a continuous source of antigen in large quantities that can be incorporated into immune complexes.[70] When antigen is in excess, each antibody can bind its own antigen, and the complexes are small. When antibody is in excess, antigen is covered with antibody, and complexes are still small. However, when antigen and antibody are present in near-equivalence, antibodies will cross link to contiguous antigen molecules and the ensuing lattice forms a large immune complex. Antibodies with low affinities release antigen quickly and tend to form small complexes regardless of their relative abundance.

Rheumatoid factors are antibodies that react with human (autologous) IgG and are a frequent component of immune complexes. These naturally occurring autoantibodies are found in almost all people. Sequences coding for many rheumatoid factors are present in the germline sequence and are expressed with little DNA rearrangement.[71,72] Their preservation in the germline DNA sequence suggests that these antibodies may have an immunoregulatory or housekeeping role.[73] Antiimmunoglobulins that react with epitopes in or near the antigen-binding site may emulate antigen signaling and have either positive or negative effects on antibody production by B cells.[74,75] They may also facilitate the clearance of effete immunoglobulin molecules.[76] In cases of chronic infection or inflammation, levels of rheumatoid factors can become quite elevated and contribute significantly to immune complexes that cause tissue injury. Cryoglobulinemia is a vasculitis triggered by complexes of rheumatoid factors and IgG that deposit preferentially in sites of reduced body temperature.

Hypergammaglobulinemia

Chronic infections such as malaria, endocarditis,[77] trypanosomiasis,[78] HIV,[79] and infections associated with cystic fibrosis[80] increase circulating immunoglobulin levels well above the normal range. The mechanism is thought to be bystander activation of B cells, because few of the antibodies produced are specific for antigens associated with the infectious agent. Epstein-Barr virus is also associated with elevated immunoglobulin levels, because the virus infects and activates a wide array of B cells, which then produce antibodies of diverse (polyclonal) specificities.[81]

Sera with very high immunoglobulin levels have higher levels of nonspecific binding in assays for specific antibodies. This high background can lead to a false-positive reading if the appropriate background subtractions are not done. Hypergammaglobulinemias are not associated with any defined pathologic process, with the exception of hyperimmunoglobulinemia D and the inherited periodic fever syndrome, in which overproduction of IgD may trigger chronic inflammation.[82]

IMMUNODEFICIENCIES

Immunoglobulin A Deficiency

Among the inherited immunodeficiencies, defects involving B cells are far more common than those involving T cells or phagocytes. The most common inherited immunodeficiency is selective IgA deficiency, which affects 1 in every 300 to 700 individuals. There are incompletely penetrant autosomal dominant and recessive modes of inheritance.

Many IgA-deficient people are relatively healthy and asymptomatic, and the true prevalence of this disease was unappreciated until serum IgA levels began to be measured systematically in blood donors. Symptoms may be minimized by compensatory transport of IgM into secretions.[6] A few IgA-deficient individuals develop recurrent sinopulmonary infections, atopy, autoimmune disorders, and malignancies. Their allergies are often directed toward dietary antigens, and it is postulated that the atopy occurs because they are unable to block absorption of environmental antigens from gastrointestinal surfaces. Individuals with the most morbidity often have a combined deficiency of both IgA and one or more of the IgG subclasses, particularly IgG2 or IgG4. Some of them go on to develop common variable immunodeficiency syndrome.

People with congenital IgA deficiency are potentially at risk for anaphylactic reactions to IgA in intravenously administered preparations of immunoglobulins or in unwashed packed red blood cells.[83] Immunoglobulin replacement does not benefit patients with IgA deficiency for two reasons. First, the preparations are IgG and do not contain IgA. Second, even if the replacement did contain IgA, intravenous IgA antibodies would not be transported into secretions. Only IgA2 antibodies produced in the submucosal lymphoid tissue are transported across epithelial barriers into the secretions.

Agammaglobulinemias

Children with inherited profound antibody deficiencies remain well for the first 6 to 9 months of life because of transplacentally acquired maternal IgG antibodies. The half-life of IgG is approximately 3 weeks, and it is not until 6 to 9 half-lives, or about 6 months, after birth that maternal IgG falls below protective levels. Thereafter, these children begin to develop infections such as sinusitis, otitis media, and pneumonias. *S. pneumoniae, H. influenzae,* meningococci, and *Mycoplasma* species are particularly common infectious agents.[84] By the time the underlying etiology is recognized and treated, these children may have already developed irreversible bronchiectasis. Intestinal infections with *Salmonella, Shigella, Campylobacter, Giardia,* and rotavirus are also common.[85,86] Rheumatologic symptoms occur in 10% to 30% of these children.[87] These children are prone to septic arthritis from common bacteria. They may also develop synovitis in the absence of infectious agents, and they are uniquely vulnerable to a chronic meningitis with enterovirus that often proves fatal.[88] Vaccination with inactivated agents is futile, because they cannot produce the desired antibodies. Live vaccines are to be avoided, because vaccine-induced poliomyelitis has occurred in patients with agammaglobulinemia.[89]

Many of the conditions that affect B-cell maturation and antibody production are X-linked. The most severe is X-linked agammaglobulinemia, which is caused by a defect in one of the cytoplasmic signal-transducing kinases in B cells. The defective protein is Bruton tyrosine kinase (Btk), and the condition is also called Bruton agammaglobulinemia. Without Btk, B cells are arrested at the pre-B stage and cannot develop into surface immunoglobulin–expressing cells. Many types of mutations of this gene have been described, but there is no apparent correlation between the specific mutation and the severity of the disease. Affected boys have less than 100 mg/dL of IgG and no serum IgM or IgA. B cells are virtually absent from bone marrow or periphery, but T cells are normal.

Hyper–Immunoglobulin M Syndrome

Hyper-IgM syndrome is usually X-linked, although there is a form that affects girls. These children have the same recurrent pyogenic infections as are seen in boys with X-linked agammaglobulinemia. Patients with hyper-IgM syndrome are also uniquely susceptible to *Pneumocystis* pneumonia. They have normal numbers of circulating B cells, low levels of IgG and IgA, and greater than normal levels of IgM. The defect is in their T cells, not B cells. They lack the CD40 ligand (CD154) that is typically expressed on the surfaces of activated T cells. Without this molecule, activated T cells cannot bind to CD40 on B cells and induce isotype switching. Therefore, the B cells continue to produce IgM but they do not switch to IgG or IgA and do not exhibit the affinity maturation that is characteristic of a secondary response. As might be predicted, the lymph nodes of these patients are populated with cells but have no organized germinal centers. Their susceptibility to *Pneumocystis* pneumonia may reflect the inability of their T cells to interact with CD40 on monocytes and activate their full microbicidal potential. These children are also prone to autoimmune hemolytic anemia, thrombocytopenic purpura, and recurrent neutropenia.[84]

Common Variable Immunodeficiency

The onset of common variable immunodeficiency is typically between 15 and 25 years of age,[90,91] which is strikingly later than with the congenital agammaglobulinemias. Although the late onset suggests an acquired etiology, there is a familial pattern that supports a genetic predisposition. These patients often, but not always, have normal numbers of B cells that express surface immunoglobulins but produce only very small quantities of circulating secreted immunoglobulin.[92] The underlying cause or causes are not yet well defined, but the defect may lie with the T cell.[93] Patients with common variable immunodeficiency are prone to malabsorption syndromes and autoimmune disorders. Some develop a sarcoid-like picture.[94] They are also at increased risk for gastrointestinal malignancies and lymphomas.[95] Relatives of patients with common variable immunodeficiency have a higher than normal incidence of IgA deficiency,[96] autoimmune disorders, and malignancies.[97]

Immunoglobulin G Subclass Deficiencies

Subclass deficiencies are an uncommon cause of susceptibility to infection. Isolated deficiency of IgG1 leads to significant morbidity, because this subclass dominates the IgG response; however, this condition is rare. IgG2 subclass deficiencies are often seen in combination with defects in IgG4, IgE, or IgA. Patients who are deficient in IgG2, IgG3, or IgG4 sometimes have recurrent bacterial infections,[98] but usually they do not.[99] If an IgG subclass deficiency is suspected, verification that the patient is deficient in the ability to produce functional antibody should be obtained before immunoglobulin replacement is proposed. This can be done by measuring the quantities of antibody before and after a booster vaccine for antigens such as diphtheria or tetanus.

Selective Immunodeficiencies

A few immunodeficiencies affect only the response to particular pathogens. Individuals with X-linked lymphoproliferative syndrome (Duncan syndrome) are unable to mount an adequate response to Epstein-Barr virus. Their B cells remain infected with the virus and continue to proliferate.

Another example of a pathogen-specific immunodeficiency is seen in people who are missing the Vκ A2 gene segment. This gene is often involved in the production of antibodies to *H. influenzae.* Presumably, the sequence of this V gene codes for an antigen-binding site that closely matches epitopes on the bacteria. Many members of the Navajo tribe are missing this V segment, and those who are without it are more prone to infections with *H. influenzae.*[100]

Combined T-Cell and B-Cell Defects

Children with severe combined immunodeficiency are unable to generate mature B cells or T cells. They are susceptible to all types of pathogens, including pyogenic bacteria, viruses, fungi, and opportunistic infections. Wiskott-Aldrich syndrome is an X-linked disease characterized by thrombocytopenia, severe eczematoid dermatitis, and deficient T- and B-cell responses. Patients have normal numbers of T and B cells, but a

defect in signal transduction impairs their ability to mount antigen-directed antibody responses. IgM and IgG levels are low, but IgE and IgA levels are high.[84] These children are vulnerable to infections with *S. pneumoniae* and *H. influenzae*, and may need immune globulin prophylaxis. Children with ataxia-telangiectasia have defects in DNA repair mechanisms that ultimately affect their immunoglobulin genes. Immunoglobulin replacement therapy should be given to children with demonstrable defects in their ability to generate antibodies to childhood vaccines.[84,101]

Malignancies

Antibody deficiencies may be seen in patients with chronic lymphocytic leukemia, multiple myeloma, or Waldenström macroglobulinemia.[102] These deficiencies may be caused by decreased numbers of B cells and suppression of normal antibody production by cellular elements of the tumor or their products.[103]

THERAPEUTIC USES OF ANTIBODIES

Passive Immunization

Passive immunization is the administration of immunoglobulin prepared from individuals known to have high levels of antibodies to the infectious agent in question. It can be used for an immunocompromised host if the ability to generate a sufficient immune response to vaccination is in doubt. For example, immunosuppressed allograft recipients may need cytomegalovirus immune serum after inadvertent exposure to this virus.

