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9 Complement and Deficiencies

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SHORT VIEW SUMMARY

- Complement comprises several soluble plasma proteins that are activated through three pathways, namely the classical, lectin, and alternative pathways. The system also consists of membrane-associated proteins that serve as receptors for activation products of complement or as inhibitors of complement.
- Complement plays a central role in innate immune defenses against invading pathogens. Activation of complement on microbes "marks" them with C3 fragments and generates anaphylatoxins such as C5a that facilitate opsonophagocytosis. Gram-negative bacteria can be killed after insertion of the membrane attack complex (C5b-9) pore.
- In addition to its function in combating infections, complement plays critical roles in several physiologic processes, including roles as a bridge between innate and adaptive immunity, in tissue regeneration and organogenesis, in disposal of immune complexes and apoptotic cells, and in metabolism.
- A fine balance exists between complement activation and its inhibition under physiologic conditions. Gain-of-function mutations of complement activators or loss-of-function mutations of complement inhibitors lead to excessive complement activation and damage of host tissues observed in conditions such as atypical hemolytic-uremic syndrome, C3 glomerulopathy, and age-related macular degeneration. Loss of glycophosphatidylinositol-anchored membrane proteins, including the complement inhibitors CD55 and CD59, results in paroxysmal nocturnal hemoglobinuria.
- Congenital deficiencies of complement protein are rare. Complete deficiencies of classical-pathway components predispose to autoimmune disorders and infections with *Haemophilus influenzae* and *Streptococcus pneumoniae*. Deficiencies of alternative or terminal complement components are associated with a several thousand–fold increase in the incidence of invasive meningococcal infections.
- Conditions associated with acquired deficiencies of complement—defective synthesis (e.g., severe liver disease), increased consumption (e.g., immune complex disease, vasculitis, C3 and C4 nephritic factors), or increased catabolism (nephrotic syndrome, protein-losing enteropathies)—are also associated with an increased risk of infection.
- Meningococcal infections in individuals with terminal complement pathway (C5 through C9) deficiencies often have a milder clinical course than in individuals with intact complement systems, which may be attributable to lower circulating endotoxin levels in the absence of membrane attack complex.
- Pharmacologic blockade of C5 (e.g., with eculizumab) is also associated with an approximately 2000-fold increase in the incidence of invasive meningococcal infection, often caused by nongroupable (unencapsulated) isolates against which capsular polysaccharide–based meningococcal vaccines are ineffective. Antibiotic prophylaxis should be considered in all persons on C5 inhibitor therapy. Clinicians should have a very low threshold for suspecting and treating meningococcal disease in this population.

Functional activity attributable to the complement system was first described in the late 1800s with the demonstration that fresh serum contained a heat-labile bactericidal factor called *alexin*.[1] Subsequently, it was shown that a heat-stable factor present in convalescent serum also contributed to bactericidal activity. At the turn of the century, Paul Ehrlich used the term *complement* to describe the heat-labile factor and *amboceptor* (antibody) to describe the heat-stable factor. With the 20th century came the recognition that complement was composed of more than one component, but it was not until 1941 that Louis Pillemer was able to separate functionally distinct components of the classical pathway from various serum fractions. In the early 1950s, Pillemer also described an antibody-independent mechanism for complement activation that he referred to as the *properdin pathway*.[1–3] However, the protein purification techniques of the time were unable to provide complement components of sufficient purity to convince others of the existence of this pathway.

With the 1960s and 1970s came the development of a mathematical model capable of describing the sequential activation of complement, and new techniques for the purification of individual complement components. The latter development led to the rediscovery of Pillemer's work, characterization of these proteins, and delineation of mechanisms that control their activity. The 1980s brought the recognition that the complement system also consists of membrane proteins—both receptors and inhibitors—that respectively mediate the cellular consequences of complement action and protect host cells from the detrimental effects of complement activation. With this advance, it was appreciated that complement functions optimally at the interface between the fluid phase and the cell surface.

Toward the end of the 20th century, an explosion in molecular biology research led to (1) cloning and structural characterization of all the complement proteins and an understanding of the molecular basis for their deficiency states, (2) characterization of a third pathway of complement activation, the lectin pathway, and (3) the use of genetically engineered mice to dissect the molecular details of complement function. Through this process, the complement system has grown to include more than 30 proteins (Table 9.1), and the diverse roles and implications of complement—as a bridge between innate and acquired immune systems, in the disposal of immune complexes and apoptotic cells, in metabolism, and in tissue regeneration and organogenesis—have been more clearly delineated.[4,5] Consequently, the view of the complement as merely augmenting host defense has been replaced by a more global one in which complement serves as one of the earliest pattern recognition systems differentiating self from nonself and provides a link between the innate and adaptive immune systems by virtue of its inflammatory and antiinflammatory actions.[4–6] The past decade has witnessed elucidation of solution and crystal structures of several complement proteins, either alone or in complex with their natural ligands, which has provided considerable insights into structure-function relationships.[7,8,9]

COMPLEMENT SYNTHESIS, CATABOLISM, AND DISTRIBUTION

Studies of cultured hepatocytes coupled with an examination of complement component polymorphisms in patients before and after orthotopic liver transplantation have established the liver as the major site of synthesis for most complement components.[10,11] The fractional catabolic

TABLE 9.1 Complement Plasma Proteins

COMPONENT	APPROXIMATE SERUM CONCENTRATION (μg/mL)	MOLECULAR WEIGHT	CHAIN STRUCTURE[a]	NO. OF GENETIC LOCI	CHROMOSOMAL ASSIGNMENT[b]
Classical Pathway					
C1q	70	410,000	(A, B, C) × 6	3 *(A, B, C)*	1p
C1r	34	170,000	Dimer of two identical chains	1	12p
C1s	31	85,000	Dimer of two identical chains	1	12p
C4	600	206,000	β-α-γ	2 *(C4A, C4B)*	6p
C2	25	117,000	One chain	1	6p
Mannose-Binding Lectin Pathway					
MBL	1–2	40,000	Homo-oligomers	1	10q
Ficolin-1 (M-ficolin)	0.04–0.1			1	9q
Ficolin-2 (L-ficolin)	3–4	34,000	Homo-oligomers	1	9q
Ficolin-3 (H-ficolin; Hakata antigen)	20	35,000	Homo-oligomers	1	1p
Collectin-11	Approximately 2			1	2p
MASPs	MASP-1, 4–30 MASP-2, 0.02–0.9 MASP-3, 2–10 MAp44, 1–3 MAp19, ND	74,000–94,000	A-B	1 per MASP	3q
Alternative Pathway					
D (adipsin)	1	24,000	One chain	1	ND
C3	1300	195,000	β-α	1	19q
B	200	95,000	One chain	1	6p
Membrane Attack Complex					
C5	80	180,000	β-α	1	9q
C6	60	128,000	One chain	1	5p
C7	55	97,000	One chain	1	5p
C8	65	150,000	Three nonidentical chains α-γ, β	3 *(A, B, G)*	α, β, 1p γ 9q
C9	60	79,000	One chain	1	5p
Control Proteins ***Positive Regulation***					
Properdin	25	220,000	Cyclic polymers of a single 57-kDa chain	1	Xp
Negative Regulation					
C1-INH	200	105,000	One chain	1	11q
C4BP	250	550,000	Seven identical chains	1	1q
Factor H	500	150,000	One chain	1	1q
Factor I	34	90,000	β-γ	1	4q
Anaphylatoxin inactivator (carboxypeptidase N)	35	310,000	Dimer of two nonidentical chains (H, L) × 2	ND	ND
S protein (vitronectin)	350	80,000	One chain	1	ND
SP-40,40 (clusterin)	50	80,000	α-β	1	8p

[a]For multichain components, parentheses indicate subunit structure; commas indicate noncovalent linkage of chains arising from separate genes; solid lines indicate covalent linkage of chains arising from posttranslational cleavage of a proenzyme molecule, with chains listed in order beginning at the amino terminus of the proenzyme molecule; a dash indicates covalent linkage of chains arising from separate genes.
[b]"p" indicates the short arm and "q" the long arm of the chromosome.
BP, Binding protein; *C1-INH,* C1 inhibitor; *H,* heavy chain; *L,* light chain; *MASP,* mannose-binding lectin–associated serine protease; *MBL,* mannose-binding lectin; *ND,* not determined.

rate for several complement components indicates that they are among the most rapidly metabolized of all plasma proteins.[12]

Fluctuations in the concentration of individual complement components largely reflect the fact that they are acute-phase reactants. Consequently, their synthesis can be modulated twofold to fivefold by a variety of immune mediators, including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF), interferon-γ (IFN-γ), and endotoxin.[13] In most cases, enhanced synthesis is mediated at the transcriptional level.

A variety of other cells also synthesize, store, and secrete a number of complement proteins. Most notable among these are neutrophils,

monocytes, macrophages, and adipocytes, but microglia, astrocytes, fibroblasts, and endothelial cells are also important sites of local complement production.[14] Complement synthesis by monocytes can be modulated by IFN-γ, endotoxin, IL-1, and TNF. Local synthesis is an important aspect of complement-mediated host defense, as evidenced by the observation that monocytes and macrophages can synthesize sufficient amounts of complement to promote opsonization, ingestion, and killing of bacteria.[13]

In healthy people, most complement is found in plasma. Concentrations of complement proteins in normal mucosal secretions are approximately 5% to 10% of serum levels, and in normal spinal fluid they are even lower, perhaps 1% or less. In the presence of local inflammation, complement concentrations in mucosal secretions and in cerebrospinal fluid increase, most likely as a result of altered vascular permeability barriers but also as a consequence of enhanced synthesis and secretion by local mononuclear cells.

Serum complement activity is reduced in preterm infants in proportion to the magnitude of their immaturity. In contrast, complement levels in healthy full-term infants range from 60% to 100% of those in healthy adults. Despite these almost normal levels, defective complement activation in either the classical or the alternative pathway has been noted in as many as 40% of such infants.[15]

COMPLEMENT ACTIVATION

Overview: C3, the Linchpin of the Complement System

The importance of C3 in the complement cascade is evident from its position at the convergence of the classical and alternative pathways, its role in activating and amplifying alternative-pathway activation, the multitude of functional activities associated with its various cleavage products, the fact that it is a major point of regulation of complement activity (Fig. 9.1), and the fact that its concentration in plasma (1.6 mg/mL) exceeds by 2- to 10-fold the concentration of all other complement components (see Table 9.1).[16]

C3 cleavage and its stable, covalent linkage to target surfaces is the critical outcome of complement activation. The crystal structure of C3 reveals that the molecule is organized into 13 domains (Fig. 9.2).[17] The reactive thioester moiety, which is required for covalent attachment to target surfaces, is protected from hydrolysis in native C3. Proteolytic cleavage of the C3a fragment from C3 is accompanied by a remarkable conformational change, where the thioester moves approximately 85 Å from its position in native C3, becomes fully exposed, and can form a covalent bond.[18] The calculated half-life of the reactive thioester after initial cleavage of C3 is less than 100 μsec[19,20]; failure to form a covalent bond within this period results in hydrolysis (reaction with an H_2O molecule), and the C3b remains in solution. The short half-life of the nascent thioester ensures that C3b is deposited on targets proximate to the site of complement activation.

C3 cleavage can occur through three general pathways of complement activation: the classical, lectin-binding, and alternative pathways. The product of each of these pathways is the formation of specific enzyme complexes (C3 convertases: C4b2a and C3bBb) capable of amplifying C3 cleavage and initiating formation of the membrane attack complex (MAC) (see Fig. 9.1).[6]

Pattern recognition molecules with properties ranging from rather broad specificity (e.g., mannose-binding lectin [MBL]) to multispecificity (e.g., properdin, immunoglobulin M [IgM]) to high specificity (IgG) trigger complement activation on binding to their respective targets. A common structural feature of MBL, properdin, IgM, and C1q is the presence of multiple binding pods, some with specificity for different targets. Engagement of these pods promotes stable binding, efficient initiation of the complement activation sequence, and expression of the many complement-mediated pathophysiologic effects early in the course of disease.[21]

Elegant studies using knockout mice established clearly that, in the absence of specific IgG antibody, complement activation via natural IgM and the classical pathway is responsible for initial C3 deposition and marking of most cells in a target population. Subsequent amplification via the alternative pathway is responsible for increasing the amount of C3 on the particle.[22]

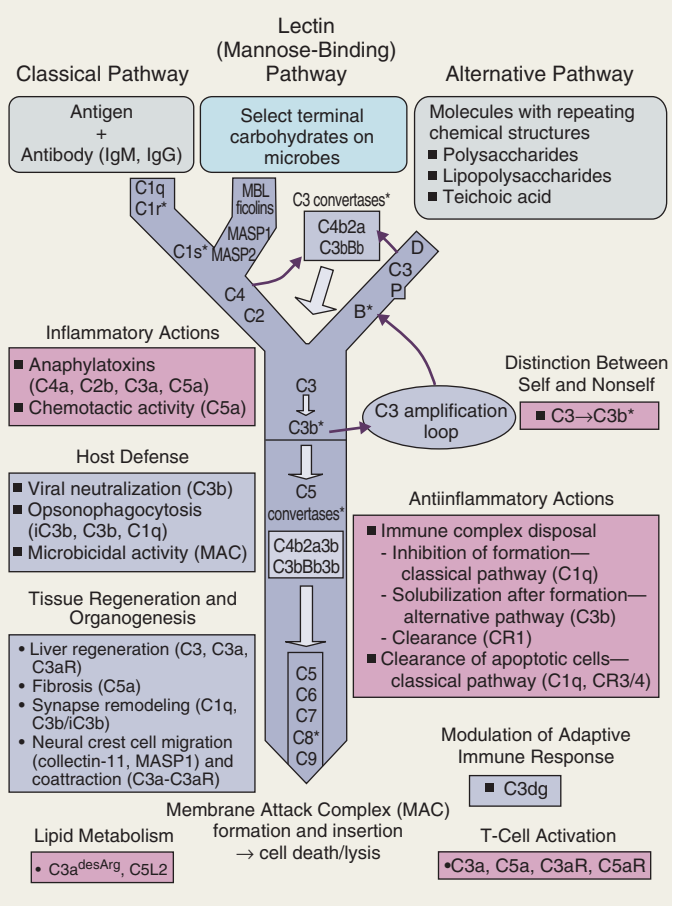

FIG. 9.1 The complement cascade. Within each pathway, the components are arranged in order of their activation and aligned opposite their functional and structural analogues in the other pathways. Rounded boxes relate to activation of the pathways; squared boxes reflect complement functions; asterisks indicate sites of downregulation of complement activity (see Table 9.2). *B,* Factor B; *C,* complement; *CR1,* complement receptor 1; *D,* factor D; *Ig,* immunoglobulin; *MAC,* membrane attack complex; *MASP,* MBL-associated serine protease; *MBL,* mannose-binding lectin; *P,* properdin.