Passive vaccination is also used if a patient cannot make antibodies in sufficient time to protect against disease. For example, *Clostridium tetani* in a contaminated wound can produce lethal quantities of toxin long before an unvaccinated host could make neutralizing antibodies to the toxin. In such a case, the patient should receive tetanus immune globulin. The patient should also be vaccinated to protect against tetanus in the event of future injuries. Simultaneous administration of antibodies (hyperimmune serum) and antigen (toxoid vaccine) will not prevent the development of an adequate immune response. In fact, dendritic cells may store immune complexes and use them to stimulate B cells over long periods. The patient who has been previously vaccinated for tetanus should have some circulating antibodies to tetanus toxin, and a booster tetanus toxoid vaccine will trigger a rapid secondary response with production of large quantities of additional toxin-specific antibodies.

Another indication for passive vaccination is antivenin for snakebite. It is not practical, and indeed may be dangerous, to immunize human volunteers with the antigen. Because individuals who have become immune through natural exposure are rarely available, it is necessary to use antisera prepared from immunized animals. Antibodies from other species are adequate because their major role, in this case, is to block the binding of toxins to cell receptors. The limitation of nonhuman immunoglobulins is that they can trigger an immune response and a type III hypersensitivity reaction, particularly with repeat administration.

Intravenous Immune Globulin Replacement

Individuals with agammaglobulinemia or hypogammaglobulinemia need lifelong replacement with antibodies that can protect them against the diverse infectious agents they will encounter in ordinary life activities. Immune globulin replacement is unlikely to be necessary until IgG levels fall to less than 200 mg/dL. Even with low overall levels of IgG, some patients may still make adequate levels of specific antibodies. This can be readily assessed with the use of clinically available tests that measure response to vaccines such as tetanus toxoid, *H. influenzae* type b toxoid conjugate, or hepatitis B. Patients with agammaglobulinemia typically require 300 to 400 mg/kg of immune globulin every 3 to 4 weeks. Preparations specifically designed for intravenous administration are available. Intravenous immune globulin (IVIG) must be free of aggregated immunoglobulin, which can act as an immune complex and trigger type III hypersensitivity reactions. The life span of infused IgG is about 21 days. With repeated infusions at 3- to 4-week intervals, the trough levels slowly rise. The goal is to ensure that levels do not drop lower than 700 to 800 mg/dL. Infusions may be accompanied by fever, chills, myalgias, headache, and nausea, but these tend to become less frequent after repeated infusions.[101] Numerous IVIG preparations are currently available, and their use in primary humoral deficiencies has been reviewed by Schroeder and Dougherty.[104]

After its success was demonstrated in treating infectious diseases, clinicians began administering IVIG for inflammatory and autoimmune conditions whose cause was unknown but was speculated to be of infectious origin. IVIG is strikingly effective treatment of idiopathic thrombocytopenic purpura and Kawasaki disease. However, IVIG is currently being used to treat a wide range of hematologic, dermatologic, and neurologic disorders.[105] It is still not understood how IVIG exerts its immunomodulatory effects in these diseases. Indeed, they represent such a wide variety of pathogenetic processes that it is likely that IVIG may work through many different mechanisms.[105,106] IVIG should contain antibodies that neutralize bacteria, toxins, or superantigens that might be responsible for the disease. IVIG preparations are made from the plasma of at least 1000 and up to 100,000 donors. The array of antigen-binding sites thus represents essentially the entire human repertoire. Within this repertoire, there may be antiidiotypic antibodies that can downregulate pathologic autoimmune responses in certain patients.[107] IVIG infusions also appear to downregulate production of inflammatory cytokines, possibly because of cytokine-specific antibodies.[108,109] In cases in which antibody-coated host cells are attacked by the immune system, IVIG may slow the process by blockading the reticuloendothelial system. Because the half-life of IgG is influenced by the serum concentration, raising the serum level of IgG with IVIG may accelerate the clearance of autoantibodies. The doses of IVIG required for immunomodulation are four to five times higher than are used for replacement therapy in humoral immunodeficiency, which suggests that some of the effects of IVIG are not mediated through the traditional effector functions of antibodies.[105,106] The antiinflammatory activity of IVIG is related to its content of sialylated IgG molecules.[110] Sialylated IgG binds to type II FcγRs such as DC-SIGN and CD23, leading to the production of IL-33. This leads, in turn, to upregulation of the inhibitory FcγRIIB on macrophages, increasing their activation threshold, and suppressing inflammation.[110] The effect of IVIG may not even be due to antibodies at all but rather to other serum proteins that are present in trace quantities. For example, CD4, CD9, human leukocyte antigen molecules, and cytokines are all present in low levels.[111,112]

Monoclonal Antibodies

Uniform and reproducible preparations of antibodies are also used as therapeutic drugs in vivo and as reagents in vitro.[113] To produce monoclonal antibodies, an animal, typically a mouse or rat, is immunized with the desired antigen. B cells from the immunized animal are fused with malignant B cells that do not produce their own immunoglobulin, with the goal of producing a hybridoma that proliferates indefinitely and produces IgG at a high rate. The fused cells are distributed, one to a well, and allowed to proliferate. The supernatant is assayed for specific antibody, and clones producing high levels of desirable antibody are selected and propagated indefinitely. In recent years, the technology for making human monoclonal antibodies has improved tremendously such that it is possible to clone the variable regions from individual B cells into expression vectors that allow unlimited production of the desired immunoglobulin. Advances in technology combined with improved production practices have led to a biologics revolution in medicine as dozens of monoclonal antibodies have now been licensed for therapy of cancer and inflammatory diseases.[113]

Although this revolution has largely bypassed the treatment of infectious diseases for multiple reasons, including microbial antigen variability, the availability of conventional antimicrobial therapy and the fact that antibody therapies tend to be effective only when used early in infection has spawned great interest in developing antiinfective monoclonal antibodies. The first monoclonal antibody approved by the US Food and Drug Administration for prevention of infection was palivizumab for respiratory syncytial virus (RSV) in 1998.[114] There are now approved monoclonals for RSV, inhalational anthrax, *Clostridioides difficile* (formerly *Clostridium difficile*), and HIV-1. Clinical trials are underway for additional monoclonal antibodies for infections with RSV and HIV-1, as well as cytomegalovirus, hepatitis B and hepatitis C viruses, *Staphylococcus aureus, Pseudomonas aeruginosa,* herpes simplex

virus type 2, influenza A, Ebola virus, rabies, Shiga toxin–producing *Escherichia coli* hemolytic-uremic syndrome (STEC-HUS), and tuberculosis.[115,116] Promising monoclonals for immunotherapy of Zika virus infection are under development.[117] The challenges of producing antiinfective monoclonals have been well reviewed,[115,116,118] particularly the challenges presented by HIV-1.[119,120] Other approaches for monoclonal antibody treatment of HIV-1 include engineering bispecific antibodies that recognize HIV envelope proteins on the surface of infected cells and recruit effector cells.[121,122]

Although native immunoglobulins have significant therapeutic activity, there are measures that can be used to enhance their effectiveness, such as conjugating them to toxins or radionuclides.[123–125]

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6 Cell-Mediated Defense Against Infection

Michail S. Lionakis[a] *and Tobias M. Hohl*

SHORT VIEW SUMMARY

- T lymphocytes represent the major constituent of cell-mediated defense against infection and express an antigen-specific and somatically recombined T-cell receptor.
- Phenotypically diverse T-cell subsets can be classified on the basis of T-cell receptor and coreceptor expression (i.e., CD4 and CD8), and undergo regulation by transcription factors to promote cytolytic, helper, regulatory, and memory functions.
- Antigen-specific T-cell memory can persist for many years in the absence of microbial antigen exposure and facilitate more rapid clearance of microbes upon secondary exposure.
- T-cell lymphopoiesis occurs primarily in the thymus and includes positive and negative selection steps to ensure T-cell binding to antigens presented by major histocompatibility complex (MHC) molecules and to exclude auto-reactive T-lymphocytes.
- All cells express MHC class I molecules, and MHC class I molecule–antigen complexes activate cytolytic CD8 T cells that play a central protective role against many viral pathogens. Antigen processing and loading on MHC class II molecules is a primary function of a heterogeneous group of antigen-presenting cells that activate CD4 T cells that play a central protective role against intracellular bacterial as well as fungal pathogens. Cooperation between CD4 and CD8 T-cell activity, and in broader terms between cell-mediated and humoral immunity, is a hallmark of immune responses against many infectious agents.
- The recognition of extracellular and intracellular pathogen-encoded molecular patterns by germline-encoded receptors links innate immune activation with cell-mediated defense against infection.
- Primary immunodeficiency disorders illustrate the functional role of molecular and cellular constituents in cell-mediated defense against infectious agents.

The principal function of the mammalian immune system is to combat infectious diseases. Immune responses to microbial pathogens are divided into those mediated by cells (i.e., cell-mediated immunity) and those mediated by antibodies (i.e., humoral immunity). Although innate and adaptive immune cells both contribute to cell-mediated immune responses, T lymphocytes represent the central player in this process by providing antigen specificity, by orchestrating antimicrobial activities of innate immune cells that generally lack antigen specificity, and by killing microbially infected host cells.

The induction of T-cell–mediated responses relies primarily on dendritic cells (DCs) that traffic from portals of microbial infection to peripheral lymphoid organs (e.g., lymph nodes) to present antigens to T cells and to provide contextual information (Fig. 6.1A).[1] T cells express T-cell receptors (TCRs) to detect pathogen-derived peptides presented on major histocompatibility complex (MHC) molecules. The somatically rearranged TCR complex confers antigen specificity to individual T lymphocytes. The primary encounter of naïve T cells with microbial antigen results in their activation, proliferation, and differentiation into effector T cells that migrate to the portal of infection (Fig. 6.1B), and exert T-cell effector functions to facilitate pathogen eradication (Fig. 6.1C). Effector T cells undergo contraction and long-term memory T cells emerge. Our understanding of T-cell responses to microbial pathogens has increased remarkably, as has our appreciation for the complexity of cell-mediated immune responses. This chapter summarizes current knowledge regarding T-cell responses to microbial pathogens.

T-CELL SUBSETS AND PHENOTYPIC DIVERSITY

Mammalian T lymphocytes are categorized on the basis of TCR and coreceptor expression. Most T lymphocytes implicated in antimicrobial defense express a heterodimeric TCR composed of an α- and a β-chain (αβ TCR).[2] These include heterogeneous populations of unconventional or "innate-like" T cells, such as invariant natural killer T (NKT) cells and mucosal-associated invariant T (MAIT) cells that typically express an αβ TCR with limited diversity (see "NKT Cells and T Cells With a Restricted αβ TCR Repertoire" later).[3,4] A small fraction of circulating human T lymphocytes, approximately 1% to 5% in humans, express a TCR composed of a γ- and a δ-chain and are referred to as γδ T cells (see "γδ T Cells" later).[5]

T lymphocytes are usually classified on the basis of CD4 or CD8 coreceptor expression since CD4 and CD8 T cells differ in terms of function, specificity, and role in antimicrobial defense. As a rule, CD4 T lymphocytes orchestrate antimicrobial defense but do not participate directly in microbial killing. The major strategy used by CD4 T cells to disable microbes is to secrete cytokines and chemokines that recruit leukocytes (e.g., monocytes and neutrophils) to and activate leukocytes (e.g., macrophages and B cells) at sites of infection. A subset of CD4 T cells, termed *regulatory T cells,* express the transcription factor forkhead box P3 (FOXP3) and can exert immune regulatory functions during microbial infections. The major strategy used by CD8 T cells is to secrete lytic proteins that destroy infected cells, depriving the pathogen of an environment that is suitable for replication and long-term survival. These functions, collectively called *T-lymphocyte effector functions,* are expressed selectively by different T-cell subsets, providing a diversity of antimicrobial mechanisms.[2,6,7]

CD4 T Cells

Naïve CD4 T cells circulate between the bloodstream and lymph nodes and undergo priming and clonal expansion upon encountering antigen-presenting cells (APCs) with cognate MHC class II–peptide complexes. Priming of naïve T cells in lymph nodes likely occurs in a stepwise fashion, with sequential interactions with distinct APCs (e.g., migratory DCs, lymph node–resident DCs) that each contribute to the phenotype of the effector CD4 T-cell response.[8] Following priming, CD4 T cells can differentiate into phenotypically distinct effector cells, a process that is determined by the inflammatory context induced by the microbe.[9] Classic studies of CD4 T-cell priming suggested that differentiation can give rise either to helper T cell 1 (Th1) cells that produce interferon-γ (IFN-γ) or to helper T cell 2 (Th2) cells that produce interleukin (IL)-4 (IL-4).[10] This model has been revised by studies conducted in the past

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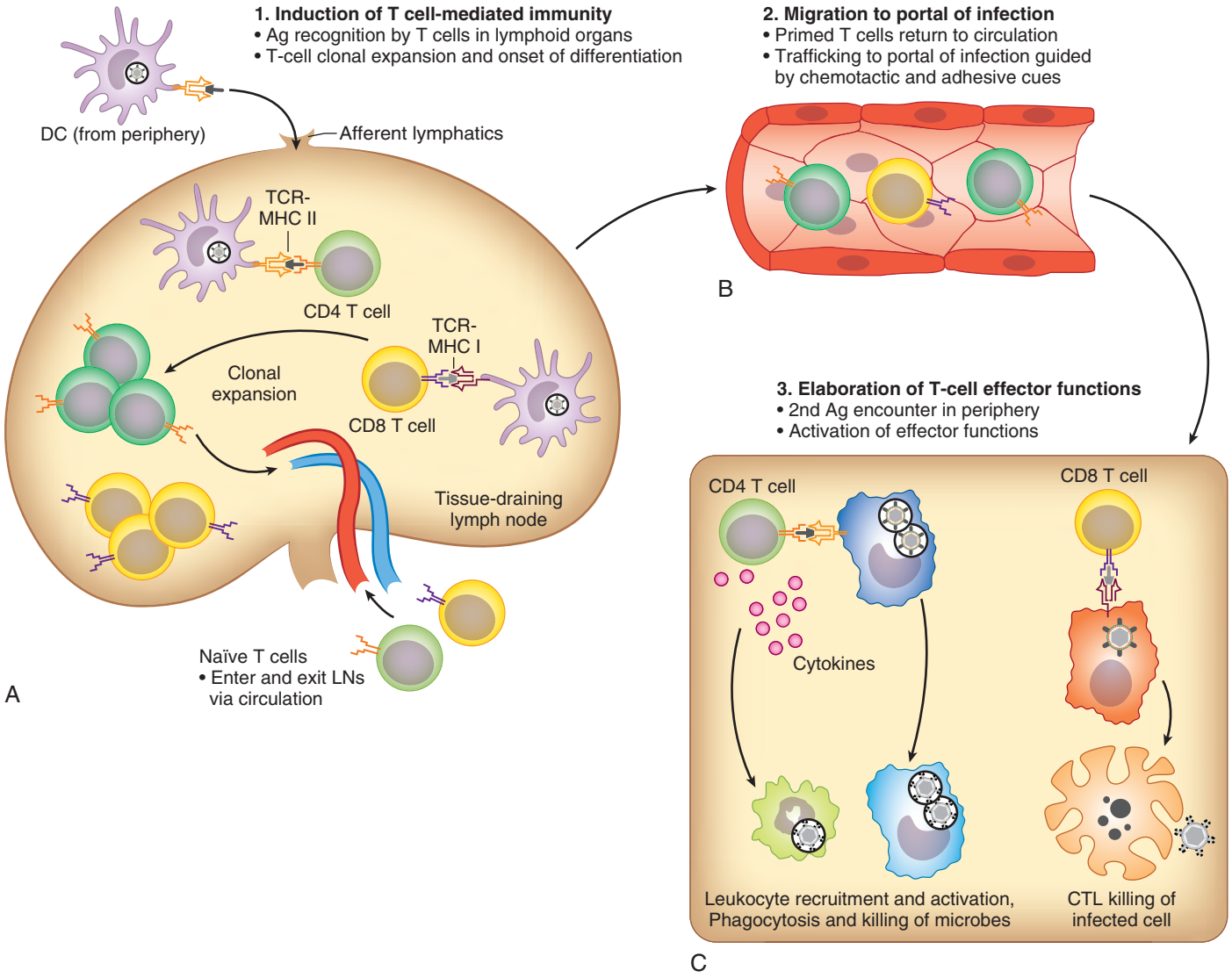

FIG. 6.1 Overview of T-cell activation and T-cell effector functions. (A) During the induction phase, naïve CD4 T cells and CD8 T cells enter the lymph node and recognize microbial-derived peptide antigens presented by dendritic cells (*DCs*) in the context of major histocompatibility complex *(MHC)* class II and MHC class I, respectively. The responding T cells undergo proliferation and begin the differentiation process into short-lived effector cells that enter the circulation. (B) T-cell trafficking follows chemokine- and integrin-mediated cues to reach the portal of infection. (C) Responding T cells elaborate effector functions, with CD4 T cells primarily recruiting and activating phagocytes to eradicate tissue-invasive microbes, and CD8 T cells directly killing infected host cells. *Ag,* Antigen; *CTL,* cytotoxic T lymphocyte; *LNs,* lymph nodes; *TCR,* T-cell receptor.

20 years that demonstrate a more diverse and flexible differentiation potential of naïve CD4 T cells.[9] Cytokines produced during infection promote the expression of distinct transcriptional regulators that determine which effector molecules will be expressed by responding CD4 T cells.

Distinct CD4 T-cell populations that respond to and control specific microbial pathogens have been well characterized based on the expression of transcriptional regulators and the secretion of specific cytokines (Fig. 6.2). *Epigenetic modifications,* including the chemical modification of histones and DNA (i.e., by acetylation, methylation, phosphorylation, ubiquination, and sumoylation) as well as the compaction and remodeling of nucleosomes and larger chromosomal segments, represent critical determinants to support CD4 T-cell lineage differentiation and specification. These mechanisms enable *cis*-regulatory elements that control helper T-cell lineage-specific genes (e.g., the *ifng* or *il4* gene) to bind to *trans*-acting factors (e.g., lineage-determining transcription factors), and regulate target genes.

Studies of in vivo CD4 T-cell priming, trafficking, and differentiation have been facilitated by the development of mice in which T-cell specificity is engineered by transgenic expression of defined TCRs. Adoptive transfer of T cells of defined specificity into normal recipient mice, followed by microbial challenge, has allowed investigators to determine the kinetics of CD4 T-cell activation and to measure trafficking from sites of T-cell priming to sites of infection. CD4 T-cell priming following respiratory challenge with influenza virus or the fungal pathogens *Aspergillus fumigatus* and *Blastomyces dermatiditis* is rapid and accompanied by differentiation into a Th1 phenotype.[11–13] Interestingly, complete differentiation of *Aspergillus*-specific CD4 T cells does not occur until CD4 T cells arrive in the lung. In contrast to these infections, CD4 T cells responding to *Mycobacterium tuberculosis* infection are not activated until at least 1 week postinfection[14,15] and do not reach the lung until 8 to 12 days postinfection. Adoptive transfer of *M. tuberculosis*–specific Th1 CD4 T cells prior to infection can provide substantial immunity, but not until the infection has progressed for roughly 1 week.[16]

Th1 T Cells

Naïve T cells are driven to differentiate into Th1 T cells by early exposure to IL-12 and IFN-γ at the time of T-cell priming. Th1 T cells secrete IFN-γ, a signature cytokine that activates macrophages and DCs and