In most instances, the recognition molecule and complement act synergistically. For example, specific antibody or MBL binding leads to more rapid and efficient complement activation and serves to direct complement deposition to nearby sites on the surface of an invading pathogen. Opsonization of infectious agents with both antibody and complement leads to more efficient ingestion and killing of these microbes than opsonization with either substance alone.[23]

Generation of the Classical-Pathway C3 Convertase

The classical pathway can be activated by multiple mechanisms, including specific antibody; MBL; ficolins; collectins; select members of the pentraxin family of proteins, such as C-reactive protein (CRP), serum amyloid P component (SAP), and pentraxin 3 (PTX3); and proteases extrinsic to the complement system per se. In the case of specific antibody, activation occurs through the formation of an immune complex as a consequence of antigen recognition by immunoglobulin, C1 binding, and sequential enzymatic activation of downstream complement components. Amino-acid and glycosylation differences in the C_H2 and C_H3 antibody regions contribute to the different complement-activating potentials among the antibody classes and IgG subclasses (IgG3 > IgG1 > IgG2; IgG4 does not activate complement).[24,25]

C1 is a trimolecular complex containing one molecule of C1q and two molecules each of C1r and C1s. C1q consists of a central core with six radiating, collagen-like fibrillar strands that terminate in globular heads that contain the antibody-binding sites. Although C1q can bind

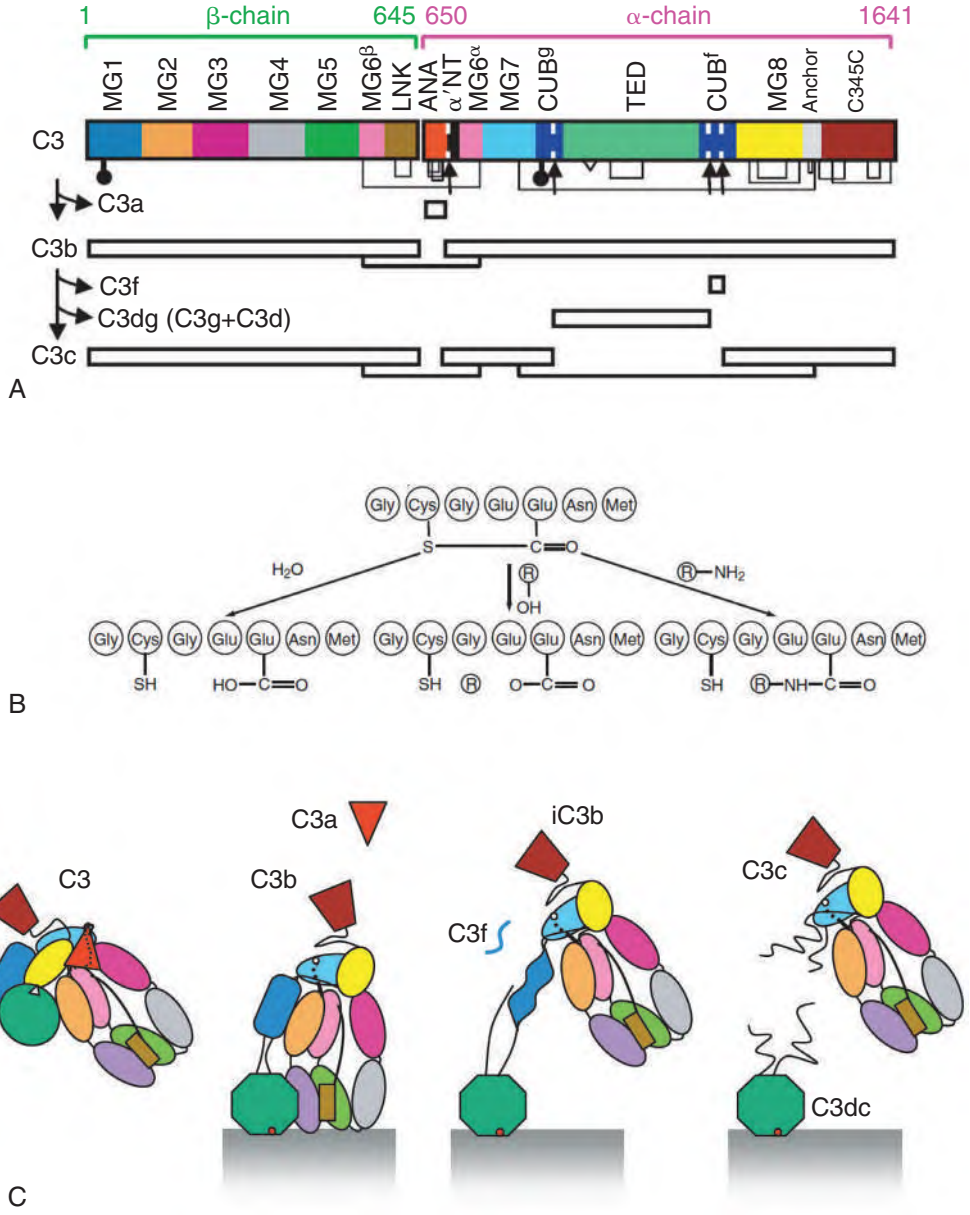

FIG. 9.2 C3 structure, activation, and fate of the internal thioester bond. (A) During activation, C3a is released from the amino terminus of the α-chain of C3. The exposed internal thioester bond becomes accessible to nucleophilic attack and can react with water or available hydroxyl or amine groups on cell surfaces (B). Analogous reactions occur with C4. Together, these reactions involving C3 and C4 are responsible for covalently linking complement deposition to the cell surface. C3 is organized into 13 domains (colors of domains in the lower panel correspond to colors of the amino-acid stretches of the α- and β-chains in A). *Arrows* indicate physiologic cleavage sites. The location of the thioester bond is indicated by the inverted white triangle. Sites of N-linked glycosylation are shown by the "inverted lollipop" symbol. Locations of disulfide bridges are also shown. Activation of C3 is accompanied by an approximately 85-Å displacement of the thioester domain, and the resulting C3b molecule can form covalent bonds with targets (C). Cleavage of C3b to iC3b also results in conformational changes that contribute to ligand specificity. *ANA,* Anaphylatoxin; *Asn,* asparagine; *CUB,* complement C1r/C1s, Uegf, Bmp1; *Cys,* cysteine; *Glu,* glutamic acid; *Gly,* glycine; *LNK,* linker; *Met,* methionine; *MG,* macroglobulin; *TED,* thioester-containing domain. *(From Janssen BJ, Huizinga EG, Raaijmakers HC, et al. Structure of complement component C3 provide insights into the function and evolution of immunity.* Nature. *2005;437:505–511; Janssen BJ, Christodoulidou A, McCarthy A, et al. Structure of C3b reveals conformational changes that underlie complement activity.* Nature. *2006;444:213–216; and Gordon DL, Hostetter MK. Complement and host defense against microorganisms.* Pathology. *1986;18:365–375.* www.tandf.co.uk/journals. *Accessed February 2009.)*

directly to negatively charged surfaces and in doing so exert functionally important effects (e.g., elimination of apoptotic cells),[26] C1q binding is classically initiated as a consequence of the recognition of antigen by antibody. In the case of IgM, functionally important C1q binding occurs after the change in configuration that accompanies binding of a single IgM molecule to multiple sites on the target particle. Elegant cryoelectron tomography studies have revealed that surface-bound antibodies are oriented as hexamers formed through noncovalent Fc:Fc interactions.[27] Fc hexamers permit engagement of each of the six globular heads of C1q in the C1 complex by Fc, which triggers classical-pathway activation. These findings explain why IgG in solution does not activate complement. Because of this topographic stipulation, many IgG molecules must be bound to a target particle to ensure sufficient density for hexamer formation. Functionally, this requirement means that complement activation by IgG is less efficient than that by IgM.[24,28] IgG variants with an enhanced ability to form hexamers could improve the therapeutic activity of antibodies that rely on complement-dependent cytotoxicity for their function.[27,29]

C1 binding by antibody results in a change in the structural configuration of the C1q molecule such that the C1r and C1s tetramer contained within the radiating pods of C1q becomes autocatalytically active. This structural alteration may involve the release of C1 inhibitor (C1-INH), which binds reversibly to proenzyme C1. Expression of enzymatic activity by C1r and C1s represents the initial activation and amplification step in the classical pathway. Many molecules of substrate are cleaved by a given enzyme complex, resulting in the fixation of subsequent complement components in the cascade in close proximity to the antibody-binding site. Therefore, antibody serves not only to activate complement in a kinetically efficient manner but also to deposit it nearby on the target surface, which includes the antibody itself (see later discussion).

Activated C1s cleaves a 9-kDa fragment, C4a, from the amino terminus of the α-chain of C4. This results in exposure of an internal thioester bond that links the sulfhydryl (SH) group of a cysteine residue with the carboxyl-terminal group of glutamic acid. This bond is subject to nucleophilic attack by hydroxyl or amino groups, leading to the formation of covalent ester or amide linkages.[30] Through this reaction, along with the analogous one involving C3 (see Fig. 9.2), the complement system acquires a chemically stable association with target surfaces. Because of gene duplication, two slightly different C4 genes exist—*C4A* and *C4B*. The product of the *C4A* gene preferentially forms amide bonds with target surfaces and is hemolytically less active than the product of the *C4B* gene, which preferentially forms ester bonds.[31] Consequently, C4A binds more effectively to proteins (e.g., antigen-antibody complexes) than does C4B.[31,32] The molecular basis for this difference is associated with an aspartic acid residue in the C4A molecule and a histidine residue in the C4B molecule, both of which influence the susceptibility of the thioester bond to nucleophilic attack by reactive groups on the target surface. This difference may play a role in the clinical presentation of patients with inherited deficiencies of C4A and C4B molecules.[32]

Activated C1s also cleaves C2 to produce a small fragment, C2b, which is released into the environment, and a larger fragment, C2a, which binds to C4b on the surface of the target particle. This complex, *C4b2a*, is the classical-pathway C3 convertase (see Fig. 9.1).

Although recognition of antigen by antibody is the historically preeminent initiator of classical-pathway activation, studies have confirmed a vital clinical role for complement activation via the lectin pathway (see Fig. 9.1). To date, five lectin molecules that can bind to a variety of terminal monosaccharides and initiate complement activation have been described. These include the collectins (collagen-containing C-type [calcium-dependent] lectins) MBL and collectin 11,[33] and ficolin-1, ficolin-2, and ficolin-3 (also called M-, L-, and H-ficolin, respectively).[34] Ficolins contain a fibrinogen-like domain combined with a collagen-like domain and therefore are not classified as collectins.

The recognition molecules of the lectin pathway are trimers that comprise three identical polypeptide subunits, each terminating in a calcium-dependent carbohydrate recognition domain. These trimers are organized into higher-order oligomers that resemble a "bouquet." MBL is structurally and functionally homologous to C1q.[35] Like C1q, it exists in serum as a complex with serine proteases, termed MBL-associated serum proteases, or MASPs. Four such molecules—MASP-1, MASP-2, MASP-3, and MAp19—are the product of two genes arising from a common ancestor shared with C1r and C1s.[36] MASP-2 plays a particular role in cleaving C4 and C2 and generating the classical-pathway C3 convertase, as described earlier.[35,36] An individual with a nonsense mutation in *MASP1* (and therefore lacking both MASP-1 and MASP-3) had a nonfunctional lectin pathway.[37] Reconstitution with MASP-1 resulted in MASP-2 cleavage and full restoration of lectin-pathway activity. MASP-1 and MASP-2 were cocomplexed with MBL, supporting a model in which MASP-1 transactivates MASP-2, analogous to C1r and C1s activation.

The carbohydrate recognition domains on MBL bind to a variety of terminal monosaccharides, including mannose, *N*-acetyl-mannosamine, *N*-acetyl-D-glucosamine, fucose, and glucose. Collectin-11 binds preferentially to L-fucose and D-mannose. The ficolins all appear to bind preferentially to acetylated sugars such as *N*-acetyl-D-glucosamine. In addition, M-ficolin binds to *N*-acetyl-D-galactosamine and select sialoglycans, such as those present in the capsule of *Streptococcus agalactiae.* Ligands reported for L-ficolin include β-(1→3)-D-glucan, *N*-acetylneuraminic acid, lipoteichoic acid, CRP, fibrinogen, DNA, and certain corticosteroids, whereas H-ficolin binds to fucose. These sugars frequently decorate microbial surfaces but rarely appear as the terminal unit on oligosaccharides or glycoconjugates on human cells. This fact has immense implications for host defense because it provides a mechanism for differentiating nonself from self and for rapidly activating the complement cascade. In these respects, the lectins share several critical features with IgM or "natural antibody": Both are polyreactive, bind to surface carbohydrates, require binding of just a single molecule to effect complement activation, and in contrast to IgG, do not require clonal expansion of a specific population of lymphocytes before recognition of antigen.[6,35]

Extrinsic proteases, such as Hageman factor (factor XII in the clotting cascade), can also activate the classical pathway.[21,38] Gain-of-function mutations of factor XII are seen in about one-third of individuals with type 3 hereditary angioedema.[39] The role of other proteases in activating downstream complement components (e.g., activation of C5 by thrombin) has been reported,[40] and their clinical significance is currently being clarified.

Generation of the Alternative-Pathway C3 Convertase

Just as several new mechanisms for generation of the classical-pathway C3 convertase have come to light, data have pointed to two mechanisms for generation of the alternative-pathway C3 convertase: the standard fluid-phase C3 "tickover" model and the newly described properdin-directed model. Several unique features characterize alternative-pathway complement activation: (1) antibody is not required, although it can facilitate the activation process; (2) activation proceeds both in the fluid phase and on cell surfaces; and (3) a component of the activation process, C3b, is also a product of the reaction, resulting in a positive feedback loop that amplifies the activation process. Consequently, C3b deposition resulting from C3 cleavage by either alternative-pathway or classical-pathway C3 convertase can initiate the alternative-pathway amplification loop (see Fig. 9.1).[30,41]

The "Tickover" Model

C3, the critical reactant of the alternative pathway, contains an internal thioester bond within its α-chain.[42] This internal thioester bond undergoes spontaneous low-rate hydrolysis to form C3(H_2O) (see Fig. 9.2). For a brief moment before its inactivation by the control proteins, C3(H_2O) can form a complex with factor B. A series of subsequent reactions yields a fluid-phase C3 convertase, C3(H_2O)Bb, that can cleave more C3 to generate metastable C3b capable of forming covalent ester or amide linkages with appropriate chemical constituents on the surfaces of nearby cells. C3(H_2O) formed in plasma may constitute an important source of intracellular C3 stores that serves to maintain T-cell homeostasis and other cellular functions,[43,44,45] as discussed later. Surface-bound C3b can bind additional factor B, which in turn can be cleaved by factor D to produce *C3bBb,* the alternative-pathway convertase (see Fig. 9.1). This convertase is inherently labile, with a half-life of approximately 90 seconds. Properdin binding to C3bBb stabilizes the complex and prolongs its half-life 5- to 10-fold,[46] thereby providing reaction conditions sufficient for further C3 cleavage and signaling initiation of the amplification phase of alternative-pathway activation.