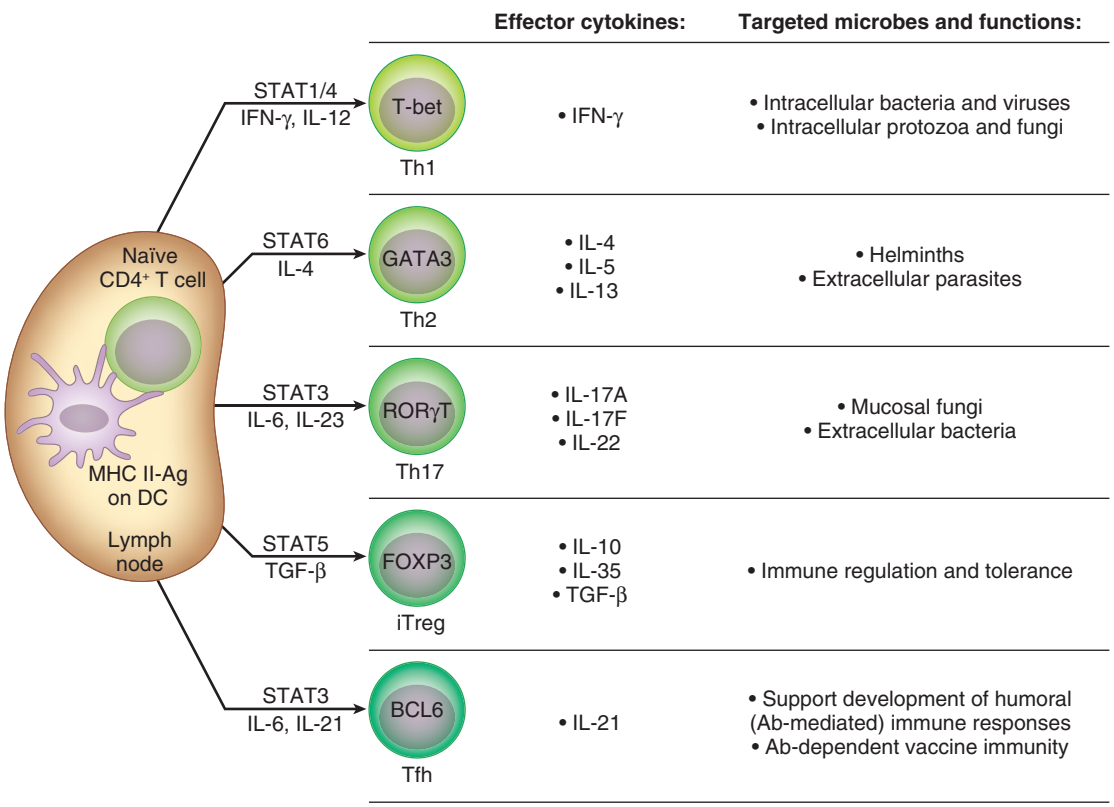

FIG. 6.2 Helper T-cell subsets. Naïve CD4 T cells proliferate and differentiate into distinct helper T-cell *(Th)* subsets following T-cell receptor (TCR) activation by cognate major histocompatibility complex *(MHC)* class II–peptide antigen *(Ag)* complexes during infectious and inflammatory states. Cytokine- and signal transducer and activator of transcription *(STAT)*–mediated signals drive Th differentiation into distinct Th effector subsets that are maintained by the expression of transcriptional activators *(T-bet, GATA3, RORγt, FOXP3)* or repressors *(BCL6)* and secrete distinct effector cytokines. Individual Th subsets demonstrate plasticity and may interconvert during infectious challenges to facilitate microbial eradication and reduce immune-mediated tissue injury. The major classes of pathogens that are targeted by individual Th subsets are indicated in the right column. *Ab,* Antibody; *BCL6,* B-cell lymphoma 6; *DC,* dendritic cell; *FOXP3,* forkhead box P3; *IFN-γ,* interferon-γ; *IL,* interleukin; *iTreg,* inducible T-regulatory cell; *LNs,* lymph nodes; *RORγt,* retineic acid receptor–related orphan nuclear receptor γt; *Tfh,* T follicular helper cell; *TGF-β,* transforming growth factor-β.

thereby enhances their ability to kill intracellular microbes and to present antigens to T lymphocytes. Th1 T cells can also secrete tumor necrosis factor (TNF), lymphotoxin, and IL-2, which contribute to antimicrobial defense as well.[9]

DCs and other myeloid cells that recognize pathogenic microbes via germline-encoded pattern recognition receptors often represent the earliest source of IL-12 during T-cell priming. Innate lymphoid cells (ILCs)—including NKT cells, unconventional T-cell populations, and neutrophils—in the setting of salmonellosis and toxoplasmosis can represent early sources of IFN-γ.[17,18] IFN-γ binds to the IFN-γ receptor on T cells, activates signaling via the signal transducer and activator of transcription (STAT) 1 (STAT1) pathway, and induces the expression of T-bet.[19] T-bet is a transcriptional regulator that promotes IFN-γ production by T cells and downregulates the expression of IL-4, a cytokine that promotes Th2 differentiation. IL-12 binds the IL-12 receptor heterodimer and signals via the STAT4 pathway, amplifying Th1 responses and enhancing IFN-γ production by responding Th1 cells.

Th1 T cells play an important role against infections by intracellular bacteria (e.g., *Salmonella typhimurium*, *M. tuberculosis*, and nontuberculous mycobacteria) and dimorphic fungi.[20] Clinical observations in humans with primary immunodeficiency disorders and experiments in mice link genetic defects in IL-12, IFN-γ, IL-12 receptor, IFN-γ receptor, or downstream signal transducers (e.g., STAT1) to susceptibility to these pathogens (Table 6.1).[21–25] Th1 CD4 T cells enhance defense against *Leishmania major* by stimulating IFN-γ–dependent macrophage killing of intracellular parasites.[26]

Th2 T Cells

Th2 cells express cytokines that influence B-cell differentiation and antibody production, eosinophil recruitment, and mucus production. The signature cytokines produced by Th2 cells are IL-4, IL-5, and IL-13, but Th2 cells can also produce IL-9, IL-10, IL-25, and amphiregulin.[27] Th2 responses are generated when naïve T cells are exposed to IL-4 at the time of T-cell priming. Following antigenic challenge, IL-4 can be produced by mast cells and basophils in the vicinity of T-cell priming[28,29] or, in the setting of low antigen concentrations, by responding T cells.[30] The IL-4 receptor transduces signals to naïve T cells via the STAT6 pathway to facilitate the expression of GATA-3, the master transcriptional regulator of Th2 differentiation.[31] This process can be enhanced by IL-4– and STAT6-independent GATA-3 activation, all of which drives the expression of additional downstream activators. For example, APCs that express the Notch ligand Delta can act on the Notch effector recombining binding protein suppressor of hairless (RBPJκ) to induce GATA-3 in the absence of STAT6 activation and to regulate *il4* gene transcription.[32]

Although Th2 cells are best known for causing or contributing to allergic diseases, Th2 cells contribute to defense against infections, particularly helminth infections of the gastrointestinal tract.[27] In this setting, eosinophil recruitment, immunoglobulin E (IgE) production, and mucus hypersecretion can enhance parasite expulsion in an IL-4– and IL-13–dependent manner, a notion that is supported by murine studies of *Nippostrongylus brasiliensis* infection.[33] The secretion of amphiregulin by Th2 cells can stimulate intestinal epithelial cell proliferation and expulsion of *Trichuris muris*, a nematode that infects mice.[34] Besides Th2 cells, tissue-resident and Th2 cytokine-secreting ILC, termed type 2 ILC, represent a significant source of IL-13 during the early stages of parasitic infection and promote expulsion.[27]

In humans, the type of disease associated with *Mycobacterium leprae* infection is tied to CD4 T-cell differentiation. Th1 differentiation is associated with tuberculoid leprosy, a paucibacillary infection in which IFN-γ–producing T cells enhance microbial killing. The induction of

TABLE 6.1 Clinical and Immunologic Characteristics of Primary Immunodeficiency Disorders That Result in Impaired Cellular Immune Responses

GENE (MODE OF INHERITANCE)	INFECTION SUSCEPTIBILITY AND NON-INFECTIOUS MANIFESTATIONS (WHERE APPLICABLE)	ASSOCIATED CELLULAR IMMUNE DEFECTS
Disorders of IL-12/IFN-γ Signaling (MSMD)		
IFNGR1 (AD or AR)	Intracellular bacterial, fungal, and mycobacterial infections	Defective IFN-γ responses by macrophages
IFNGR2 (AD or AR)	Intracellular bacterial, fungal, and mycobacterial infections	Defective IFN-γ responses by macrophages
IL12RB1 (AR or AD)	Intracellular bacterial, fungal, and mycobacterial infections	Defective IL-12–dependent IFN-γ production by Th1 cells
IL12B (AR)	Intracellular fungal and mycobacterial infections	Defective IL-12–dependent IFN-γ production by Th1 cells
STAT1 (AR)	Mycobacterial and viral infections, including herpes simplex encephalitis	Defective IFN-γ responses by macrophages
STAT1 (AD)	Mycobacterial, viral, and intracellular fungal infections; invasive mold infections; CMC; hypothyroidism; squamous cell carcinomas; autoimmunity	IFN-γ tachyphylaxis (intracellular infections); defective Th17 differentiation (CMC)
IRF8 (AD or AR)	Mycobacterial infections; CMC	Defective IL-12 responses or decreased DCs and monocytes (intracellular infections), or both; decreased Th17 cells (CMC)
ISG15 (AR)	Mycobacterial infections; intracranial calcifications	Defective IFN-γ production by Th1 cells
Anhidrotic ectodermal dysplasia with immune deficiency caused by mutations in: *NEMO/IKBKG* (X-linked)	Bacterial, mycobacterial and viral infections, including herpes simplex encephalitis; PJP; CMC; anhidrotic ectodermal dysplasia	Severe lymphopenia and decreased Th1 cells
RORC (AR)	Mycobacterial infections; CMC	Defective IFN-γ production by Th1 cells; defective Th17 differentiation (CMC)
GATA2 (AD)	Mycobacterial, viral, and invasive mold and intracellular fungal infections; myelodysplasia; lymphedema	Lymphopenia; decreased numbers of monocytes and DCs; neutrophil defects
Disorders of IL-17 Signaling		
IL17F (AD)	CMC; asthma	Defective IL-17F responses
IL17RA (AR)	CMC; skin staphylococcal infections; pulmonary bacterial infections; atopic dermatitis	Defective IL-17 responses
IL17RC (AR)	CMC	Defective IL-17 responses
ACT1 (AR)	CMC; skin staphylococcal infections; atopic dermatitis	Defective IL-17 responses
Job (hyper-IgE) syndrome caused by mutations in *STAT3* (AD)	Skin and pulmonary bacterial infections; CMC; invasive mold and intracellular fungal infections; eczema; skeletal abnormalities; aneurysms	Defective RORγt-dependent Th17 differentiation
DOCK8 (AR)	Skin viral infections; molluscum contagiosum; CMC; malignancies	Defective Th17 differentiation
STK4 (AR)	Bacterial and viral infections; CMC; lymphoproliferative disorder	Defective Th17 cell responses
APECED syndrome caused by mutations in *AIRE* (AR or AD)	CMC; endocrine and nonendocrine autoimmunity; ectodermal dystrophy	Autoantibodies against Th17 cytokines
Disorders of Pathogen Recognition Receptors and Associated Downstream Signaling		
MYD88 (AR)	Invasive pyogenic bacterial infections	Defective TLR/IL-1R signaling
IRAK4 (AR)	Invasive pyogenic bacterial infections	Defective TLR signaling
TLR3 (AD or AR)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
TRIF (AD or AR)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
UNC93B1 (AR)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
TBK1 (AD)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
TRAF3 (AD)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
CLEC7A (AR)	Vaginal candidiasis; dermatophytosis	Defective Th17 responses
CARD9 (AR)	CNS candidiasis; CMC; deep-seated dermatophytosis; extrapulmonary aspergillosis; phaeohyphomycosis	Decreased numbers of Th17 cells; defective neutrophil recruitment and fungal killing
MALT1 (AR)	Bacterial and viral infections; CMC; bronchiectasis	Defective T-cell activation; hypogammaglobulinemia
BCL10 (AR)	Mycobacterial and viral infections; CMC	Lymphopenia; hypogammaglobulinemia
IFIH1 (AR)	Severe respiratory viral infections	Defective MDA5-dependent dsRNA sensing and downstream IFN-α/β responses

TABLE 6.1 Clinical and Immunologic Characteristics of Primary Immunodeficiency Disorders That Result in Impaired Cellular Immune Responses—cont'd

GENE (MODE OF INHERITANCE)	INFECTION SUSCEPTIBILITY AND NON-INFECTIOUS MANIFESTATIONS (WHERE APPLICABLE)	ASSOCIATED CELLULAR IMMUNE DEFECTS
Disorders of Lymphopenia or Lymphocyte Activation		
SCID caused by mutations in: *ADA, JAK3, IL7RA, RAG1, RAG2, ARTEMIS* (AR); *IL2RG* (X-linked)	Viral, bacterial, and invasive (PJP) and mucosal (CMC) fungal infections	Severe T, B, and/or NK lymphopenia
XLA caused by mutations in *BTK*	Bacterial infections	Significantly decreased or absent B cells; hypogammaglobulinemia
Classic or functional NK cell deficiency caused by mutations in: *MCM4, RTEL1, FCGR3A* (AR); *GATA2* (AD)	Severe viral infections; malignancies (GATA-2 additional features as above)	Absent NK cells or defective NK cell function
IL21R (AR)	Respiratory infections; cryptosporidiosis; CMC; PJP; liver fibrosis	Defective T-cell activation
CD40L (X-linked)	Bacterial, mycobacterial, fungal (PJP), and parasitic infections; inflammatory bowel disease	Defective T-cell activation
Bare lymphocyte syndrome type II caused by mutations in: *CIITA, RFX5, RFXANK, RFXAP* (AR)	Bacterial, mycobacterial, fungal, and viral infections; chronic diarrhea; failure to thrive	Absent MHC class II expression on mononuclear cells; defective T-cell activation
Disorders of the CD8 T Cell–MHC Class I Complex Interface		
Bare lymphocyte syndrome type I, caused by mutations in: *TAP1, TAP2* (AR)	Respiratory bacterial infections; bronchiectasis; skin ulcerative granulomas	Defective processing and presentation of MHC class I molecules
CD8A (AR)	Respiratory bacterial infections; bronchiectasis; skin ulcerative granulomas	Decreased or absent CD8 T cells
B2M (AR)	Respiratory bacterial infections; bronchiectasis; skin ulcerative granulomas	Defective processing and presentation of MHC class I molecules
ZAP70 (AR)	Bacterial, viral, and fungal (PJP) infections; failure to thrive	Decreased CD8 T cells; defective proliferation of CD4 T cells

AD, Autosomal dominant; *APECED,* autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy; *AR,* autosomal recessive; *CMC,* chronic mucocutaneous candidiasis; *DC,* dendritic cell; *dsRNA,* double-stranded RNA; *IFN,* interferon; *IgE,* immunoglobulin E; *IL,* interleukin; *IL-1R,* interleukin-1 receptor; *MDA5,* melanoma differentiation-associated gene 5; *MSMD,* mendelian susceptibility to mycobacterial disease; *NK,* natural killer; *PJP, Pneumocystis jirovecii* pneumonia; *SCID,* severe combined immunodeficiency disorder; *Th1,* helper T cell 1; *Th17,* helper T cell 17; *TLR,* Toll-like receptor; *XLA,* X-linked agammaglobulinemia.

type I interferon and IL-10 signaling in innate immune cells during leprosy can antagonize IFN-γ–dependent protection.[35] Th2 differentiation is associated with high tissue densities of *M. leprae* and more robust, but ineffective, antibody responses.[36,37]

Th17 T Cells

Helper T cell 17 (Th17) cells produce the signature cytokines IL-17A and IL-17F, often termed IL-17, since they can form homo- and heterodimers. Although Th17 T cells were initially characterized as the principal CD4 T-cell effectors that promote inflammatory diseases (e.g., rheumatoid arthritis, psoriasis, and multiple sclerosis),[38] the roles of Th17 T cells and IL-17–dependent signaling pathways in defense against microbial infection, particularly against extracellular bacteria and mucosal fungi, are now well established.

CD4 T-cell differentiation into Th17 cells requires early exposure to transforming growth factor-β (TGF-β) and IL-6 at the time of priming, IL-21–driven amplification, and IL-23–driven stabilization.[9,39–43] The combination of robust TCR stimulation and IL-6/TGF-β exposure induces IL-23 receptor expression and STAT3-dependent expression of the transcription factor retineic acid receptor–related orphan nuclear receptor γt (RORγt).[44] RORγt, together with RORα,[45] promotes IL-17A, IL-17F, IL-21, and IL-22 release. Th17 cell activation leads to the expression of chemokine receptors (i.e., CCR4, CCR6) that facilitate their migration to inflammatory lesions at mucosal sites, specifically the intestines, lung, and skin.[46]

Microbial factors that promote the differentiation of Th17 T cells include *M. tuberculosis*–derived complete Freund adjuvant, specifically trehalose dimycolate (i.e., cord factor) and peptidoglycan that synergize to recapitulate Th17-promoting adjuvant activity of mycobacteria.[47,48] The fungal cell wall components β-glucan and α-mannan trigger C-type lectin receptor signaling and stimulate caspase recruitment domain-containing protein 9 (CARD9)–dependent signals in DCs that enhance Th17 T-cell differentiation[49] (see "C-Type Lectin Receptors" later).

Th17- and IL-17–dependent functions in antimicrobial immunity are best understood at mucosal surfaces. IL-17–responsive epithelial cells produce chemokines that recruit proinflammatory cells, primarily neutrophils, to the site of infection, and release antimicrobial peptides for local control of extracellular pathogens.[50,51] Clearance of murine pulmonary infection with *Klebsiella pneumoniae* is dependent on cytokines secreted by Th17 T cells, including IL-17 and IL-22.[52] Patients with hyper-IgE syndrome, also known as Job syndrome, have impaired differentiation of Th17 T cells.[53] This defect results from mutations in STAT3, a signaling molecule that is essential for Th17 T-cell development, and accounts for a markedly increased risk of pulmonary infections, mucocutaneous candidiasis, and staphylococcal infections. Mendelian susceptibility to chronic mucocutaneous candidiasis and dermatophytosis maps to components of the IL-17A and IL-17F signaling pathway and underscores the importance of these Th17-derived cytokines against mucosal fungal infections (see Table 6.1).[20]

Th17-dependent and IL-17–dependent immune functions are not synonymous, since RORγt$^+$ type 3 ILCs, and unconventional and γδ T cells, can provide early sources of IL-17 and IL-22 prior to Th17 T-cell differentiation and trafficking to sites of infection.[54] In the intestine, colonization of germ-free mice with commensal segmented filamentous bacteria is sufficient to induce Th17 cells in the lamina propria.[55] In this model, tissue-resident type 3 ILCs trigger IL-22–dependent epithelial production of serum amyloid A proteins that in turn act on poised Th17 cells to amplify the production of effector cytokines.[56] These data suggest that intestinal commensal microbes can stimulate Th17-mediated mucosal protection against pathogenic microbes.

T-Regulatory Cells

T-regulatory cells (Tregs) can either emerge from the thymus as natural Tregs (nTregs), or they can be induced during T-cell priming of naïve T cells, in which case they are referred to as inducible Tregs (iTregs).[57] Tregs were first identified as a CD4$^+$CD25$^+$ T-cell subset in normal mice

and constituted roughly 10% of the CD4 T-cell population.[58] This T-cell population had the remarkable ability to prevent autoimmune inflammatory bowel disease when transferred into susceptible mice.

Similar to conventional CD4 and CD8 T lymphocytes, nTregs are selected in the thymus on epithelial cells.[59] iTregs, on the other hand, are induced in the periphery when naïve T cells are primed in the presence of TGF-β, which induces expression of FOXP3, a transcriptional regulator that induces iTreg differentiation.[60] nTregs emerging from the thymus also express FOXP3.[59] The human IPEX syndrome (*i*mmunodysregulation, *p*olyendocrinopathy, and *e*nteropathy that is *X*-linked) results from a mutation in the gene encoding FOXP3, and gives rise to a profoundly inflammatory disease characterized by the absence of Tregs.[61–63]

Both nTregs and iTregs contribute to and expand the TCR repertoire implicated in regulatory T-cell function, suppress the activation of autoimmune T lymphocytes, and mediate immune tolerance.[64] TGF-β and IL-10 have been implicated in this process, and, depending on the organ site, both can limit the development of inflammatory disease. In the large intestine, short-chain fatty acids (i.e., butyrate, propionate, and acetate) accumulate to concentrations of 50 to 100 mM in the colonic lumen due to the fermentation of dietary fiber by commensal anaerobic bacteria.[65] These microbial metabolites are sensed by G protein–coupled receptor signaling pathways (e.g., GPR43) that act to promote the formation of iTregs.[66]

The role of Tregs in defense against microbial infection is significant and varies based on the microbial pathogen and the type of inflammatory response, resulting in a positive or a negative role. For example, in murine infection with *L. major,* Tregs at the site of parasite inoculation prevent complete Th1-mediated microbial clearance, which enables long-term CD4 T-cell memory to be maintained.[67] Similarly, Tregs enable CD8 T-cell memory responses to West Nile virus in mice.[68]