Although antibody is not required for activation of the alternative pathway, it acts synergistically with properdin to facilitate the activation process.[47] Facilitation depends on the Fab portion of the antibody molecule rather than the Fc fragment responsible for classical-pathway activation.[47,48] The molecular basis for facilitation is uncertain but may depend on the identity of the carbohydrate moieties present on IgG. The hydroxyl groups in these moieties can serve as sites for ester bond formation with C3.[48] Moreover, the alternative-pathway C3 convertase generated on IgG is relatively resistant to inactivation by regulatory proteins.[48–50]

The Properdin-Directed Model

The properdin-directed model, articulated by Hourcade and coworkers, is exciting for several reasons, not the least of which is that it provides direct support for Pillemer's original postulate for the existence of an

alternative pathway of complement activation, which he called the properdin pathway.[51–53]

Properdin is a positively charged molecule made up of identical subunits, each of which is composed of six globular thrombospondin type 1 repeats that associate to form dimers, trimers, and tetramers. These multimers can bind directly to a variety of cell surface molecules, particularly sulfated glycosaminoglycans (GAGs), such as heparan sulfate proteoglycans and chondroitin sulfate E. Properdin is synthesized by immune cells, especially those of phagocytic origin. Discharge of properdin from these cells increases its local concentration and could focus alternative-pathway activation on specific targets. Properdin bound directly to target cells may serve as a preferential platform for binding of fluid-phase–generated C3b. Subsequent binding of factor B and its cleavage generate the already stabilized alternative-pathway C3 convertase, *C3bBb.*[51,53] Purified properdin has a propensity to aggregate with freeze-thawing or prolonged storage, which may result in artifactual binding to biologic surfaces. Thus, experimental data with unfractionated properdin should be interpreted with caution; at this time, the role of properdin as an initiator of the alternative pathway remains controversial.[54,55]

Assembly of the Membrane Attack Complex

Of the various forms of C3, *only* C3b can perpetuate complement activation. C3b binding to the C3 convertases generates new complexes, the C5 convertases (C4bC2aC3b*n* and C3bBbC3b*n*),[56] which are responsible for cleaving C5 and initiating assembly of the MAC. C5 is the structural homologue of C4 and C3, except that its α-chain does not contain an internal thioester bond. Analogous to C4 and C3, activation of C5 proceeds via cleavage of an 11.2-kDa fragment, C5a, from the amino terminus of its α-chain. The remaining C5b binds noncovalently to the surface of the target particle.[57] The remaining terminal complement components—C6, C7, C8β, C8α-γ, and C9—share a high level of structural organization at both DNA and protein levels.[58,59] Unlike the early components of the classical and alternative pathways, these proteins lack enzymatic activity but as a group are characterized by their amphipathic properties. They circulate in plasma in hydrophilic form, undergoing hydrophobic transformation on binding to the nascent MAC.

Assembly of the MAC begins when binding of C5b to hydrophobic sites on the cell surface exposes binding sites for C6 and C7, leading to the formation of a stable trimolecular complex, C5b-7. Incorporation of C7 results in hydrophilic-to-amphiphilic transitioning of the assembling complex and promotes direct insertion into cell membranes. Subsequently, C8 binds to C5b at a site on its β-chain.[60] In the final step, C8 initiates polymerization of C9 through a binding site on C8α-γ.[61] A current model of this process suggests that the function of C5b-8 is to create a discontinuity in the membrane lipid bilayer, thereby establishing an environment for the stepwise unfolding, insertion, and polymerization of monomeric C9.[62] In its completely assembled state, the MAC consists of a single molecule each of C5b, C6, C7, and C8 and multiple (1–18) molecules of C9, with an internal pore diameter of about 110 Å.[57,63]

Fully inserted and polymerized C9 has a tubular shape and the properties of an integral membrane protein. Previously thought to form a closed ring structure similar to pores formed by perforin and other cholesterol-dependent cytolysins, electron cryomicroscopy studies have shown that the MAC adopts a "split-washer" configuration because C6 and the final C9 molecules do not make extensive contact.[63] The inner aspect of this tubular structure is hydrophilic and allows the passage of water and ions, whereas the outer surface is hydrophobic and causes varying degrees of membrane disorganization during insertion. Both of these effects contribute to the microbicidal and cytolytic properties of the MAC.[57,62]

REGULATION OF COMPLEMENT ACTIVATION

A major feature of the complement cascade is its controlled production of an inflammatory reaction that is sufficient to enhance host defense and the immune response, yet not so potent as to lead to host injury. Upregulation of this process is achieved by the inherent property of enzymes to turn over multiple substrate molecules rapidly and by stabilization of enzyme complexes (e.g., by properdin). Downregulation is achieved in a temporal fashion through the short half-lives of the enzymatic complexes and the anaphylatoxins, and in a spatial manner through direction of complement activation to the target surface (e.g., by antibody, MBL, properdin). Modulation of the potentially injurious effects of indiscriminate complement activation is achieved by specific regulatory proteins that act at three major levels: activation (C1), effector initiation (C3), and cytolysis (MAC). Unique disease entities that result from deficiencies of these control proteins are testimony to the importance of complement regulation.

Regulation of C1 Activation

C1 esterase inhibitor (i.e., C1-INH) binds reversibly to pro-C1, thereby preventing its spontaneous activation.[28] Binding of C1q to antibody subverts this control by causing dissociation of C1-INH from pro-C1 and allowing autocatalytic cleavage to proceed. At some point after C1 activation, C1-INH binds covalently to the active sites on C1r and C1s, inactivating their catalytic function and dissociating them from C1q. C1-INH neither prevents nor inhibits initial activation; rather, its roles are to prevent amplification of fluid-phase C1 activation and to limit excessive activation on the target cell. Complete C1 inactivation requires the binding of four molecules of C1-INH, one per catalytic site. In contrast to its binding to pro-C1, C1-INH binding to C1r and C1s is irreversible; it prevents cleavage of C4 and thereby controls the initial amplification step of classical-pathway activation.[28,64] C1-INH also limits the activity of MASP-2 and several proteases of the coagulation-anticoagulation system, including factor XI, factor XII, plasma kallikrein, plasmin, and tissue plasminogen activator.[65]

Regulation of the C3 Convertases

The classical-pathway and alternative-pathway C3 convertases are functionally analogous molecules (see Fig. 9.1). Control of their activity occurs via three basic mechanisms that use functionally identical or shared regulator proteins (Table 9.2)[30,41]:

1. *Spontaneous decay:* Both convertases (C4b2a and C3bBb) are inherently labile and undergo spontaneous decay, with the loss of C2a or Bb from their respective complexes.
2. *Accelerated decay:* Spontaneous decay can be accelerated by C4b-binding protein (C4BP) and especially by factor H. These regulatory proteins compete with C2a and Bb for binding sites on C4b and C3b. In doing so, they inhibit new convertase formation and enhance the rate of dissociation of already formed convertases.
3. *Facilitated inactivation:* Exposed C4b and C3b molecules are highly susceptible to enzymatic cleavage by factor I. C4BP and factor H serve as cofactors to promote factor I–mediated cleavage and production of inactivated C4b (iC4b) and C3b (iC3b), respectively. Inactivation eliminates the ability of these molecules to re-form the C3 convertases.[66,67] Under typical circumstances, the functional half-life of C3b is just 90 seconds, whereas its cleavage product, iC3b, has a half-life of approximately 35 minutes.

Several additional points have emerged from the many studies on C3 convertase regulation. First, control of C3 convertase activity is expressed both in the fluid phase and on host cell surfaces. C4BP and factor H modulate convertase activity in both locations, whereas membrane-bound proteins (complement receptor 1 [CR1], membrane cofactor protein [MCP], and decay-accelerating factor [DAF]) primarily control convertase activity on cell surfaces. Second, all control proteins either accelerate the decay of the C3 convertases (DAF) or promote factor I–mediated cleavage of C4b or C3b (MCP), or both (CR1, C4BP, factor H). Third, C4BP and factor H, unlike their membrane-bound counterparts, exhibit relative specificity for the classical-pathway and alternative-pathway C3 convertases, respectively.[64,67] Of these two serum proteins, factor H plays the dominant regulatory role.

These regulatory molecules contain a structural motif called *short consensus repeats (SCRs).*[68] SCRs are tandem repeats of approximately 60 amino acids that share a conserved consensus sequence. The number of repeats varies considerably among the control proteins, from a low

TABLE 9.2 Plasma and Membrane Proteins That Regulate or Mediate Complement Activity

LOCATION AND PROTEIN	SPECIFICITY	FUNCTIONS
Plasma		
C1-INH	C1r, C1s	Binds to and inactivates C1r and C1s in the C1 complex
C4BP	C4b	Inhibits assembly and accelerates decay of C4b2a; cofactor for C4b cleavage by factor I
Factor H	C3b	Inhibits assembly and accelerates decay of C3bBb; cofactor for C3b cleavage by factor I
Factor I	C4b, C3b	Proteolytic inactivation of C4b and C3b
Properdin	C3bBb	Stabilizes alternative-pathway C3 convertase
S protein (vitronectin), SP-40,40 (clusterin)	C5b-7	Binds fluid-phase C5b-7; prevents attachment of alternative C5b-7 and C5b-9 to membranes
Carboxypeptidase N	C4a, C3a, C5a	Inactivates these anaphylatoxins by removal of carboxyl-terminal arginine
Cell Membranes		
CR1 (CD35)	C3b, C4b, iC3b	Inhibits assembly and accelerates decay of C3 convertases; binds immune complexes to erythrocytes; phagocytosis
Membrane cofactor protein (CD46)	C3b, C4b	Cofactor for cleavage of C4b/C3b by factor I
Decay-accelerating factor (CD55)	C4b2a, C3bBb	Promotes decay of C3 convertases
CR2 (CD21)	C3d, C3dg	Phagocytosis; modulates B-cell responses; Epstein-Barr virus receptor
CR3 (CD11b/CD18)	iC3b	Phagocytosis
CR4 (CD11c/CD18)	C3dg, C3d	Phagocytosis
CD59	C8 in C5b-8	Binds to C8; inhibits polymerization of C9
C3a/C4aR	C3a, C4a	Vasodilation
C5aR	C5a, $C5a^{desArg}$	Chemotaxis, cell activation, cytokine secretion
C1qR	C1q	Phagocytosis
CRIg	C3b, iC3b	Phagocytosis; soluble form inhibits C3-containing convertases

BP, Binding protein; *CR,* complement receptor; *i,* inactive; *INH,* inhibitor.

of 3 in MCP and DAF to a high of 59 in C4BP. SCRs constitute the binding domains for C3b and other molecules. Two to four SCRs are typically required to form a complete binding site, but the number of SCRs and which ones in a sequence form a specific binding site differ among the proteins and their binding ligands.[68,69]

As noted, control of C3 convertase activity, and in particular amplification of alternative-pathway convertase activity, is expressed both in the fluid phase and on cell surfaces. Convertase control under these two circumstances is achieved through differential interaction among several C3b-binding sites and polyanion-binding sites on factor H. Fluid-phase regulation proceeds rapidly and depends on the interaction of C3b with SCRs 1 through 4 at the amino terminus of factor H. In contrast, regulation of convertase activity on cell surfaces depends on SCRs 16 through 20 at the carboxyl terminus of factor H. These SCRs bind both cell surface polyanions (e.g., sialic acid, GAGs, heparan sulfate) and C3b. The simultaneous recognition of surface polyanions and C3b by the same factor H molecule greatly increases the affinity of factor H for C3b, thereby enhancing convertase control. These findings are particularly relevant to our understanding of the pathophysiology of the hemolytic-uremic syndrome and factor H deficiency (see later discussion).[70,71,72]

In summary, the C3 convertases represent the major site of both complement amplification and complement regulation. The membranes of host cells contain both specific proteins that act to downregulate the C3 convertases and polyanions that enhance the affinity of fluid-phase factor H for surface-bound C3b and promote its regulatory activity. The chemical microenvironment on microbial surfaces is also a major determinant of the outcome of the competition between C3b and C4BP with C4b in formation of the classical-pathway convertase, and between Bb and factor H with C3b in formation of the alternative-pathway convertase. Nonpathogenic microbes typically possess an activating surface environment, whereas pathogenic microbes usually manifest a nonactivating environment (see later discussion).[73]

Regulation of the Membrane Attack Complex

MAC assembly is controlled in two ways—by proteins that bind to the C5b-7 complex and by proteins that inhibit C9 incorporation and polymerization within the MAC. Nascent C5b-7 molecules have the potential to insert into any cell membrane and are not restricted to the surface on which complement is activated. By binding to this trimolecular complex, S protein (vitronectin) and clusterin abrogate the ability of C5b-7 to insert into cell membranes and consequently its hemolytic potential.

Although several proteins inhibit C9 incorporation and polymerization within the MAC, by far the most potent is CD59. CD59 is very widely distributed. Its presence as a membrane protein probably accounts for homologous restriction,[74] a phenomenon whereby cells are not lysed by complement from the same species but can be (although are not always) lysed by complement from a different species. Although it is clear that CD59 binds to C9, the site of binding and the mechanism by which it prevents C9 incorporation and polymerization within the MAC are unclear.[66]

Nucleated eukaryotic cells are resistant to complement-mediated cytolysis, even in the face of a nonhomologous complement source. Resistance is associated with the capacity of the cell to maintain high synthetic rates of membrane lipids and the ability to shed MAC from the cell surface.[75,76] Insertion of the MAC in eukaryotic cell membranes is accompanied by a rapid influx of calcium, generation of multiple signals, and stimulation of arachidonic acid metabolism.[77,78] These events probably promote normal physiologic functions and contribute to host cell injury.

Basis for Discriminating Between Host and Microbial Cell Surfaces

The potential of C4 and C3 to form covalent bonds with reactive groups on cell surfaces makes them inherently incapable of distinguishing between host and microbial cells. It follows that the basis for discrimination between self and nonself must depend on other factors.[73] One of these elements is the presence of complement regulatory proteins in the membranes of host cells but not on the cells of microbial organisms.[73] Another element is the chemical composition of the cell surface. Because covalent bond formation is nondiscriminatory, the basis for discrimination must lie in the capacity for chemical differences on the cell surface to affect the outcome of the competition between factor B and factor H for the binding site on C3b. Typically, this is accomplished by modulation of the affinity of factor H, not that of factor B, for C3b. Enhanced affinity for and binding of factor H favors C3 convertase decay and diminishes activation of the alternative pathway and its amplification on the cell surface. Decreased affinity and binding of factor H results in the opposite effect (see earlier discussion). Cells in the former category (e.g., "self") are nonactivators, whereas those in the latter category (e.g., "nonself") are activators with respect to the alternative pathway. C3b bound to the surface of a nonactivating particle binds factor H with about 100-fold greater affinity than does C3b bound to an activator particle. Consequently, factor B binding and subsequent amplification of complement activation are favored on the latter particle.[30,41,70–72]

Chemical constituents in cell membranes that contribute to a microenvironment that enhances factor H binding include polyanions such as sialic acid and sulfated acid mucopolysaccharides (e.g., heparan sulfate). These molecules, which are present on most human cells, bind to the anion-binding sites on factor H and enhance its affinity for C3b, thereby contributing to the nonactivator status of host cells.[70–72,79,80] Structural data confirm that simultaneous binding of surface-bound C3b and GAGs to factor H domains 19 and 20, respectively, enhances the avidity of factor H binding.[81,82] Avid factor H binding increases the rate of C3 convertase decay over the rate of its formation, thus rendering the surface a "nonactivator." In contrast, loss of GAGs reduces the affinity of factor H and favors the rate of C3 convertase formation over its decay, thereby enhancing complement activation and effector functions.

COMPLEMENT RECEPTORS

Complement receptors have been described primarily on peripheral blood cells, including erythrocytes, neutrophils, monocytes, macrophages, B and T lymphocytes, eosinophils, mast cells, and platelets. They fall into two broad categories: (1) those that bind diffusible complement fragments released during activation of the complement cascade and (2) those that bind complement components deposited on cell surfaces such that the component serves as a bifunctional ligand, or bridge, linking the target cell to the receptor (see Table 9.2).