In contrast, Treg depletion during herpes simplex virus and respiratory syncytial virus infections resulted in adverse outcomes as a result of disorganized early innate immune cell recruitment[69] or immunopathology linked to respiratory syncytial virus–specific CD8 T-cell responses.[70] During malaria infection, Tregs can restrict immune responses to create a lethal outcome, and Treg depletion enhances survival.[71] Similarly, Treg depletion in filarial infection models markedly enhances pathogen clearance.[72] During tuberculosis, pathogen-specific Tregs expand early during pulmonary infection and delay the arrival of effector CD4 and CD8 T cells in the lung,[73] yet undergo eventual elimination in response to IL-12–mediated signals.[74]

In humans, Tregs influence acute and chronic viral infections.[75] Although hepatitis A virus does not infect T cells, Tregs express the hepatitis A virus cellular receptor 1 (HAVCR1), and viral binding blocks its ability to recognize phosphatidylserine on apoptotic cells. At the peak of viral infection, patients lack Treg immunosuppressive function, yet clear the virus in the context of high IL-22 and low TGF-β levels that act in concert to limit hepatic injury.[76] Tregs may suppress the proliferation of acutely human immunodeficiency virus (HIV) –infected CD4 T cells, limiting HIV spread at early stages of disease.[77] In contrast, high Treg levels correlate with disease progression during chronic hepatitis B infection.[78] Similarly, high circulating Treg frequencies are found in patients with extrapulmonary tuberculosis.[75] In sum, although Tregs have a well-established role in preventing autoimmune diseases, their functional characteristics appear either beneficial or detrimental to infectious outcomes, depending on the specific microbial pathogen and the impact of their regulatory properties on inflammatory and memory responses.

Helper T-Follicular Cells

Helper T-follicular (Tfh) cells represent a distinct CD4 T-cell population that regulates antibody responses to T cell–dependent antigens in germinal centers, a specialized B-cell zone in secondary lymphoid organs.[79,80] Tfh cells produce IL-21 and promote immunoglobulin class switching and affinity maturation. Tfh cells are characterized by high expression of programmed cell death protein 1 (PD-1; CD279), inducible costimulator (ICOS), and the chemokine receptor CXCR5 for targeting to splenic B-cell zones. ICOS-dependent signals regulate the induction of a transcriptional repressor and Tfh master regulator, B-cell lymphoma 6 (BCL6).[80,81] Although not exclusive to Tfh cells, genetic defects in ICOS-1 lead to a marked reduction in circulating and germinal center Tfh cells and represent a rare monogenetic cause of common variable immunodeficiency.[82]

Plasticity of Helper T-Cell Subsets

The paradigm of distinct helper T-cell subsets has been critical in understanding CD4 T-cell–driven immunity to a wide range of microbial pathogens. However, the expression of master regulator genes and cytokine expression patterns is not as exclusive or enduring as initially proposed.[83] The process of switching CD4 helper T-cell effector functions is referred to as *trans*-differentiation. For example, IL-4–producing Th2 cells specific for murine lymphocytic choriomeningitis virus (LCMV) switch to IFN-γ production upon adoptive transfer and ensuing infection in mice.[84] During helminth infection, Th1 cells can convert into Th2 cells.[85] Th17 cells can be reprogrammed to secrete Th1 cytokines through IL-12 exposure.[86] In the resolution phase of inflammation, Th17 cells can convert into Tregs via TGF-β and aryl hydrocarbon receptor signaling.[87] These data are consistent with the notion that effector T cells remain responsive to external cues during the progression of microbial infections to optimize microbial eradication and to limit immunopathology.

CD8 T Cells

In contrast to CD4 T cells, which can differentiate into different subsets, naïve CD8 T cells typically differentiate into cytotoxic T lymphocytes (CTLs).[7] The principal effector function of these short-lived effector CD8 T cells is lysis of pathogen-infected cells, a process triggered by MHC class I–dependent presentation of microbial antigens. This function is particularly effective in defense against viral infections because lysis of infected host cells prevents further viral replication.[2] The most rapid cytolytic mechanism involves release of perforin granules by antigen-activated CD8 T cells. Perforin lyses the target cell membrane and enables granzymes, which are also released by cytolytic T cells, to enter the target cell and initiate the apoptotic pathway. In addition to perforin/granzyme–mediated lysis, CD8 T cells can mediate target cell death by expressing Fas ligand, which engages Fas on the target cell surface, resulting in Fas-mediated death. CD8 T-cell–mediated cytolysis generally targets the host cell and not the microbe. Human T cells produce a cytolytic protein called *granulysin* that can kill bacteria directly, including *M. tuberculosis* and *M. leprae*.[88,89] In most infections, pathogen-specific CD8 T cells secrete IFN-γ and TNF, which enhances macrophage- and neutrophil-mediated microbial killing. CD8 T cells also produce chemokines (e.g., CCL3-5) that contribute to the recruitment of inflammatory cells to sites of infection.

Studies in animal models have provided the clearest picture of pathogen-specific T cell responses. CD8 T cell responses are induced rapidly after viral (e.g., influenza, LCMV) and bacterial infections (e.g., *Listeria monocytogenes*). Pathogen-specific T cells are detectable 5 days after infection and expand rapidly to peak frequencies approximately 8 days after infection. Studies using adoptively transferred T cells of defined specificity have shown that antigen-specific T cells undergo rapid proliferation during this phase of the immune response, dividing at a rate of once every 6 hours.[90,91] In a matter of a few days, the frequency of antigen-specific T cells can increase from 1 in 100,000 CD8 T cells to 1 in 2 CD8 T cells in some viral infections.

During the early immune response, a rapid *expansion phase* of activated, antigen-specific CD8 T cells occurs (Fig. 6.3). The differentiation of quiescent naïve CD8 T cells to short-lived effector cells involves metabolic remodeling to a program of anabolic cell growth that depends on aerobic glycolysis, a process in which glucose is converted to lactate, to meet the demand for nucleic acid, protein, and lipid synthesis and to maintain cellular redox balance.[92] In addition, glycolytic intermediates activate the pentose phosphate pathway and serine, glycine one-carbon metabolism that in turn fuel nucleotide biosynthesis required for clonal expansion.[92,93] In contrast, quiescent naïve T cells primarily rely on pyruvate as metabolic fuel, generating energy via mitochondrial oxidative phosphorylation[92]; this process is also increased during their conversion to activated effector T cells.

During clonal expansion, cytokine receptors (e.g., the IL-7 receptor) that transmit homeostatic signals to naïve T cells are downregulated.

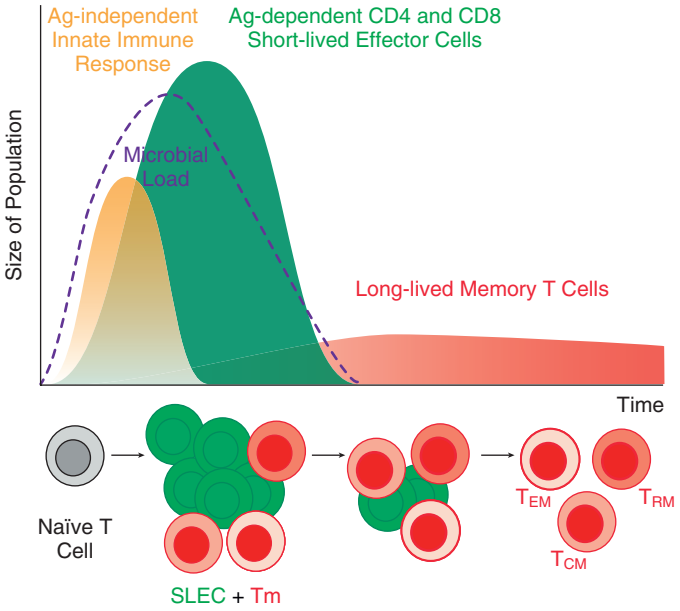

FIG. 6.3 Generation of T-cell memory. During a primary response to a microbe, the innate immune response provides rapid antigen-independent defense against microbial tissue invasion and growth. Antigen-dependent formation of CD4 T cell and CD8 T cell effectors occurs after several days and generally peaks within 1 to 2 weeks. Only 5% to 10% of short-lived effector cells (*SLEC*s) develop into long-lived memory T cell *(Tm)* populations that include central memory *(T_{CM}),* effector memory *(T_{EM}),* and resident memory *(T_{RM})* subsets. In the event of a secondary infection with the same microbe, memory T-cell populations give rise to short-lived effector cells more rapidly and in a higher magnitude than in the primary response. *Ag,* Antigen.

In contrast, the high-affinity IL-2 receptor is upregulated,[94] in part because IL-2, together with growth factors, promotes the metabolic switch to glycolysis by inducing the expression of nutrient transporters and activation of the mammalian target of rapamycin (mTOR) signaling complexes.[95] Upon initial stimulation, responding T cells are programmed and undergo proliferation and differentiation independent of further encounters with antigens or with the inflammation associated with infection.[96,97] During CD8 T cell expansion, antiapoptotic signaling pathways dominate, exemplified by glucocorticoid-induced TNF receptor–related protein (GITR) activity during acute influenza.[98]

As CD8 T cells complete the expansion phase, a small percentage (i.e., 5%–10%) survive and ultimately give rise to memory T-cell populations (see Fig. 6.3). Similar to the expansion phase, the *contraction phase* of CD8 T-cell responses is antigen and inflammation independent. During the contraction phase, effector CD8 T cells are prone to apoptosis because the proapoptotic receptor Fas is upregulated on the cell surface and the balance of the proapoptotic protein BCL-2–like protein 11 (BIM) and the antiapoptotic protein B-cell lymphoma 2 (BCL2) is shifted in favor of BIM-dependent contraction.[99]