Receptors in the first category mediate many of the clinical manifestations of the inflammatory response in that they bind C4a, C3a, and C5a, the complement-derived inflammatory mediators. Of these, the high-affinity C5a receptor (CD88) has been best studied. This G protein–coupled receptor is present on neutrophils, monocytes, and macrophages, and its perturbation causes migration (chemotaxis) of these cells in the direction of increasing C5a concentration. Studies have furnished interesting examples of bidirectional crosstalk between engagement of macrophage Fcγ receptors (FcγRs) and C5a receptors (C5aRs).[83] Engagement of FcγR initiates C5 release; C5a generated by cleavage of this C5 binds to its receptor on phagocytes and stimulates increased synthesis and expression of FcγRs that possess an immunoreceptor tyrosine-based activation motif (ITAM), thereby resulting in enhanced efficiency of macrophage function.[23] On the other hand, engagement of FcγRIIb, which is the only FcγR that possesses an immunoreceptor tyrosine-based inhibition motif (ITIM),[84] inhibits signaling by C5aR. A second receptor for C5a called C5L2 (GPR77) is a G protein–independent receptor. The functions of C5L2 remain to be fully elucidated,[85] but some studies have suggested that it serves as a decoy receptor with regulatory functions. Experimental evidence has confirmed the presence of receptors for C3a on B lymphocytes, guinea pig ileum, vascular endothelium, adipocytes, and mast cells.[86] Several studies in animal models have begun to elucidate the complex role of anaphylatoxins and their receptors in the outcome of polymicrobial sepsis,[87] airway hypersensitivity,[88] modulation of signaling through Toll-like receptors (TLRs),[89] and in T-cell expansion and differentiation,[90–94] often with conflicting results.

The second category of receptors includes C1qR, CR1, CR2, CR3, CR4, and CRIg (*c*omplement *r*eceptor of the *i*mmuno*g*lobulin superfamily). C1qR is a carbohydrate-rich protein expressed on phagocytic cells and lymphocytes that modulates phagocytosis, cytokine release, cytotoxicity, and interactions with endothelial cells. Functional ligands in addition to C1q that are recognized by C1qR include MBL, surfactant protein A, and conglutinin, all of which exhibit structural homology with C1q.[95]

Receptors for the cleavage products of C3 and C4 (CR1, CR2, CR3, and CR4) have been studied more extensively. CRIg is the newest member of the complement receptor family.[96] Although they recognize closely related ligands, each of these receptors is structurally distinct and exhibits a unique pattern of distribution across peripheral blood cells or tissue macrophages. A portion of these receptors are linked to the cellular cytoskeleton, an association that is important for signal transduction.[95,97,98]

CR1, the C3b/C4b receptor, is present on erythrocytes, neutrophils, monocytes, B cells, subpopulations of T cells, follicular dendritic cells (DCs), and glomerular podocytes. Four polymorphic variants differ in size (190–280 kDa) and in the number of C3b/C4b-binding sites.[99] The number of CR1 molecules per cell is determined genetically but varies with cell type and disease activity. CR1 mediates immune complex binding and clearance, promotes ingestion of C3b/C4b-bearing particles, modulates certain lymphocyte functions,[95,97] and carries certain blood group antigens.[100]

CR3 and CR4 are members of the integrin family of heterodimeric proteins.[101] They recognize iC3b as their major binding ligand. CR3 also binds to C3b and C3dg and bears a lectin-like domain that recognizes specific carbohydrates on microbial surfaces. The three–amino-acid sequence arginine-glycine-aspartic acid (Arg-Gly-Asp, also known as the RGD motif), which is present in C3 and other ligands, represents an important binding motif for CR3.[102] Together, CR3 and CR4, particularly the former, recognize the various combinations of C3b, iC3b, and C3dg present on the surfaces of microbial cells and play a major role in their elimination by all types of phagocytic cells.[103] In addition, CR3 plays an important role in adherence-related functions of neutrophils (see Chapter 8).

CR2 is present on B lymphocytes and follicular DCs and serves to recognize C3d and C3dg. The association of CR2 and CD19 in the B-cell membrane constitutes an important mechanism for B-cell activation.[104] CR2 acts to target C3dg-bearing particles or immune complexes to lymphocyte-rich areas in the spleen and lymph nodes, thereby driving antigen activation of these cells and promoting long-term immunologic memory.

CRIg was first identified on Kupffer cells (resident macrophages in the liver) and binds to C3b and iC3b.[96] The Kupffer cells in the liver of CRIg knockout mice, compared with wild-type mice, were impaired in their ability to clear C3-opsonized pathogens, such as *Listeria monocytogenes* and *Staphylococcus aureus,* from the circulation. Although CRIg knockout mice had lower bacterial burdens in the liver, significantly higher bacterial loads were seen in the bloodstream and other organs, such as the spleen and lungs, which accounted for the increased mortality compared with wild-type mice. On binding to C3b present in alternative-pathway C3 and C5 convertases, soluble CRIg prevents the association and cleavage of C3 and C5, respectively, thereby functioning as an inhibitor of the alternative pathway.[105,106]

FAMILIES OF COMPLEMENT PROTEINS

The complement cascade represented in Fig. 9.1 emphasizes features shared by the three pathways with respect to activation and regulation. Complement components can also be grouped into several different protein families[107]:

- The serine protease family (C1r, C1s, MASP-1 through MASP-3, MAP19, C2, factor D, factor B, and factor I).
- Disulfide-linked, multichained molecules with homology to an ancestral protein that contained an internal thioester bond (C4, C3, and C5).
- Proteins that are the products of class III major histocompatibility complex (MHC) genes located on chromosome 6 (C2, factor B, C4A, and C4B).
- Proteins that bind C3 and C4 fragments and belong to a closely clustered supergene family located on the long arm of chromosome 1 (C4BP, factor H, DAF, MCP, CR1, and CR2); these proteins share a common SCR motif (see earlier discussion) with other complement components that bind to C3 and C4 (e.g., C2, factor B) and with some other complement and noncomplement proteins that do not bind these two components.[68,69]
- Proteins that share homology with the low-density lipoprotein (LDL) receptor (C6, C7, C8α, C8β, and C9); the large number of disulfide bonds in these cysteine-rich molecules is thought to convey a tertiary structure that facilitates the hydrophilic-hydrophobic transition that occurs when they interact with lipid membranes during assembly of the MAC.[58,59]

The MHC III genes are located between the class I and class II loci on the short arm of chromosome 6[107] and merit discussion. The genetic material in this region appears to have undergone two duplication events, resulting on the one hand in the structurally and functionally related proteins C2 and factor B, and on the other in the C4 and 21-hydroxylase

A and B variants.[107] Recombinant events in this region of the chromosome tend to be suppressed, leading to the usual inheritance of the entire region intact from each parent.[108] The polymorphic variants of the complement components encoded by these genes in a given individual are referred to as *complotypes.*[109] The association of specific complotypes with specific products of the MHC I and II genes probably contributes to the association of specific complotypes with certain disease states (e.g., systemic lupus erythematosus [SLE]).

COMPLEMENT-MEDIATED FUNCTIONS

Complement plays major roles in the distinction between self and nonself, development of an inflammatory response, elimination of microbial pathogens, modulation of the adaptive immune response, limitation of the potential for an injurious inflammatory response (through the disposal of immune complexes and apoptotic cells), metabolism, angiogenesis, tissue regeneration, and organogenesis (see Fig. 9.1).[4,6]

Whether initial formation of a C3 convertase is followed by its enhanced decay or by its amplification is the event that distinguishes self from nonself, respectively, as described earlier.[6]

Small, diffusible peptide fragments released from C4, C3, C5, and probably C2 during their activation initiate and modulate the inflammatory response.[86] Collectively, C4a, C3a, and C5a are referred to as *anaphylatoxins,* and together they stimulate histamine release from mast cells (C3a), promote vascular dilation (C3a, C4a), increase endothelial permeability (C3a), and stimulate neutrophil responses (C5a). In addition to these proinflammatory activities, C3a acts via its receptor on B cells to downregulate cytokine synthesis and antibody production.[110] Carboxypeptidase N–mediated removal of the carboxyl-terminal arginine from the anaphylatoxins abrogates their functional activity by preventing their interaction with specific receptors.[104]

Elimination of Pathogens

Complement activation promotes the elimination of microorganisms in conjunction with phagocytic cells by opsonophagocytosis or, in the case of certain gram-negative pathogens, by direct bactericidal attack. Complement-mediated opsonophagocytosis promotes uptake via complement receptors, predominantly CR1 and CR3, that recognize C3b and iC3b, as described previously (see Table 9.2). In the case of bacteria, opsonization with C3b or iC3b, especially in conjunction with IgG, promotes ingestion of the organism and triggers the microbicidal mechanisms of phagocytic cells (see Chapter 8). Ingestion appears to be more efficient when the organism is opsonized with iC3b than with C3b.[111] Complete activation of the complement cascade, with assembly of the MAC and its effective insertion into cell membranes, results in the death and eventual lysis of the cell. Death and lysis are independent events, and in the case of prokaryotes, evidence suggests that a metabolic response is required by the organism before the lethal effects of the MAC can be expressed.[112] For some organisms, assembly of the MAC through C8 is sufficient for killing,[113] but in all cases the incorporation of C9 accelerates this process. Complement-mediated virucidal activity has also been well described and in some cases seems to require deposition of only the early components of the classical pathway.[114]

Modulation of Adaptive Immune Responses

Substantial data indicate that C3 modulates the adaptive immune response.[115–117] This evidence includes (1) the absolute requirement for C3 binding to effect antigen localization within splenic and lymphoid germinal centers; (2) the presence of complement receptors, especially CR2, on B lymphocytes, follicular DCs, and other antigen-presenting cells; (3) impaired antibody responses in animals or humans who lack one of the complement components (C1, C2, C4, C3) required for classical-pathway C3 convertase formation and restoration of the immune response by replacement of the missing component; and (4) the association of these deficiencies in humans with depressed concentrations of IgG4 and IgG2.[118,119] In general, studies have demonstrated that soluble C3 fragments (especially C3a) inhibit adaptive immune responses, whereas C3 fragments (especially C3d) covalently linked to target particles enhance these responses. Complement activation via the classical pathway and engagement of CD21/CD35 receptors promote differentiation of naïve B cells and elimination of self-reactive cells.[115] Ligation of C3d to its receptor, CR2, leads to its association with CD19 in the B-cell membrane and constitutes an important signal for the activation of these cells.[104] Antigen-bound C3d acts as an immune adjuvant, lowering the stimulation threshold necessary for B-cell activation. This adjuvant role is particularly critical in enhancing the response to antigens with a low affinity for the B-cell receptor.[120–122] In addition to its adjuvant role, C3d facilitates isotype switching, anamnestic responses after secondary antigenic exposure, and B-cell survival and long-term immunologic memory. Similarly, C3 fragments promote the expansion of $CD8^+$ T cells after viral infection, and costimulation of CD3 and CD46 (MCP) promotes development of regulatory T cells (Tregs). Thus, complement activation contributes to the development of both B-cell and T-cell acquired immune responses.[115,116,123]

Local production and activation of complement and signaling through the anaphylatoxin receptors can determine the outcome of T-cell responses.[93] Engagement of TLRs on DCs results in secretion of alternative-pathway components and upregulation of C3aR and C5aR. C3a and C5a act on their cognate receptors on the DCs and induce secretion of IL-6, IL-12, or IL-23. Stimulation of CD28 on T cells induces expression of C3aR and C5aR; engagement of the latter by the anaphylatoxins generated by DCs induces IL-12R expression and a series of signaling events that result in IFN-γ and IL-2 production. The interleukins secreted by the DCs then determine whether responses are skewed toward Th1 or Th17. In the absence of activation of DCs through pattern recognition receptors, local complement production ceases and the lack of signaling through C3aR and C5aR is associated with increased production of transforming growth factor-β (TGF-β) and induction of suppressive $Foxp3^+$ Treg cells.[94] During this process, upregulation of C5L2 sequesters any local C5a, ensuring absence of C5aR stimulation. These events depend on the production of complement locally, not on systemically circulating complement.

Some experts believe that an ancient form of C3—part of a primitive complement system in simple multicellular pathogens such as members of the phylum Porifera (sponges)[124,125]—functioned intracellularly to regulate metabolic processes and also to fight intracellular pathogens. The more familiar extracellular functions of C3 may have evolved as organisms became more complex and developed circulatory systems, when C3 was secreted into the vasculature. However, the intracellular functions of complement likely have been retained over the course of evolution. As discussed later, intracellular C3 fragments play a role in cellular survival and metabolic reprogramming of T cells. In addition to T cells, intracellular complement may function in most cells of the body.

A recently elucidated novel recycling pathway describes a source for intracellular C3. $C3(H_2O)$, the hydrolytic product of native C3, is taken up by cells and constitutes an important source of intracellular C3.[43] About 80% of $C3(H_2O)$ is recycled back to the extracellular space.[43] A fraction of intracellular $C3(H_2O)$ is cleaved by lysosomal cathepsin L to generate C3a and C3b. C3a binds to C3aR on lysosomes and activates the metabolic-checkpoint kinase, mammalian target of rapamycin (mTOR), at a low level that permits survival of T cells.[126] Activation of the T-cell antigen receptor (TCR) and costimulation through the coreceptor CD28 results in redistribution of C3aR and cathepsin L from their intracellular locations in the resting state to the cell surface. Cleavage of C3 at the cell surface generates C3a and C3b, which signal through C3aR and CD46, respectively, resulting in upregulation of growth factor receptors and secretion of cytokines characteristic of Th1 (such as IFN-γ) and Th17 (such as TNF and IL-17) responses, but not Th2 cytokines (IL-4 and IL-5). Expansion of Th1 cells is accompanied by high local levels of IL-2; signaling via IL-2R and CD46 induces IL-10 production and initiates contraction of the Th1 cell population.

Metabolic reprogramming determines the fate of T-cell differentiation. Activation of $CD4^+$ T cells results in autocrine activation of C3aR and CD46 by C3a and C3b, respectively, and sustained activation of mTOR complex (mTORC1), which stimulates glycolysis and oxidative phosphorylation and induction of a Th1 response.[127] Accordingly, individuals with CD46 deficiency show impaired production of IFN-γ but intact Th2 cytokine responses.[125] Proteolytic cleavage of intracellular C5 generates C5a, which activates C5aR1, and results in generation of reactive oxygen species, which activates the NLRP3 inflammasome. NLRP3 activation cleaves pro-IL-1β to active IL-1β, which in an autocrine fashion promotes differentiation to Th1 cells.[128]

Clearance of Immune Complexes and Apoptotic Cells

The incorporation of complement in immune complexes enhances clearance and helps to minimize the potential for tissue damage.[129,130] This process includes inhibition of immune complex precipitation, solubilization of immune complexes, and clearance of C3b-bearing immune complexes via the CR1 receptor. Under conditions of antibody excess or antibody-antigen equivalence, the probability that both antigen-binding sites on a single antibody will bind to epitopes on a single antigen and the probability that multiple antibody molecules will bind to a given molecule of antigen are increased. This situation promotes antibody–antibody interactions via Fc fragments and subsequent immune complex precipitation.[129] C1q binding to the Fc portion of antibody inhibits Fc–Fc interactions and leads to covalent binding of C3b to the immune complex. Subsequent recruitment of the alternative pathway via the C3b amplification loop promotes further C3b deposition within the immune complex lattice, thereby reducing the forces that hold the lattice together and causing separation (solubilization) of smaller complexes from the lattice network. Thus, classical-pathway activation inhibits immune complex precipitation, whereas the alternative pathway promotes immune complex solubilization.[129,130] However, in the context of disease pathogenesis, it must be stressed that complement is 10 times more efficient in inhibiting immune complex precipitation than in solubilizing precipitated complexes. This property probably contributes greatly to the close association of classical-pathway component deficiencies with immune complex disease formation (e.g., SLE).

In healthy people, most immune complexes bearing C3b are bound to cells bearing C3b receptors (CR1). The number of these receptors per cell varies from a low of 950 for erythrocytes to a high of 57,000 for neutrophils.[131] However, because there are 1000 times more erythrocytes than leukocytes, 95% of the CR1 receptors in the peripheral circulation are located on erythrocytes. Consequently, immune complexes bearing C3b are 500 to 1000 times more likely to be cleared from the circulation by erythrocytes than by leukocytes.[131] These complexes are removed from the erythrocyte, along with the CR1, during passage through the liver and the spleen. This extraction probably involves fixed macrophages that line the sinusoids of these organs.[132]

Recent studies have extended our understanding of the antiinflammatory role played by complement in promoting the clearance of apoptotic cells. Under steady-state conditions, the billions of host cells that die every day are eliminated with minimum induction of an inflammatory or immune response. Despite the number of cells involved and completion of the apoptotic cycle over a period of several hours, few apoptotic cells are identified in tissues or in the circulation. Rapid complement-dependent phagocytic removal of apoptotic cells by macrophages appears to account for this apparent paradox. During apoptosis, the cell membrane bulges to form blebs that contain macromolecular complexes of proteins and nucleic acids, a finding that may be a significant contributing factor in the development of SLE (see later discussion). The exposed surface of the bleb contains several unique phospholipids that have been translocated from the inner to the outer leaflet of the lipid bilayer of normal cell membranes. Some of these phospholipids, especially phosphatidylserine, bind C1q directly to activate the classical pathway. In addition, data have suggested that the phospholipid-binding proteins annexin 2 and annexin 5 may also serve as ligands for C1q on apoptotic cells.[133] An analogous process occurs on ischemic cells on reperfusion; exposed GAGs promote properdin-directed alternative-pathway activation.[53] Unlike viable eukaryotic cells, apoptotic cells and ischemic cells "permit" both C3 convertase formation and amplification. These cells can then be eliminated via C1qR, CR3, and CR4 on mononuclear cells, fixed macrophages, and DCs. The importance of CR3 in clearing apoptotic cells is underscored by the observation that a variant allele (R77H) in CD11b (α-chain of CR3) that impairs phagocytosis is one of the strongest risk factors for SLE.[134–137] Elimination in this fashion minimizes the inflammatory potential of injured cells.[138,139]

Complement and Metabolism

One of the characteristics of the metabolic syndrome is increased inflammation, including activation of the complement system.[140] Insulin resistance and abdominal obesity are associated with higher serum concentrations of C3. Adipocytes are the main source for factor D (also known as adipsin) and also synthesize C3 and factor B. Local activation of the alternative pathway results in production of C3a that is rapidly converted to $C3a^{desArg}$. $C3a^{desArg}$ (also known as acylation-stimulating protein [ASP])[141] stimulates triglyceride synthesis in fat cells by increasing the activity of diacylglycerol acyltransferase. C5L2 also appears to play a role in lipid clearance by $C3a^{desArg}$, although it is not clear whether C5L2 and $C3a^{desArg}$ interact directly.

Complement and Cancer

Complement plays complex and multifaceted roles in the pathogenesis of cancer, both protective and pathogenic.[142,143] The conventional view has been that complement plays a (protective) role in immune surveillance against cancer cells by promoting antibody-dependent cellular cytotoxicity (ADCC) and lysis of cells by the MAC. Many tumor cells, however, express high amounts of membrane inhibitors of complement, such as CD46, DAF, and CD59, and in addition can recruit soluble complement inhibitors, such as factor H and factor H–like protein 1 (FHL-1; an alternatively spliced variant of factor H), to their surface and thus resist direct lysis by complement.[144] Complement also promotes angiogenesis and may therefore contribute to tumor growth.[145] C3a and C5a stimulate the secretion of vascular endothelial growth factor (VEGF) and facilitate neovascularization. This effect contributes to the choroidal neovascularization seen in age-related macular degeneration (AMD; see later) and forms the basis for the use of VEGF inhibitors in the treatment of this disease. The ability of complement to degrade extracellular matrix may increase tumor invasion and migration. C5a generated by the classical pathway in the tumor microenvironment enhanced the growth of cervical cancers in mice. C5a attracts myeloid-derived neutrophil- and monocyte-like suppressor cells to the tumor, which generate reactive oxygen and nitrogen species that interfere with the ability of T cells to respond to tumor antigens.

Complement in Tissue Regeneration and Organogenesis

Complement proteins can also modulate diverse developmental processes, such as cell survival, growth, and differentiation in various tissues.[146] For example, C3 and C5 have been implicated as mediators of lens and limb regeneration in lower vertebrates.[147] Impaired liver regeneration was observed in both C3 and C5 knockout mice after partial hepatectomy; infusion of C3a and C5a into these mice restored hepatic regeneration. Enhanced signaling through the canonical Wnt pathway is associated with aging-associated decline in tissue regeneration. Aging mice have higher serum levels of C1q; C1q binds to Frizzled receptors, whereas C1s cleaves the Wnt coreceptor low-density lipoprotein receptor–related protein 6 (LRP6), which activates the Wnt pathway and impairs skeletal muscle regeneration.[148]

Studies in mice have provided evidence for a critical role of the classical pathway in synaptic remodeling.[149] C1q expression is upregulated when neurons are exposed to immature astrocytes, which results in deposition of C3 fragments and elimination by macrophages or microglia. Mice deficient in C1q or C3 could not eliminate unwanted synapses. Neuronal pentraxins that bear 20% to 30% structural homology to the immune pentraxins PTX3 and CRP were cited as possible C1q binding molecules in this process. Complement activation may contribute to neuronal pathologic conditions in adults. A strong genetic link exists between C4A alleles associated with high levels of C4A expression and schizophrenia. Increased complement activation associated with higher C4 levels may result in synapse elimination in the prefrontal cortex, which is characteristically seen in the brains of individuals with schizophrenia.[150] Studies in mice suggest C1q-dependent complement activation initiated by soluble β amyloid oligomers, accompanied by microglial pruning of complement-coated synapses, may contribute to the pathology of Alzheimer disease.[151] Studies in *Xenopus* embryos revealed a role for the C3a-C3aR interaction in neural crest migration. A role for the lectin pathway, specifically collectin 11 and MASP-1, in neural crest migration and craniofacial development is discussed later.

The context in which complement activation occurs is important. Although complement may have salutary effects in development and some aspects of tissue regeneration, complement activation contributes

to ischemia-reperfusion injuries related to acute myocardial infarction, stroke, and organ transplantation, and to the pathologic features of diseases such as hepatic, pulmonary, and renal fibrosis, Alzheimer disease, Parkinson disease, and multiple sclerosis.

MICROBIAL INTERACTIONS WITH THE COMPLEMENT SYSTEM

The demonstration by Roantree and Rantz[152] that gram-negative bacteria isolated from blood were almost always resistant to complement-mediated killing, whereas two-thirds of those isolated from mucosal surfaces were serum sensitive, was one of the first findings to suggest an important clinical role for complement-mediated bactericidal activity in host defense. This suggestion was borne out by subsequent studies of people with complement deficiencies (see later discussion) and by elucidation of the strategies and the extremes to which microorganisms go to escape host defense mechanisms. In the case of complement, these strategies parallel those used by host cells to circumvent injury during the inflammatory response—that is, they are focused on decreasing complement activation, accelerating convertase decay, and inhibiting the formation or insertion of the MAC.[153] In many instances, the microbial proteins responsible for these effects share molecular, structural, immunologic, and functional homology with their human counterparts.

As noted earlier, sialic acid is a well-characterized modulator of alternative-pathway activity, the action of which is expressed through enhanced factor H binding. Sialylation of the lipooligosaccharides (LOSs; lipopolysaccharide [LPS] molecules that lack O-antigenic repeats) of the pathogenic Neisseriaceae, *Haemophilus influenzae* (both typeable and nontypeable), and *Campylobacter jejuni* may enhance interactions of factor H with surface-bound C3 fragments,[154] or directly with the bacterial surface as reported with *Neisseria gonorrhoeae.*[155] Sialic acid is also a prominent chemical constituent of capsular polysaccharides on type 3 group B streptococci, K1 *Escherichia coli,* and groups B and C meningococci.[73,80] Consequently, the capsules of these organisms are nonactivators of the alternative pathway and are poor stimuli for antibody production. In this context, it is noteworthy that K1 *E. coli*, group B streptococci, and group B meningococci are prominent causes of neonatal and infant sepsis and meningitis. The frequent absence in these young patients of specific antibody to activate the classical pathway, coupled with bacterial sialic acid–mediated inhibition of alternative-pathway activity, may provide the ideal clinical setting for infection with these organisms.

Elegant experiments correlating virulence with LPS composition and complement activation in three isogenic *Salmonella typhimurium* variants, which differed only in the chemical structure of their LPS side chains, demonstrated the importance of limited complement deposition on the surfaces of bacteria. The greatest rate of C3 consumption and extent of C3b deposition were initiated by the least virulent strains. Subsequent experiments demonstrated that discrete differences in O-antigen structure were expressed at the level of alternative-pathway amplification, as manifested in the greater affinity of factor B for C3b on the surface of the least virulent compared with the most virulent strains. In contrast, the affinity of factor H for C3b was the same in all strains.[156,157]

Appropriation of complement regulatory proteins is a strategy used by a wide range of microbial pathogens, as has been demonstrated for many pathogenic bacteria, viruses, fungi, and worms.[158–160] These organisms express any of a number of complement regulator–acquiring surface proteins (CRASPs) on their surfaces. A chief binding target of these proteins is factor H, although the site to which factor H binds varies slightly among the CRASPs.[161] M protein in group A streptococci functions as a CRASP. Its α-helical coiled-coil repeats promote binding, not just of factor H but also of C4BP and MCP. The fact that group A streptococci bind multiple proteins to regulate complement at the level of C3 attests to the critical importance of C3 activity for survival of the organism, and likewise for host defense against the organism. Through these interactions, M protein not only limits complement deposition on the streptococcal surface but also promotes adhesion to keratinocytes.[161,162] Meningococci bind factor H in a human-specific manner through two surface molecules, factor H–binding protein (fHbp) and neisserial surface protein A (NspA).[163–165] fHbp is a key antigen in two licensed vaccine preparations against group B meningococcal disease (Bexsero [GlaxoSmithKline {Philadelphia, PA}] and Trumenba [Pfizer {New York, NY}]).[166]

Other organisms use related strategies. The envelope of type 1 herpes simplex virus contains a virus-specific protein, gC-1, that interferes with properdin-dependent stabilization of the alternative-pathway C3 convertase, thereby limiting complement-mediated effects. Deletion mutants lacking gC-1 are exquisitely sensitive to complement-mediated lysis. Natural mutants have not been isolated with any frequency in surveys of clinical specimens, attesting to the importance of this protein and perhaps this mechanism in the pathogenesis of infection. Vaccinia virus bears a C4BP structural and functional homologue that accelerates the decay of the classical-pathway convertase.[153,167,168]

A variation on this theme occurs in serum-sensitive gonococci isolated from patients with symptomatic local genital disease. These organisms possess a sialyl transferase but lack the ability to synthesize cytidine monophospho-*N*-acetyl neuraminic acid (CMP-NANA). Consequently, they are incapable of endogenous sialylation of their LOS; rather, they appropriate host CMP-NANA for this purpose. Exogenous sialylation confers serum resistance to these gonococci by reducing the binding of bactericidal antibody[169] and by enhancing binding of factor H (see earlier); it also reduces phagocytic uptake and may alter C3 cleavage and intracellular survival. *Trypanosoma cruzi* accomplishes the same effect by means of a *trans*-sialidase that removes terminal sialic acid residues from host glycoconjugates and transfers them to acceptor molecules on the parasite's surface.[170]

The striking metamorphosis undergone by protozoa during transformation from insect-infective to human-infective forms is accompanied by the acquisition of resistance to complement-dependent killing. This phenomenon has been studied most extensively in *T. cruzi,* in which surface proteins have been identified that block assembly and promote decay of the alternative-pathway C3 convertase.[153,170] These proteins function in a manner identical to human CR1 and DAF (see Table 9.2). *Ixodes* tick saliva contains a protein, Salp15, that, when bound to *Borrelia burgdorferi,* inhibits MAC assembly and prevents complement-mediated lysis of the organism.[171] Gametes of *Plasmodium falciparum* bind to human factor H through a surface protein called PfGAP50, which protects them from complement-mediated lysis in the mosquito midgut.[172]

The growth of DNA sequence data banks and investigations into molecular pathogenesis have served to focus attention on virus-complement interactions and the mechanisms by which these organisms elude complement-mediated attack. Studies of the human immunodeficiency virus (HIV) are particularly illustrative. During viral replication, the virus is assembled and released from the infected cell by budding, a process that incorporates host cell membrane proteins into the viral envelope. Host cell DAF and CD59 incorporated into the viral envelope in this manner function efficiently to limit amplified complement deposition on HIV and its subsequent lysis. In addition, the HIV-specific envelope proteins gp120 and gp41 both contain factor H–binding domains, which, in the case of the latter protein, demonstrate significant homology with C3. Factor H passively absorbed from serum and secretions serves to further limit complement deposition on the virus.[167,173]

Other organisms owe their serum resistance to functional homologues of CD59, the protein that interferes with MAC assembly on host cell membranes. For example, the galactose-specific adhesin of *Entamoeba histolytica* functions in this manner and also shares DNA sequence homology and antigenic cross-reactivity with CD59.[174] Plasmids in *S. typhimurium* and *Yersinia enterocolitica* contain the *rck* and *ail* genes, respectively, which encode products of a family of virulence-associated outer membrane proteins. By preventing C9 polymerization, these proteins function similarly to CD59 and mediate serum resistance.[175,176] Ail has recently been shown to recruit factor H and C4BP, whereas Rck binds to factor H, providing evidence that a single protein can block complement using multiple mechanisms.[177–181] Akin to protein Ail, select gonococcal porin molecules can bind both factor H and C4BP, thereby regulating the alternative and classical/lectin pathways, respectively.[182,183]

Bacterial capsular polysaccharides modulate the effects of complement deposition on the organism and interaction of the organism with the host (see earlier discussion). In the absence of specific antibody, the

capsule, by masking C3 deposited on subcapsular structures, blocks C3 interaction with complement receptors on phagocytic cells. These effects contribute to the antiphagocytic properties of capsular polysaccharides. In addition, capsular polysaccharides and outer membrane blebs are shed during organism growth and complement attack. Shedding serves to divert complement attack from the intact organism to these shed complexes. The ability of capsular-specific antibody to reverse these effects is testimony to the importance of antibody in redirecting complement deposition to a relevant site on the surface of the organism.[184]

Gonococci isolated from patients with disseminated gonococcal infection are resistant to the bactericidal activity of normal human serum.[185] The serum resistance of these strains is multifactorial. In the absence of bactericidal antibody, the MAC is assembled on the organism's surface but fails to insert properly into the outer membrane.[186] MAC insertion and killing occur normally in the presence of anti-LOS IgG found in the convalescent serum of some patients with this infection.[187] However, some sera also contain IgG specific for gonococcal outer membrane protein 3.[188] This antibody competes with bactericidal antibody for binding sites on the surface of the organism, thereby blocking its bactericidal effect. Although the blocking antibody promotes complement deposition on the organism, it apparently does so at sites that do not lead to killing of the organism.[189] Blocking antibody also appears to account for the resistance of meningococci to killing by the serum of some adults who acquire this infection.[190,191–193] Antibodies that block killing of *Pseudomonas aeruginosa* and *Brucella abortus* have also been described.[194–196] A study in HIV-infected adults in Africa showed that antibodies directed against the LPS of nontyphoidal *Salmonella* blocked killing by otherwise bactericidal antibodies directed against outer membrane proteins.[197] Thus, blocking antibodies may represent a fairly ubiquitous yet relatively underappreciated mechanism of complement evasion by bacteria. These findings illustrate the influence of the composition of the outer membrane of gram-negative bacteria in determining sensitivity to complement-mediated killing and the importance to the host of specific antibody in overcoming the resistance of these organisms to killing.[198]

Redundancy in complement evasion mechanisms by pathogens appears to be the rule, rather than the exception. This is well illustrated by the following examples with *S. aureus*.[199] Staphylococcal protein A (SpA) and staphylococcal binder of immunoglobulins (Sbi) engage IgG on the bacterial surface in a manner that precludes activation of the classical pathway. Staphylokinase cleaves IgG and releases it from the bacterial surface. Several small molecules secreted by *S. aureus* interfere with complement activation at various steps. Staphylococcal complement inhibitor (SCIN) and its homologues SCIN-B and SCIN-C bind to the classical- and alternative-pathway C3 convertases and block their function. Extracellular fibrinogen-binding protein (Efb) and extracellular complement-binding protein (Ecb; also called Efb homologous protein or Ehb) also target and block the activity of the C3b-containing convertases (C3bBbC3b and C4bC2aC3b). The chemotaxis inhibitory protein of *S. aureus* (CHIPS) binds to the C5aR and formyl peptide receptor (FPR) on neutrophils and inhibits chemotaxis.