NKT Cells and T Cells With a Restricted αβ TCR Repertoire

As discussed earlier, T-lymphocyte populations bear diverse TCRs that determine antigen binding specificity. A small subset of T lymphocytes, generally termed *NKT cells,* are heterogeneous and express a limited repertoire of TCRs that includes a single TCR α-chain (Vα24-Jα18 in humans) and a limited number of Vβ-chains. In addition to the TCR, these cells express natural killer (NK) cell markers and are either $CD4^+$ or double negative ($CD4^-/CD8^-$). NKT cells are of particular interest because they respond to bacterial, mycobacterial, and fungal lipid and glycolipid antigens presented on CD1 molecules, rather than MHC class I or II molecules.[100,101] Alternatively, NKT cell activation can occur in a cytokine-driven fashion, as has been observed in the context of viral infections,[102] or by sensing endogenous host lipids. While NKT cells primarily reside in peripheral tissues, they can also circulate in the blood. NKT cells may play a role in immune defense against *Borrelia burgdorferi, Streptococcus pneumoniae, Chlamydia* spp., tuberculosis, and viral diseases that include hepatitis B, HIV, influenza, and dengue.[103] NKT cells primarily secrete copious amounts of cytokines (e.g., IFN-γ, IL-4, granulocyte-macrophage colony-stimulating factor) and chemokines that activate or recruit, or both, NK cells, DCs, and macrophages to portals of infection.[104]

Additional populations of human T lymphocytes with a restricted TCR α-chain repertoire include MAIT cells that predominately reside in the intestine, express an invariant TCR α-chain (Vα7.2-Jα33 in humans) coupled to a limited TCR β-chain repertoire, and are activated by microbial riboflavin metabolites presented on MR1, an MHC class I–related molecule.[105,106] Circulating $CD4^+$ and $CD8^+$ MAIT cells are activated in humans following exposure to tuberculosis and postulated to play a role in clearance.[107] Human CD4 germline-encoded, mycolyl lipid–reactive (GEM) T cells express a limited TCR α-chain diversity and respond to *M. tuberculosis*–derived mycolic acids restricted by CD1b (see "CD1 and Antigen Presentation" later).[108]

TCR α-restricted lymphocytes, γδ T cells (see next section), and ILCs, including NK cells and lymphoid tissue inducer cells,[109] straddle the traditional boundaries between innate and adaptive immune cells. In general terms, all of these innate-like T-lymphocyte and innate lymphoid cell subsets produce cytokines that amplify and orchestrate antimicrobial defenses, primarily at barrier surfaces, and engage in tissue repair functions.

γδ T Cells

γδ T cells are predominately tissue-resident lymphocytes that are enriched in the intestinal mucosa and in epidermal layers of the skin. γδ T cells constitute a minor fraction (1%–5%) of T cells that circulate in the blood or are stationed in secondary lymphoid organs. γδ T cells encode a TCR that consists of a γ-chain and a δ-chain heterodimer; both chains undergo somatic V(D)J gene segment recombination. In specific subsets of γδ T cells, the γδ TCRs are invariant, or nearly invariant, though other γδ TCRs are quite diverse initially and later are selected by microbial infections.

Unlike αβ T cells that recognize discrete pathogen-specific epitopes (e.g., peptides presented in the context of the MHC), γδ T cells recognize peptide and nonpeptide ligands that vary in size, chemical composition, and structure.[5] Furthermore, the MHC-like Ib proteins (T10 and T22) can bind to the γδ TCRs absent of peptide antigens and activate a small subset (0.1%–1%) of murine γδ T cells. γδ T-cell antigens include surface and soluble proteins that are not obligately presented by the MHC or MHC complex-like molecules.[110] For example, *Clostridium tetani* tetanus toxoid, staphylococcal enterotoxin, and *L. monocytogenes* listeriolysin O all elicit γδ T-cell responses. γδ T-cell activation can also occur via lipid-derived mycobacterial antigens restricted by CD1 family members. Additional γδ T-cell ligands include phosphoantigens such as hydroxymethyl-but-2-enyl-pyrophosphate (HMBPP), an intermediate in bacterial and protozoan isoprenoid biosynthetic pathway, and isopentenyl pyrophosphate, an intermediate in the conserved mevalonate metabolic pathway found in bacteria and vertebrates and that is commonly activated by virus-infected cells. Heteromeric pairing interactions between butyrophilin family members, members of the B7 superfamily, are thought to facilitate phosphoantigen presentation to γδ T cells. In humans, butyrophilin family members are critical for the activation of circulating $Vγ9^+Vδ2^+$ cells.[111] Thus γδ T cells have the capacity to sense metabolic products that are associated with microbial infection.

During microbial infections, γδ T cells appear poised to amplify inflammatory responses and to exert cytolytic activity at portals of microbial invasion.[112] Following *Staphylococcus aureus* infection, murine epidermal $Vγ5^+$ γδ T cells produce IL-17 and facilitate neutrophil recruitment to resolve cutaneous bacterial lesions.[113] During tuberculosis, γδ T cells produce IL-17, TNF, and IFN-γ and contribute to disease containment. Beyond the release of proinflammatory cytokines, γδ T cells can produce bacteriostatic and lytic molecules such as granulysin and defensins (i.e., regenerating islet-derived protein 3α [REG3α] in humans) that contribute to their host defense functions.[5]

T-CELL MEMORY

Antigen-specific memory is a hallmark of the adaptive T-lymphocyte response.[114] T cells with specificity for a particular pathogen can persist in the host for many years after the pathogen has been eliminated, in the absence of continuous antigen exposure.

A single naïve CD8 T cell can give rise to both terminal effector and memory cells in a broad range of infections.[115,116] Thus memory fate decisions and formation occur independent of the priming antigen, the responding APC populations, and time of CD8 T-cell priming. Transcriptional responses that govern CD8 T-cell memory formation appear conserved in the context of LCMV and *Listeria monocytogenes* infections, despite differences in inflammatory responses elicited by viral and bacterial pathogens.[117] In the case of CD8 T cells, the size of the memory compartment appears to expand with immunologic experience.[118]

While naïve CD8 T cells depend on MHC- and IL-7–dependent stimuli for long-term survival, memory CD8 T cells survive and undergo homeostatic proliferation in an antigen-independent but cytokine-dependent manner, largely in response to IL-7 and IL-15.[119,120] Memory CD4 T-cell populations show a similar dependency on IL-7 and IL-15 signals,[121] indicating that in vivo maintenance of naïve and memory T cells is fundamentally different. Despite similarities in cytokine requirements for the maintenance of CD4 and CD8 memory T-cell populations, antiviral CD8 memory T-cell populations typically persist at relatively constant levels over time, while virus-specific CD4 memory T cells decrease in frequency.[122] Similarly, antigen persistence is required for long-term maintenance of *L. major*–specific CD4 memory T cells.[67] The concept of T-cell memory extends to the Treg CD4 T-cell subset, with the identification of long-term persistence of antigen-specific Treg cells with potent immunosuppressive properties.[123]

Several models of CD8 memory T-cell generation have been proposed.[124,125] First, researchers have proposed a concept of separate precursors for different memory cell populations based on signals that occur during thymic development. Most experimental evidence does not support this model because elegant adoptive transfer experiments of a single or of bar-coded naïve CD8 T cells yield heterogeneous effector and memory T-cell descendents.[115,116] Second, researchers have advanced the concept that signals (i.e., antigen, costimulatory molecules, and cytokines) encountered during the primary immune response stimulate the differentiation of naïve T cells first into effector T cells and then into memory T-cell populations.[126] In support of this model, loss of cytokine responsiveness via IL-21, IL-10, and STAT3 signaling disrupts the formation of virus-specific CD8 memory T cells, but not the formation of CD8 terminal effector differentiation.[127] Comparison of the TCR repertoire of effector and memory T cells suggests that these two populations share a common ancestry.[128] Memory T cells appear to have expressed genes encoding effector proteins, consistent with the model that memory T cells derive from effector T cells, though not all effector T cells have the equal potential to form memory T cells.[124]

Studies in mice have advanced several ideas to explain this form of T-cell diversification during primary immune responses. These include the formation of heterogeneous effector cell populations based on (1) the overall strength of three signals triggered by antigen, costimulation, and cytokines (signal-strength model), (2) the repetitive stimulation of naïve T cells by these three signals (decreasing potential model), and (3) asymmetrical cell division in which a single precursor T cell can divide into functionally distinct daughter cells with greater terminal effector or greater memory potential based on proximity to or distance from the APC.[124,125,129] This asymmetrical cell fate model predicts that daughter cells inherit immunologic synapse components in a variable manner and differ functionally in sensing the three signals. Asymmetrical cell division is relevant for the induction of Th1-like effector features versus CD4 T cell memory-like features during acute influenza.[130] Transcriptional studies have identified gene clusters whose expression correlates with the memory potential of responding CD4 and CD8 T cells.[117,131]

Detailed characterization of memory T lymphocytes reveals that they can differ with respect to expression of activation markers, homing properties, and effector capacities.[114,132,133] This discovery has led to the division of memory T cells into three major populations: central memory (T_{CM}), effector memory (T_{EM}), and resident memory (T_{RM}) T cells (see Fig. 6.3).

Human T_{CM} cells express high levels of CD45RO, CD62L, and CCR7 (i.e., a lymph node–homing chemokine receptor) and intermediate levels of CD44, do not express effector functions, and traffic preferentially to secondary lymphoid tissues (i.e., lymph nodes, splenic white pulp).[134] T_{CM} cells have a high proliferative capacity in recall responses, produce copious amounts of IL-2 upon activation, and provide superior protection in mice challenged with bacterial and viral pathogens compared to other memory CD8 T-cell populations.[135] Upon antigen encounter, T_{CM} cells give rise to heterogeneous progeny that are multipotent, capable of both terminal differentiation and self-renewal across serial adoptive transfers in mice,[136] and migrate to peripheral tissues to eliminate pathogens in concert with other memory T-cell subsets.[137]

Human T_{EM} cells express high levels of CD45RO and CD44 and low levels of CD62L and CCR7; they actively exert effector functions (e.g., cytokine synthesis and cytolytic activity), and traffic between peripheral tissues, the lymphatic system, secondary lymphoid tissues, and the circulation. The discovery that a subset of memory T cells can remain constrained in peripheral tissues following infection led to the characterization of human and murine T_{RM} cells. T_{RM} cells reside within the gut, skin, lung, genital tissues, and other mucosal tissues with minimal turnover[138–140] and are readily identified in the context of respiratory virus, herpes simplex virus (HSV), and parasitic infections.[141–143] T_{RM} cells respond to secondary pathogen encounters by releasing cytokines (e.g., TNF, IFN-γ, and lymphotoxin) that activate macrophages and NK cells and by exerting direct cytolytic activity. The distinct anatomic distribution of CD4 and CD8 memory T-cell subsets is maintained during the human life span.[144]

The generation and maintenance of T-cell memory is influenced by the chronicity of a microbial infection. Persistent infections result in CD8 T-cell populations that lose effector functions in a process referred to as *exhaustion,* a process that may be driven by sustained antigen presentation.[145] Exhausted CD8 T cells express PD-1 and cytotoxic T lymphocyte antigen-4 (CTLA-4; CD152), surface proteins that transmit an inhibitory signal to proliferating CD8 T cells.[146–148] Blocking PD-1 and CTLA-4 signaling (i.e., immune checkpoint blockade) with specific antibodies rescues exhausted T cells, enabling them to reexpress effector functions.[149] Immune checkpoint blockade therapy is a highly promising therapy for a number of human cancers (e.g., melanoma) in which regulatory mechanisms promote an exhaustion phenotype in tumor-infiltrating T lymphocytes. Monoclonal antibodies that target PD-1 and CTLA-4 do not appear to be associated with a significant increase in infectious complications in melanoma patients.[150] Early studies of patients with persistent HIV or hepatitis C virus infection demonstrate PD-1 expression on exhausted CD8 T cells, which is associated with disease progression.[151–153] In a mouse model of cryptococcosis, anti–PD-1 antibody therapy enhanced clearance of persistent lung infection.[154] The extent to which immune checkpoint blockade therapies may ameliorate chronic infections in humans remains to be explored.