Appreciation has been growing for the number of intracellular pathogens that use complement receptors to gain entry into cells.[200,201] Organisms vary as to whether entry in this fashion initiates an appropriate signal transduction response and whether it is sufficient to establish effective intracellular infection. For example, gp350 on Epstein-Barr virus serves as a ligand for CR2 to initiate viral entry into B cells. The resulting cellular transformation probably contributes to the polyclonal gammopathy observed early in infectious mononucleosis.[200,201] In addition to its factor H–binding domain, HIV gp120 contains several C3b-binding regions. Cells harboring latent HIV can be activated by the uptake of additional HIV or other particles via CR3. Interactions via this receptor can induce the cellular transcription factor, nuclear factor kappa B (NF-κB), which in turn binds to promoter regions in the virus to stimulate generation of progeny virus.[6,35] The pathogenic mycobacteria (*Mycobacterium tuberculosis, Mycobacterium leprae,* and *Mycobacterium avium*) can bind to (preformed) C2a, which results in the formation of a C3 convertase on the bacterial surface. Subsequent C3 cleavage and C3b deposition facilitate bacterial uptake by macrophages through CR1.[202] C3 fragment deposition mediated by natural IgM facilitates uptake of *Francisella tularensis* by human neutrophils through CR1 and CR3, whereas uptake by macrophages occurs through engagement of CR3 and CR4.[203]

Over the past decade, our concept of extracellular organisms has been modified by the recognition that many of these organisms can enter and survive inside epithelial cells. Both the meningococcus and the gonococcus, like the measles virus, use CD46 (see Table 9.2) to gain entry to such cells, activating intracellular signaling cascades that are important for their infectivity.[204,205] Primary human cervical epithelial cells synthesize all components of the alternative pathway and also express CR3.[206] Gonococcal pilin and porin can interact with the I-domain of CR3, which, in cooperation with iC3b deposited on bacteria, facilitates gonococcal invasion into epithelia.[207]

COMPLEMENT DEFICIENCY STATES

Incidence

Complement deficiency states can be acquired or inherited. Acquired deficiency can occur acutely, as part of an abrupt insult such as infection, or in conjunction with chronic rheumatologic or autoimmune disease. The frequency of inherited complement deficiencies in the general population is about 0.03%. Because these states are rare, the usefulness of screening tests is greatest in populations that bear the clinical correlates of abnormal complement inheritance—that is, persons with rheumatologic disease or recurrent bacterial infection.[208–210] The frequency of complement deficiencies reported among people with these disorders is affected by both methodologic and biologic factors.[211] The most important methodologic variables are sample size and degree of ascertainment. The most important biologic considerations are the ethnic makeup of the population and the incidence of the target disease in that population.

One such study, using immunologic and functional assays, detected a single individual with homozygous C2 deficiency among 545 patients with rheumatologic disease.[211] This frequency (0.2%) is approximately 10-fold greater than that in the general population. Studies using DNA typing methodologies have found the frequency of homozygous C2 deficiency in whites with SLE to be about 1.7%.[212] These studies provide clear support for the association of complement deficiency states with certain rheumatologic disorders, particularly SLE.[213]

Reports of an association between systemic meningococcal and gonococcal infections and inherited deficiency of C5, C6, C7, or C8 led to several studies of the prevalence of such deficiencies among patients with these infections. These studies found that as few as 0 (<2%) of 47 and as many as 8 (50%) of 16 individuals presenting with a first episode of documented meningococcal disease had a complement deficiency.[209,211] Analysis of these studies demonstrated an inverse relationship between the prevalence of complement deficiency among people with meningococcal disease and the incidence of the disease in the general population—that is, a high prevalence of complement deficiency was found in populations with hypoendemic disease and a low prevalence in populations with epidemic disease (Fig. 9.3). This finding suggests that the overall prevalence of complement deficiencies is relatively constant (0.03%–0.11%) but that among populations in which the level of protective antibody is low and meningococcal disease is epidemic, a greater number of healthy than complement-deficient persons will be infected because the number of healthy persons who lack specific antibody is significantly greater than the number of those who are complement deficient. As the level of immunity in the population increases, the incidence of meningococcal disease falls; however, because the prevalence of complement deficiency in the general population is relatively stable, the frequency of infectious states among those individuals with meningococcal disease increases—that is, complement deficiency becomes a proportionately greater determinant of the risk of infection.[211] The best estimate of inherited complement deficiency states among patients with *endemic* neisserial disease (approximately 10 cases per 1 million population; see Fig. 9.3) is about 5% to 10%. However, the likelihood of complement deficiency is increased dramatically (to 31%) among patients who have had more than one episode of meningococcal infection or who have a family history of meningococcal disease.

Although the number of infections caused by *Streptococcus pneumoniae* and *H. influenzae* appears to be increased in patients with

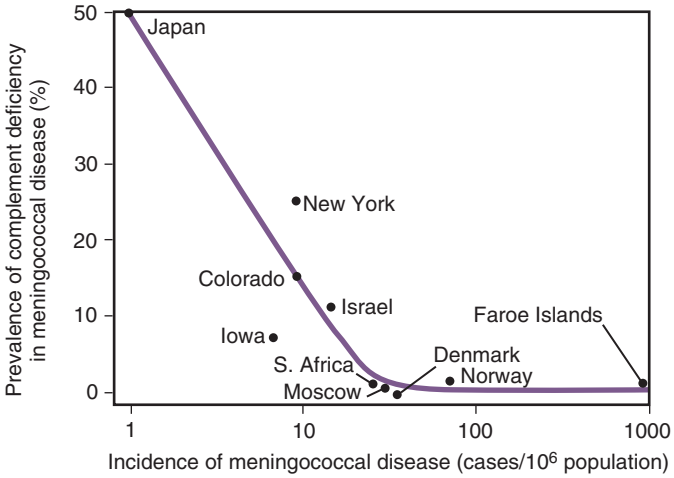

FIG. 9.3 Relationship between the prevalence of complement deficiency and the incidence of meningococcal disease. *(From Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies.* Clin Microbiol Rev. *1991;4:359–395.)*

complement deficiencies, the prevalence of complement deficiency among persons with systemic infection caused by these organisms does not appear to be markedly different from that in the population at large.[214] The basis for this apparent paradox, especially for *H. influenzae,* which shares many features with meningococci, is unknown.

General Aspects of the Molecular Basis for Complement Deficiencies

As with most other inherited conditions, the basis for complement deficiency states exhibits substantial heterogeneity, especially among people who represent different ethnic or racial ancestries. Within a defined ethnic or racial population, the probability that a single molecular defect will predominate is substantially increased. A corollary of this principle is that the same functional deficiency occurring among persons of different backgrounds is likely to exhibit molecular heterogeneity. The latter generalization is useful in the selection of patients whose deficiencies, when characterized at the molecular level, are likely to yield new information. Current studies are focusing increasingly on the contribution of various component polymorphisms to the pathophysiology of different disease states.

Classical-Pathway Deficiencies

Clinical Aspects

Immune Disorders

The association of immune disorders, in particular SLE, with complement deficiency states is most evident in persons lacking C1, C4, C2, or C3 (Table 9.3). Even though these inherited deficiencies are present in a small minority of patients with SLE, their association with this disorder is convincingly underscored by the very high penetrance of the disease in these patients. "The strength of the association of complement deficiency with SLE itself and with the severity of the disease is inversely correlated with the position of the deficient protein in the activation sequence of the classical pathway."[6] Uncertainty exists as to whether both homozygous and heterozygous complement deficiencies, or only the former states, constitute a risk factor for SLE. Early studies that used immunologic and functional assays suggested an association in both homozygous- and heterozygous-deficient persons. More recent studies using DNA typing techniques strongly suggested that, at least in C1q and C2 deficiency, the association exists only for the homozygous state.[213]

Assessment of this issue in patients with partial C4 deficiency is complicated by the existence of separate *C4* genes, *C4A* and *C4B.* As a result, complete C4 deficiency (i.e., the absence of the products of all four C4 genetic loci) is extremely rare. Conversely, heterozygous C4 deficiency is very common, occurring in approximately 25% of the general population.[215,216] In addition, as a result of linkage disequilibrium, the C2 and C4 null genes occur as part of distinct, extended haplotypes. These haplotypes include MHC I, MHC II, complement, and TNF genes, all of which are candidate disease susceptibility genes. The specific haplotype associated with C2 deficiency occurs in about 93% of affected persons. In the case of C4 deficiency, multivariate analysis of HLA-DR and C4 genotypes has confirmed an independent contribution of C4AQ0 and DR alleles to the development of SLE. The C4B null gene *(C4BQ0)* was not associated with SLE.[217–219] The chemical preference of the internal thioester in C4A to form amide bonds and to react with immune complexes may contribute to the effect that the C4A null gene has on the development of SLE.[31]

Pathophysiologic roles for complement in systemic lupus erythematosus. The pathophysiologic basis for the association between these classical-pathway deficiencies and SLE is incompletely understood, but our emerging understanding of the role of complement in the clearance of apoptotic cells (see earlier discussion) has provided additional insight and an increasingly compelling logic for this association.[138,139] Despite the significant insights afforded by studies of these individuals and of genetically engineered complement-deficient mice, it remains clear that predisposition to SLE is genetically multifactorial.[220]

The key clinical features of SLE in complement-deficient persons include early age at onset (median, 7 years), increased severity (often with impressive photosensitivity), central nervous system involvement, and increased frequency of glomerulonephritis.[221] A striking hierarchy of decreasing penetrance of SLE is seen among classical-pathway deficiencies: 93%, 57%, 57%, 75%, and 10% for C1q, C1r, C1s, C4, and C2 deficiencies, respectively. This hierarchic relationship is also generally evident in the severity of SLE and in the frequency and type of autoantibodies present in the sera of these patients. SLE is more severe in patients with C1q, C1r, C1s, or C4 deficiency.[219,221–223] In persons with C2 deficiency, the severity of SLE appears to be similar to that in the general population, and in C3-deficient persons SLE may be less severe.[214,219,223]

Additional evidence of the importance of complement deficiency as a predisposing factor for SLE comes from studies of the occurrence of SLE in some patients with acquired hypocomplementemia. Among these findings, C1-INH deficiency is especially interesting because the absence of this regulatory protein is associated with uncontrolled consumption of the classical-pathway complement components, which is particularly severe and prolonged in a small number of patients. It appears that it is these patients who are at risk for development of SLE.[221]

The unique functional roles played by the classical pathway, impairment of which seems to play a role in the development of SLE, are elimination of apoptotic cells and clearance of immune complexes.

As noted earlier, membrane blebs on apoptotic cells express phosphatidylserine, annexin 2, and annexin 5, to which C1q can bind, thereby activating the classical pathway and promoting opsonophagocytic elimination by complement receptors (e.g., through cC1qR and its signaling partner, CD91) on macrophages and mononuclear cells. Engulfment of apoptotic cells via these receptors appears not to initiate a prominent inflammatory response, in part because apoptotic cells also bind to complement inhibitors such as C4BP and factor H.[138,139] Sunlight is very effective in inducing apoptosis of dermal cells,[26] an observation that probably helps explain photosensitivity in SLE, and the positive lupus band that is demonstrated in skin biopsy specimens of people with SLE, regardless of disease activity. The inability of macrophages to clear apoptotic cells effectively is associated with an increased display of apoptotic cell debris on the surfaces of DCs, where it may be presented to autoreactive lymphocytes as an early step in the development of autoantibodies and autoreactive T cells. Particularly intriguing is the observation that membrane blebs on apoptotic cells contain the very macromolecular complexes of ribonucleic proteins to which SLE autoantibodies are directed and that the relevant epitopes on these complexes become exposed during incorporation into the blebs.[6,138,139]

Approximately one-third of all patients with SLE develop autoantibodies to C1q that lead to secondary C1q deficiency. In contrast to primary C1q deficiency in which the deficiency is strongly linked to the development of SLE, secondary deficiency in SLE is a result of the disease

TABLE 9.3 Complement Deficiency States

COMPONENT	NO. OF REPORTED PATIENTS (APPROXIMATE)	MODE OF INHERITANCE	FUNCTIONAL DEFECTS	DISEASE ASSOCIATIONS
Classical Pathway				
C1qrs C4	40 26	ACD ACD	Impaired immune complex handling, delayed C′ activation, impaired immune response	CVD, 48%; infection (encapsulated bacteria), 22%; both, 18%; healthy, 12%
C2	100	ACD		
Mannose-Binding Lectin Pathway				
MBL	Many	ACD	Impaired complement activation	Pyogenic infection; meningococcal disease
MASP-2	9	ACD	Defective association with MBL	Ulcerative colitis, CVD, pneumococcal disease
Ficolin-3	4		Impaired activation of the lectin pathway	Recurrent lower respiratory infections (*Haemophilus influenzae* and *Pseudomonas aeruginosa*), recurrent digital warts, bilateral cerebral abscess (nonhemolytic streptococcus)
Collectin-11 MASP-1	10 9		Impaired activation of the lectin pathway Impaired activation of the lectin pathway	3MC syndrome (craniofacial-ulnar-renal syndrome); facial dysmorphism, cleft lip/palate, craniosynostosis, learning disability; genital, limb, and vesicorenal anomalies
Alternative Pathway				
D P	4 70	ACD XL	Impaired C′ activation in absence of specific antibody	Infection (meningococcal), 74%; healthy, 26%
Junction of Classical and Alternative Pathways				
C3	19	ACD	Impaired immune complex handling, opsonophagocytosis; impaired granulocytosis, chemotaxis, immune response, and absent SBA	CVD, 79%; recurrent infection (encapsulated bacteria), 71%
Terminal Components				
C5 C6 C7 C8	40 80 80 75	ACD ACD ACD ACD	Impaired chemotaxis; absent SBA Absent SBA Absent SBA Absent SBA	Infection (*Neisseria,* primarily meningococcal), 58%; CVD, 4%; both, 1%; healthy, 25%
C9	165	ACD	Impaired SBA	Healthy, 91%; infection, 9%
Plasma Proteins Regulating C′ Activation				
C1-INH	Many	AD; Acq	Uncontrolled generation of an inflammatory mediator on C′ activation	Hereditary angioedema
Factor H	25	ACD	Uncontrolled alternative-pathway activation→low C3	CVD, 40%; CVD plus infection (encapsulated bacteria), 40%; MPGN; healthy, 20%
Factor I	30	ACD	Uncontrolled alternative-pathway activation→low C3	Infection (encapsulated bacteria), 100%
Membrane Proteins Regulating C′ Activation				
Membrane cofactor protein (CD46)	9	ACD/AD	Uncontrolled alternative-pathway amplification on host cells	aHUS
Decay-accelerating factor (CD55) Homologous restriction factor (CD59)	Many	Acq	Impaired regulation of C3b and C8 deposited on host red blood cells, polymorphonuclear neutrophils, platelets→cell lysis	Paroxysmal nocturnal hemoglobinuria
CR3	>20	ACD	Impaired PMN adhesive functions (i.e., margination), chemotaxis, iC3b-mediated opsonophagocytosis	Infection (*Staphylococcus aureus, Pseudomonas* spp.), 100%

TABLE 9.3 Complement Deficiency States—cont'd

COMPONENT	NO. OF REPORTED PATIENTS (APPROXIMATE)	MODE OF INHERITANCE	FUNCTIONAL DEFECTS	DISEASE ASSOCIATIONS
Autoantibodies				
C3 nephritic factor	>59	Acq	Stabilizes alternative-pathway C3 convertase→low C3	MPGN, 41%; PLD, 25%; infection (encapsulated bacteria), 16%; MPGN plus PLD, 10%; PLD plus infection, 5%; MPGN plus PLD plus infection, 3%; MPGN plus infection, 2%
C4 nephritic factor		Acq	Stabilizes classical-pathway C3 convertase→low C3	Glomerulonephritis, 50%; CVD, 50%

ACD, Autosomal codominant; *Acq,* acquired; *AD,* autosomal dominant; *aHUS,* atypical hemolytic-uremic syndrome; *C',* complement; *C1-INH,* C1 inhibitor; *CVD,* collagen vascular disease; *iC3b,* inactive C3b; *MASP,* mannose-binding lectin–associated serine protease; *MBL,* mannose-binding lectin; *MPGN,* membranoproliferative glomerulonephritis; *PLD,* partial lipodystrophy; *PMN,* polymorphonuclear neutrophils; *SBA,* serum bactericidal activity; *XL,* X-linked.
Data from Ross SC, Densen P. Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. Medicine (Baltimore). *1984;63:243–273; and Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies.* Clin Microbiol Rev. *1991;4:359–395.*

process itself. Nonetheless, autoantibody to C1q impairs complement consumption via the classical pathway, potentially leading to further impairment in the clearance of apoptotic cells, which in turn may reinforce the SLE disease process.[223]

Patients with high concentrations of antibody to C1q may also develop hypocomplementemic urticarial vasculitis. Clinically, these patients are often young females who present with chronic urticaria and associated leukocytoclastic vasculitis. Although called urticaria, the rash is nonpruritic and persistent. They may also develop angioedema, airway obstruction, glomerulonephritis, arthralgia, and neuropathy. Their laboratory findings are characterized by profound hypocomplementemia (as measured by total hemolytic complement [CH_{50}]) and marked depression of C1q, C4, and C2 concentrations, and a moderate reduction in C3.[223] Normal levels of C1 inhibitor distinguish this disease from hereditary angioedema. These individuals characteristically lack serologic markers for SLE, such as antinuclear antibodies or anti–double-stranded DNA (dsDNA) antibodies. Histologically, the skin lesions show perivasculitis or a leukocytoclastic vasculitis.

Approximately one-third of patients with SLE develop antibodies to phospholipids, again presumably as a result of the exposure of these molecules in apoptotic cell blebs. These patients may present with recurrent thrombotic events or spontaneous abortion—the clinical hallmarks of the antiphospholipid syndrome. Fetal wastage in these patients has been shown to be causally related to both placental infarction and massive complement consumption in the fetus. The factors that determine which patients with SLE develop which autoantibodies (e.g., anti-C1q, antiphospholipids) are unknown.[223,224]

In addition to altered apoptotic cell elimination, complement-deficient patients with SLE display several distinct abnormalities in immune complex clearance that have been documented by elegant in vivo experiments.[225–228] These abnormalities include (1) increased size of immune complexes, consistent with the key role of C1q in inhibiting complex formation (see earlier discussion); (2) decreased numbers of CR1 on erythrocytes, consistent with their increased removal in association with immune complexes during passage through the spleen[229]; (3) an increased proportion of immune complexes circulating unbound in plasma rather than bound to red blood cells, consistent with both less C3 bound to the complexes and fewer CR1 on erythrocytes; and (4) altered rate and pattern of immune complex clearance by the liver and spleen—a greater number of complexes being removed more rapidly by the liver, with a portion of these being released back into the circulation after a short delay.[131,132] The altered clearance reflects the greater proportion of complexes circulating in an unbound state and their uptake by IgG receptors on hepatic macrophages. A portion of these receptors exhibit relatively low affinity for IgG and release the complexes over time. In contrast, splenic removal of immune complexes is almost totally C3 and CR1 dependent. Each of these abnormalities, with the exception of CR1 number, is reversed by replacement of the missing complement component,[230] and reduction in the number of circulating immune complexes as a consequence of effective treatment for SLE is associated with an increase in the number of CR1 on circulating erythrocytes. Studies in genetically engineered mice suggest that tissue injury reflects a greater role for events consequent to the engagement of immune globulin receptors and also for the phlogistic activities of complement anaphylatoxins, especially C5a.[223]

Infectious Diseases

The relatively low frequency of infection (20%) in persons with a deficiency of C1, C4, or C2, compared with other component deficiencies (see Table 9.3), has been attributed to the presence of an intact alternative pathway in these patients. Bacterial infection, when it occurs, is usually caused by encapsulated bacteria, especially *S. pneumoniae,* and may be recurrent. The most common sites of infection are the sinopulmonary tree, meninges, and blood.[208,209]

Molecular Aspects

C1q is the product of three separate genes (*A, B,* and *C;* see Table 9.1). Mutations in each of these genes have been associated with C1q deficiency. A predominant mutation has not yet emerged from the few patients whose defect has been characterized, nor is there any apparent difference in the clinical picture found among persons with these various defects.[231]

C2 deficiency is probably the most common of all the total complement component deficiencies. It occurs predominantly in white persons of northern European extraction and is inherited in association with a distinct haplotype. The molecular basis for this defect in more than 90% of the cases (type I C2 deficiency) is a 28–base-pair gene deletion that causes skipping of exon 6 during messenger RNA (mRNA) splicing. Exon skipping in turn results in the generation of a premature stop codon and the synthesis of a nonfunctional protein.[232,233] The remainder of cases (type II C2 deficiency) result from a point mutation that encodes a dysfunctional polypeptide that is retained intracellularly.

The predominant molecular basis for C4A and C4B deficiencies involves large deletions that encompass both the respective C4 and associated 21-hydroxylase genes.[217] A 2–base-pair insertion in exon 29 of the *C4A* gene has been reported in association with the haplotype HLA-B60 DR6.[234]

Lectin-Pathway Deficiencies

Deficiency of Mannose-Binding Protein

Clinical Aspects

In 1976, a group of children with recurrent infection and failure to thrive were described; their serum failed to opsonize *Saccharomyces cerevisiae.* This defect was subsequently found in 5% to 7% of the general population. In 1989, MBL deficiency was identified in a substantial proportion of these patients.[235] Complete deficiency of MBL has not been reported; genetic variations that result in low protein levels (see later) are reported as MBL deficiency in the literature. Although the association with infection has been best documented in children, it spans the entire age range and has been confirmed in multiple ethnic

populations. One study that used genetic techniques found MBL deficiency in 42% of 345 children admitted with infection to one hospital, compared with 24% of 272 children admitted to the same hospital for other reasons. The prevalence of an abnormal *MBL* gene among infected children was almost twice that among uninfected children (23% vs. 13%), and homozygous deficiency was strikingly prevalent (3%) in the entire group of hospitalized children. In this study, affected patients with MBL deficiency presented with a wide variety of infections caused by a broad range of organisms. Common diagnoses included sinopulmonary infection (31%), meningococcal disease (13%), and fever of unknown origin (10%). The basis for the incomplete penetrance of infection among affected individuals with MBL deficiency is unknown but most likely reflects polymorphisms in other genes, in addition to environmental effects.[236,237]

Molecular Aspects

MBL is encoded by a single gene that contains four exons. The first exon encodes the signal peptide and the collagen-like region, the second exon encodes the remainder of the collagen region, the third encodes the "neck" region, and the fourth encodes the carbohydrate recognition domain. The three known MBL deficiencies are caused by mutations clustered in the first exon. Each of these point mutations results in an amino-acid substitution that interferes with oligomerization of three single chains to form the mature protein, and each is associated with reduced serum concentrations of MBL.[6,36]

In addition to mutations in the coding portion of the gene, three polymorphic sites are found in the promoter region of the gene: H/L, X/Y, and P/Q. Four of the total possible polymorphic combinations (LXP, LYP, LYQ, and HYP) account for most of the observed promoter haplotypes. These polymorphisms affect transcription of the gene through alterations in the binding of transcriptional factors, with LXP being associated with the lowest MBL serum concentration and HYP with the highest. These polymorphisms also exist in linkage disequilibrium with the three structural mutations in exon 1 to create haplotypes that differ markedly in their frequency among various populations. Therefore, MBL serum concentrations reflect the aggregate effects of promoter polymorphisms, structural gene mutations, and interaction between these two factors. Low MBL concentrations are associated with an increased risk of pyogenic infection, as noted earlier; however, they may protect against mycobacterial infection. Conversely, high MBL serum concentrations may increase the risk of mycobacterial infection.[6,36]

Deficiency of Ficolin-3

A case of complete ficolin-3 was reported in a 32-year-old man with a history of repeated lower respiratory tract infections resulting in bronchiectasis, recurrent digital warts, and bilateral frontal cerebral abscesses caused by nonhemolytic streptococci. The defect was a deletion at position 1637 of the *FCN3* gene (*FCN3+1637delC*) that resulted in a missense mutation. About 1.1% of healthy whites are heterozygous for this mutation in *FCN3* and do not appear to be at an increased risk of infections.[238] Ficolin-3 deficiency appears to be associated with necrotizing enterocolitis (NEC) among preterm infants.[239] A case of total ficolin-3 deficiency was reported in a preterm infant with group B streptococcal infection.[240]

Deficiencies of Collectin 11 (CL-K1) and MASP-1

Clinical Aspects

Deficiencies of both proteins are considered together because of their association with the 3MC syndrome, a term used to unify four overlapping rare autosomal recessive disorders: Mingarelli, Malpuech, Michels, and Carnevale syndromes. The 3MC syndrome is characterized by developmental abnormalities, including characteristic facial dysmorphism (high-arched eyebrows, ptosis, asymmetrical skull as a result of cranial synostosis, cleft lip and/or palate, and downturned mouth); learning disability; and genital, limb, and vesicorenal anomalies. Elegant gene-knockdown studies in zebrafish embryos showed that collectin 11 and MASP-1 both served to guide migration of neural crest cells during development.[241] The reported cases of 3MC syndrome have not been associated with an increased risk of infections. The serum of a 9-year-old girl with a nonsense mutation in *MASP1* and with clinical features consistent with the 3MC syndrome showed a nonfunctional lectin pathway; an episode of severe urinary tract infection was the only infection reported in this patient.[37] In contrast to this MASP-1–deficient individual who had a functional alternative pathway, *Masp1* knockout mice could not cleave pro–factor D to factor D and therefore lacked alternative-pathway function.[242] The reasons for the apparent differences in the outcomes of MASP-1 deficiency in mice and humans are unclear but could be the result of additional or alternative mechanisms of pro–factor D cleavage in humans.

Molecular Aspects

All individuals with clinical features of the 3MC syndrome have homozygous mutations of either *COLEC11* or *MASP1*. *COLEC11* mutations include deletions of exons 1 through 3, frameshift mutations in exon 2 (encodes the N-terminal domain) or exon 6 (neck domain), or missense mutations in exon 8 (carbohydrate recognition domain). The *MASP1* defects were all missense mutations in regions encoding for the serine protease domain. One family harbored a missense mutation in a *MASP1* exon that uniquely splices to *MASP3* (2059G→A, leading to 687G→R).

Deficiency of Mannose-Binding Protein–Associated Serine Protease 2 (MASP-2)

A single patient with MASP-2 deficiency has been described. This patient manifested ulcerative colitis at age 13, erythema multiforme bullosum at age 29, and three episodes of severe pneumococcal infection at 28 to 30 years of age. Severe hypocomplementemia and antibodies to C1q were documented at 35 years of age, along with low concentrations of MASP-2 and defective association between MBL and MASP-2. MASP deficiency in this patient was shown to be caused by a point mutation that resulted in the replacement of an aspartic acid residue with glycine. Recombinant MASP-2 protein bearing this amino-acid substitution displayed altered binding to MBL that mirrored what was observed in the patient's serum.[243]

Alternative-Pathway Deficiencies

Clinical Aspects

Inherited deficiencies of components of the alternative pathway appear to be less common than those of other complement proteins. To date, no individuals with homozygous factor B deficiency have been identified (see Table 9.3). In the presence of specific antibody, persons with alternative-pathway defects activate the classical and lectin pathways normally, but in the absence of specific antibody, a defect in the alternative pathway can lead to a significant impairment in complement activation and serum bactericidal activity. Infection in such persons might be expected to have dire consequences, a prediction supported by the poor outcome in patients with properdin deficiency, three quarters of whom develop meningococcal infection. Such infection is frequently characterized by a fulminant course and a high mortality rate (Table 9.4). Similarly, factor D deficiency is also associated with severe invasive meningococcal infection.[244,245]

Molecular Aspects

Three properdin-deficient variants have been described: type 1, characterized by extremely low concentrations (<0.1 μg/mL) of properdin and absent properdin function[246,247]; type 2, characterized by a low concentration (approximately 2 μg/mL) of antigenically detectable but functionally altered properdin; and type 3, characterized by a normal concentration (approximately 25 μg/mL) of antigenically detectable properdin but absent function.[248–250] Type 1 deficiency stems from various mutations, all of which result in premature stop-codon formation and the production of truncated proteins, which are presumably neither functional nor secreted.[251] Type 2 deficiency arises from mutations leading to amino-acid substitutions that may affect molecular charge. The altered molecules are secreted normally but appear to have an accelerated rate of extracellular catabolism.[251] Type 3 deficiency results from an amino-acid substitution that affects neither the secretion of the molecule nor its extracellular catabolism. Instead, folding of the molecule seems to be altered, and

TABLE 9.4 Comparison of Meningococcal Disease in Persons Deficient Versus Nondeficient in Late Complement Components or Properdin

CHARACTERISTICS	NONDEFICIENT	DEFICIENT IN C5, C6, C7, OR C8	DEFICIENT IN C9	PROPERDIN DEFICIENT[a]
No. of homozygotes	—	250	165	54–70
No. with meningococcal disease	—	146	15	25–37
Frequency of infection (%)	0.0072	58	9.1	46–53
Median age at first episode (yr)	3	17	16	14–11.5
Recurrence rate (%)	0.34	44	0	2–1.4
Relapse rate (%)	0.6	7.9	0	0
Mortality per 100 episodes (%)	19	1.5	0	12–51.4
Infecting serogroup				
No. of isolates	3184	67	2	16
B (%)	50	19.4	50	18.7
Y (%)	4.4	32.8	0	37.5