T-CELL DEVELOPMENT AND LYMPHOID TISSUE ORGANIZATION

Thymic Selection of CD4 and CD8 T Cells

Mature CD4 and CD8 T cells are generated in the thymus,[155] though T-cell maturation can occur in children who underwent partial or complete surgical thymectomy in infancy, albeit at diminished levels.[156] In mice, extrathymic T-cell maturation can occur in the liver and in the intestine, though in the absence of thymopoiesis, T cells show a bias toward the γδ T-cell lineage and a relative paucity of αβ T cells.[157] Thymic selection has evolved to ensure that peripheral T cells are able to interact with self-MHC molecules, through the process of *positive selection,* but not to respond to self-peptides, through the process of *negative selection.* T-lymphocyte progenitors originate in the bone marrow and travel to the thymus, where they enter the cortex at the *double-negative* stage, defined by the absence of CD4 and CD8 coreceptors on the cell surface. In the thymic cortex, the TCR β-chain gene undergoes VDJ recombination, which, if successful, gives rise to the TCR β-chain protein. This gene recombination event is catalyzed by the *RAG1* and *RAG2* genes.[158] The TCR β-chain first complexes with the invariant

pre-TCR α-chain and travels to the cell surface.[159,160] If this process is successful, the endogenous α-chain locus undergoes VJ recombination and replaces the pre-TCR α-chain in subsequent TCR complexes. At this stage, the cell upregulates the expression of CD4 and CD8 on its cell surface and is referred to as a *double-positive thymocyte.* Positive selection occurs first through a process in which the double-positive thymocyte interacts with a cortical thymic epithelial cell (cTEC). The consensus model is that low-avidity interactions between the TCR on the double-positive thymocyte and MHC–self-peptide complexes on the cTEC drive cell proliferation and survival, whereas high-affinity interactions or no interaction result in programmed cell death, termed *death by neglect* if the double-positive thymocyte does not interact with the uniquely large repertoire of peptides presented by cTECs.[161] On completion of positive selection, the double-positive thymocyte down-regulates either CD4 or CD8, depending on whether positive selection occurred on MHC class II or MHC class I.

At this stage, thymocytes enter the thymic medulla, where they come into contact with medullary thymic epithelial cells, medullary DCs, and a small number of B cells that express a range of self-antigens. High-affinity interaction with self-antigens results in deletion of the single positive thymocyte, providing a second opportunity to eliminate potentially autoreactive T lymphocytes. Collectively, these interactions expose thymocytes to a complete representation of the proteins encoded in the genome.[162] Studies have identified a transcriptional regulator, the autoimmune regulator (AIRE), that promotes the ectopic expression of certain peripheral antigens in the thymus.[163,164] Individuals lacking AIRE develop severe multiorgan autoimmune disease (see Table 6.1).[165]

Upon completion of positive and negative selection, thymocytes exit the thymus in a process that is regulated by sphingosine-1-phosphate.[166,167] Thymic emigrants undergo postthymic maturation and become part of the peripheral T-cell compartment.[168] Naïve T cells generally express high levels of CD62L (also called L-selectin) and the chemokine receptor CCR7,[169] a combination of adhesion molecule and chemokine receptor that targets cells for the T-cell zone of lymph nodes and spleen. The diversity of T cells in the peripheral compartment is enormous. One estimate assumes that naïve human T cells express approximately 25 million distinct TCRs (i.e., clonotypes),[170] although technical and methodologic limitations impart a significant degree of uncertainty with regard to actual clonotype diversity.[171]

Thymic selection is crucial for the development of peripheral T lymphocytes. In patients with DiGeorge syndrome, caused by a deletion of a small region of chromosome 22, there is a congenital absence of thymic tissue and a corresponding absence of peripheral T lymphocytes.[172] Transplantation of thymic tissue into these patients corrects this problem.[173,174]

In humans, thymic function diminishes with aging. Young children have active thymic function and produce large numbers of naïve T lymphocytes, whereas older adults have a markedly smaller thymus that produces few new T lymphocytes.[175] However, adults in their 50s continue to have active thymic tissue and recent thymic emigrants in the bloodstream, suggesting the thymus continues to function despite its relatively small size.[176] Many endogenous and exogenous factors (e.g., glucocorticoids, chemotherapy) can injure thymic function. This issue is of particular importance in allogeneic hematopoietic stem cell transplantation, wherein long-term survival depends on reconstitution of the peripheral T-cell compartment. Studies that measure TCR excision circles, an indication of recent thymic emigration, and the kinetics of TCR repertoire reconstitution have shown that thymic function returns and TCR repertoire diversity increases in the posttransplantation setting.[177,178] Exogenous administration of IL-22 can enhance thymopoiesis by promoting the proliferation of thymic epithelial function and may restore thymic function following radiation- or chemotherapy-induced injury.[179]

Lymphoid Anatomy

Studies in humans and mice have provided a detailed picture of cell-mediated immunity and the cooperation between T-cell subsets that reside primarily in lymphoid tissues with those in peripheral and nonlymphoid sites.[180] In the classical view, T cell–mediated responses are predominately initiated in secondary lymphoid tissues (i.e., in lymph nodes and in the spleen). While lymph nodes survey an area of peripheral tissue defined by afferent lymphatic drainage, the spleen surveys the bloodstream for evidence of systemic infection. Following activation at these sites, activated lymphocytes traffic to nonlymphoid sites of infection. Because lymphoid tissues play this vital role in adaptive immunity, understanding lymph node and splenic anatomy is essential to understanding the comingling of antigen with its cognate immune cell.

Lymph Nodes

Lymph nodes serve as a nexus for lymphocyte interaction with APCs, overcoming the problem of bringing together rare, antigen-specific lymphocytes with small quantities of antigen.[181] Located at the interface between the blood and lymphatic systems, lymph nodes receive afferent lymphatic drainage from peripheral tissues as well as blood flow through arteries that enter the hilum of the node. The flow of cells, proteins, inflammatory mediators, and in some instances microbial pathogens to the lymph nodes (Fig. 6.4) from distant sites of infection via afferent lymphatic channels facilitates the activation of cell-mediated immunity.[181,182]

In the periphery, microbial antigens are taken up by migratory DCs that, after receiving signals through innate immune receptors, mature and travel in the afferent lymph fluid to a draining lymph node.[183] Inflammatory cytokines produced at the site of infection travel in lymph fluid to the draining lymph node concurrently.[184] Upon reaching the subcapsular space of the draining lymph node, the cellular and molecular immigrants follow different paths.

Afferent lymph is filtered slowly through the capsular sinus and the cortical sinus, and converges on the medullary sinus in the hilum before exiting via an efferent lymph vessel.[185] Lymph-borne DCs enter the subcapsular sinus, actively traverse an endothelial and fibroblastic layer of cells that forms the floor of the subcapsular sinus, and reach the lymph node cortex to access the collection of lymphocytes in the paracortical cords. Small protein molecules, such as cytokines and chemokines, and small microbe-derived molecules, do not traverse this barrier and fail to enter lymph node parenchyma.[186] Instead, these molecules are channeled into size-selective conduits that are both produced and enclosed by a tight network of fibroblastic reticular cells and contain an internal matrix consisting of collagen bundles. The collagen fibers provide structural support to the lymph node and define its architecture. Despite the enclosed nature of these conduits, leukocytes within the lymph node are able to sample soluble material from the lymphatic content. Lymphatic conduits associated with these collagen fibers provide connections between the subcapsular and cortical sinuses and the high endothelial venules (HEVs), enabling fluid flow and substrate transport from peripheral tissues via the subcapsular sinuses to the HEV, the site of cellular entry into the lymph node from the bloodstream.[181,185]

Endothelial cells in the HEVs can produce chemokines on their luminal surface and, since most fibroblastic, reticular cell–bounded conduits end in the HEV, intravascular T cells can respond to lymph-borne chemokines as well. Both processes trigger lymphocyte extravasation into the lymph node parenchyma.[186] To enter lymph nodes, lymphocytes undergo a four-stage process that involves rolling, activation, adhesion, and transmigration through the endothelium. On traversing the HEV, lymphocytes enter the perivenular channel, a narrow space bounded by fibroblastic reticular cells, that provides entry into the labyrinthine corridors of the paracortical cord. As lymphocytes move through this labyrinth side-by-side, they have ample opportunities to contact antigen-bearing DCs that have entered the cord from the subcapsular sinus.

Advances in live-imaging microscopy have enabled investigators to visualize interactions between T cells and DCs in lymph nodes over several hours and to monitor their trafficking patterns under homeostatic and inflammatory conditions.[181,187] T-cell movement within the paracortical cords occurs in a meandering fashion at a speed of approximately 10 to 12 μm/min. *Chemokinesis* is thought to be the underlying mechanism; the term refers to the ability of chemokines, in many instances bound on sessile DCs and stromal elements, to increase the general mobility of lymphocytes, analogous to a random walk.[181,188] T-cell chemokinesis enables sessile DCs to interact with an estimated 5000 T cells per hour.[189,190] This high frequency of DC–T cell interactions