[a]If a range is given, the first number refers to documented cases, and the second number refers to documented plus probable and possible cases.
From Densen P. Human complement deficiency states and infection. In: Whaley K, Loos M, Weiler JM, eds. Complement in Health and Disease. *Dordrecht, The Netherlands: Kluwer Academic Publishers; 1993:173–197.*

this appears to lead indirectly to a decreased affinity of properdin for C3b.[252]

Mutations in *CFD* (gene encoding factor D) include (1) mutation of the codon for Ser42 that resulted in a premature stop codon in a Dutch family[245] and (2) two simultaneous homozygous mutations (Val213Gly and Cys214Arg) in a Turkish family, which resulted in undetectable factor D levels.[253]

C3 Deficiency

Clinical Aspects

Primary C3 deficiency is uncommon (see Table 9.3). As might be expected from the central position of C3 in the complement cascade and its key functions, virtually all persons with this defect are seriously ill.[208,209] Approximately three-quarters develop SLE or a related rheumatologic syndrome. Moreover, the inability to use either the classical or the alternative pathway results in a multitude of severe defects in host defense, including impairments in opsonization, immune response, and neutrophil chemotaxis and an inability to generate serum bactericidal activity. Consequently, severe and recurrent pneumococcal, *H. influenzae,* and meningococcal infections involving the sinopulmonary tree, meninges, and bloodstream are common, occurring in about 70% of such patients.[208,209]

Secondary C3 deficiency is observed in patients with an inherited absence of either factor H or factor I because of unregulated alternative-pathway activation leading to C3 consumption and low serum C3 concentrations (<10%). Autoantibodies to C3 (C3 nephritic factor, or C3 nef) or to factor H also lead to low C3 concentrations via a similar mechanism. These patients exhibit varying degrees of susceptibility to infection, especially to meningococcal disease, as a consequence of impaired host defense mechanisms. In addition, some patients with C3 nef develop partial lipodystrophy, a disorder characterized by the gradual onset of bilateral and symmetrical loss of subcutaneous fat from the face, neck, upper extremities, thorax, and abdomen, in a "cephalocaudal" sequence, and which spares the lower extremities (see Table 9.3).[254] C3 nef is seen in more than 80% of cases of partial lipodystrophy.[255] Complement activation may target adipose tissue because fat cells are a major reservoir of factor D (also known as adipsin), the activating enzyme of the alternative pathway. It is worth noting that about 30% of cases of partial lipodystrophy are associated with membranoproliferative glomerulonephritis type II (MPGN II) (discussed later), further supporting a common link in their pathogenesis.

Molecular Aspects

Primary C3 deficiency is uncommon, but cases have been recognized in ethnically diverse populations. The molecular basis for this deficiency arises from two different splicing defects: one causing a substantial deletion and the other a single base-pair change that leads to a defect in C3 secretion.[256]

Late Complement Component Deficiencies

Clinical Aspects

People who have a deficiency of one of the terminal complement components exhibit a striking susceptibility to systemic neisserial infections, especially meningococcal disease. Despite the chemotactic defect associated with C5 deficiency, the clinical manifestations of meningococcal disease in people with this defect and in those with other terminal-component deficiencies are remarkably similar.[211] Therefore the basis for the occurrence of meningococcal disease in these persons appears to be their inability to assemble the MAC and express complement-dependent bactericidal activity.

This conclusion was supported by detailed population-based epidemiologic studies that demonstrated an approximate 5000-fold increased risk of meningococcal disease in C7-deficient compared with complement-sufficient Japanese patients. In contrast, C9-deficient Japanese patients experienced an approximate 700-fold increase in risk.[257] The increased risk of meningococcal disease in persons deficient in C5, C6, C7, or C8, compared with those deficient in C9, is consistent with in vitro studies demonstrating that C9-deficient sera can kill meningococci, albeit at a slower rate.[113] This dose-response relationship, coupled with the fact that the structural genes that encode these proteins are located on multiple chromosomes, provides strong evidence for a cause-and-effect relationship between the absence of complement-dependent bactericidal activity and the increased susceptibility of these patients to meningococcal disease.

Meningococcal Disease in Complement Deficiency

Meningococcal disease is the single most common infection sustained by persons with complement deficiency, accounting for 75% to 85% of etiologically identified infections.[208,209] Although meningococcal disease has been reported in people with a deficiency of any of the plasma complement proteins, it is most common in those with a deficiency of properdin, C5, C6, C7, or C8, of whom 50% to 60% experience at least one episode of infection during their lifetime. This striking association confirms the importance of the complement system in host defense against meningococci.

Meningococcal disease in patients with these complement deficiencies exhibits several unique features that help to distinguish it from that in complement-sufficient persons (see Table 9.4). These features provide important clinical clues that should suggest the underlying presence of a deficiency and the need to screen for a complement deficiency state. These features are unlikely to result solely from ascertainment bias

for the following reasons. First, they have been confirmed in multiple studies in varied populations around the world. Second, each feature has been borne out by investigations of complement-deficient families after exclusion of the proband from the analysis. Third, at least in the case of the late complement component deficiencies, family studies have failed to reveal undiagnosed infection or unexplained or premature death.

Data compiled from the literature and from a detailed population-based study suggest that these complement deficiency states increase the risk of meningococcal disease 5000- to 10,000-fold. In the general population, the median age at onset of meningococcal infection is 3 years, and 56% of infections occur before 5 years of age; in contrast, the median age of first infection in complement-deficient patients is 17 years, and only 10% of infections occur before 5 years of age. Therefore, most deficient persons pass through the age of life when the deficiency might be expected to increase maximally their susceptibility to meningococcal disease without evidence of that susceptibility. The basis for this observation is unknown, but it likely relates to the relatively greater importance of specific antibody in protection against this infection. As specific antibody develops during childhood as a result of asymptomatic carriage, the contribution of complement deficiency states as a risk factor for systemic meningococcal disease increases relative to the contribution of antibody. Consequently, more cases of complement deficiency are detected among older individuals with meningococcal disease.

Meningococcal disease in these patients is caused by uncommon serogroups—particularly groups Y, W135, and X—more often than in persons without complement deficiencies.[208,209,258] Likewise, the prevalence of complement deficiencies is increased among patients with meningococcal disease caused by these serogroups.[259] The physiologic basis for this observation is not known with certainty, but factors that may be important include (1) the critical requirement for anticapsular antibody for prevention of disease in deficient compared with nondeficient persons, (2) better elimination of group B isolates by phagocytic cells in the absence of capsular antibody,[260] and (3) the propensity for uncommon meningococcal serogroup organisms to cause disease in older persons.[261]

Recurrent meningococcal disease, defined as a new infection that occurs more than 1 month after a previous episode, occurs in about 40% to 45% of persons deficient in C5, C6, C7, or C8. This recurrence rate is approximately 100 to 150 times greater than that in the general population. Results of a statistical analysis of the number of patients with a specified number of episodes of meningococcal disease were consistent with the interpretation that the risk of meningococcal disease in complement-deficient persons is independent of previous infection.[209] Previous disease did not reduce the risk of subsequent meningococcal infection in these patients; the estimated probability of each infection was 0.39.[209] A similar analysis, using a slightly different statistical approach, led to an identical conclusion and produced an estimated probability of infection of 0.6.[262,263] The latter analysis also demonstrated that the interval between infections (4–5 years) did not differ, again suggesting that the risk of subsequent meningococcal infection was not reduced by previous episodes of disease. The explanation for the failure of previous infection to reduce the risk of subsequent episodes of meningococcal disease in these complement-deficient persons appears to lie in their critical dependence on capsular antibodies for protection and the fact that infection constitutes a relatively poor stimulus for production of these antibodies. These antibodies are highly efficient in promoting opsonophagocytic elimination of meningococci. In contrast, antibodies to subcapsular antigens, although bactericidal and protective in the nondeficient host, are relatively poor opsonins and afford little protection in complement-deficient patients who lack the effector proteins necessary for the expression of bactericidal activity.[264]

Relapse of meningococcal disease, defined as infection with the same serogroup occurring less than 1 month after the initial infection, is observed in 7.6% of patients with meningococcal disease and who are deficient in C5 through C8, as reported in the literature. This frequency, which is approximately 10 times greater than in the general population, suggests that meningococci may be sequestered intracellularly, where they are relatively protected from antibiotics.[209]

One of the most striking aspects of meningococcal disease in persons with late complement component deficiencies is that, despite a several thousand–fold increase in the risk of infection, they experience a 5- to 10-fold reduction in the chance of dying from the disease, compared with healthy persons.[209] Therefore the same defect that predisposes to infection appears to provide protection from the lethal consequences of the disease. This remarkable observation suggests that the host's exuberant response to the organism is as much responsible for the clinical manifestations and outcome as is the organism itself. This deduction is supported by the report of Brandtzaeg and coworkers[265] of a close correlation between the extent of complement activation and mortality in meningococcal disease and suggests that the latter is in part dependent on the assembly of the MAC.

The basis for the lower mortality rate of meningococcal disease in persons who are deficient in late complement components is unknown, but variables that may be relevant include milder disease,[262] the possibility that fewer organisms are required to initiate infection, and the ability to better tolerate a given endotoxin load with less host cell injury.

The possibility that fewer organisms may be required to establish systemic meningococcal disease in deficient than in nondeficient persons is attractive, but data that address this point are conspicuously absent in the literature. Such an effect would account for the increased number of infections and also for the milder disease and the decreased case-fatality rate, in that mortality is directly related to the number of organisms in the bloodstream.[266] A reduction in organism load might translate into a lower concentration of circulating endotoxin and less systemic inflammation. Alternatively, because insertion of the MAC into the outer membrane of gram-negative organisms results in release of free endotoxin, the inability of persons with late complement component deficiencies to assemble the MAC may be associated with a reduction in the quantity of circulating endotoxin for a given load of organisms. This reduction, in turn, might lessen ongoing complement activation and decrease secretion of various cytokines linked to the development of septic shock in meningococcal disease.[265,267,268] Finally, insertion of the MAC into host cell membranes might occur in vivo as a consequence of exuberant complement activation in the vicinity of innocent bystander cells or as a consequence of endotoxin binding to these cells and subsequent complement activation on their surfaces. Such an event might stimulate adverse cellular responses. For example, MAC insertion activates leukocytes, stimulating release of a plethora of potentially noxious mediators[269] and increased expression of procoagulant molecules on endothelial cells.[270] Interruption of these processes in the patient with a late complement component deficiency would result in an improved ability to tolerate a given load of organisms and endotoxin.

Molecular Aspects

The basis for C5 deficiency is molecularly heterogeneous, with multiple but different defects in whites and African Americans. In one study, all the African-American patients were compound heterozygotes who possessed nonsense mutations in exons 1 and 36.[271] Of 109 patients diagnosed with meningococcal disease in the Western Cape region of South Africa, 3 (2.8%) were homozygous for the A252T mutation in the *C5* gene and displayed very low (1%–2% of normal) circulating C5 levels; all three individuals were of native African descent.[272]

Worldwide, complete C6 deficiency is molecularly diffuse. This deficiency is particularly common among native Africans and individuals of mixed ethnicities from the Western Cape in South Africa.[273] Several distinct types of C6 deficiency have been described, including complete deficiency and two different subtotal deficiencies (SDs), one of which occurs in association with a C7 SD. The structural genes for C6 and C7 are tightly linked on chromosome 5 (see Table 9.1). Combined C6/C7 SD states represent novel combinations of the *C6* SD allele with a *C7* SD allele and various *C6* and *C7* null alleles. These partial deficiencies typically come to clinical attention when some initiating event generates production of the C5b6 complex, which in turn converts the partial deficiency into a total one. Persons in whom the two SD alleles are shared in combination with a *C6* null allele are functionally *C6* SD. Their serum contains a near-normal concentration of C7, despite the presence of the *C7* SD allele, because the markedly reduced C6 activity is insufficient to lead to consumption of the existing C7. Complement consumption converts the subtotal state to complete C6 deficiency. Similarly, persons in whom the *C6* SD allele is paired with two different

C7 SD alleles are functionally C7 deficient, despite the expression, albeit reduced, of functional C7, because the C5b6 complex consumes the C7.[274,275]

Three genes (see Table 9.1) encode the C8 molecule, but defects have been reported in only the *A* and *B* genes. C8β deficiency has been reported predominantly in whites, especially those of Russian descent. Ninety-five percent of all the C8β null alleles characterized to date have been caused by cytosine to thymine (C→T) transitions, including 85% in exon 9 and 10% in other locations. It is unclear why C→T transitions should be such a common underlying mechanism for this deficiency.[276]

C9 deficiency is particularly common in the Japanese population. In this population, a C→T transition in exon 4 that converts an arginine codon to a stop codon is the dominant mutation associated with this deficiency.[277]

Deficiencies of Complement Regulatory Proteins

Hereditary Angioedema: C1 Inhibitor Deficiency

People who lack C1-INH present with a distinctive clinical picture historically referred to as hereditary angioneurotic edema (HAE).[278] The hereditary form of this disease was recognized more than 100 years ago, and an acquired variant was identified more recently as a distinct entity. HAE is an autosomal dominant disorder, but about 20% of newly identified patients lack a positive family history and reflect spontaneous mutations. Type 1 HAE accounts for 75% to 85% of cases and is characterized by the presence of low (5%–30%) levels of normal C1-INH protein arising from the intact allele. Type 2 HAE is characterized by the presence of normal to increased levels of antigenic C1-INH, representing a mixture of functional and dysfunctional gene products.[64,278,279] In a recently described third form of HAE, the serum from affected individuals contains normal amounts of functional C1-INH. Most of these patients have a gain-of-function mutation in the coagulation factor XII (Hageman factor) gene that may lead to enhanced bradykinin generation during contact activation (see later discussion).[279]

The acquired forms of this disorder (AAE) are considerably less common. Historically, two variants have been recognized; one occurs in association with B-lymphocyte disorders, and the other because of the presence of an autoantibody to C1-INH. Studies have suggested that this distinction may be inaccurate and that autoantibodies may account for both types of AAE. Antibody binding does not interfere with cleavage of C1-INH by C1s, but rather prevents the formation of a covalent linkage between the enzyme and the cleaved inhibitor. This alteration effectively converts C1-INH from an inhibitor to a substrate and permits C1s action to continue unchecked. This leads to complement consumption in the fluid phase and associated low levels of C1s, C1r, C4, and C2 that are the hallmark of the disease.[280,281]

Because HAE is inherited as an autosomal dominant trait, the serum from all of the patients contains some normally functioning C1-INH.[64] In contrast, persons with the acquired variants have markedly reduced or absent functional C1-INH activity in their serum. As a consequence of this basic difference, the serum from patients with HAE contains normal amounts of C1 and C1q but reduced levels of C4 and C2, whereas serum from those with AAE contains strikingly reduced amounts of C1, C1q, C4, and C2.[64,139,279]

The health of patients with HAE or AAE is punctuated by attacks of nonpitting, nonpruritic, and nonpainful edema of the extremities, face, or larynx. Angioedema of the larynx is the most severe complication of the disorder and is a common cause of death in these patients. The gastrointestinal tract may also be affected, and such attacks manifest as episodes of acute, crampy abdominal pain frequently associated with nausea, vomiting, and occasionally diarrhea. In HAE, attacks typically begin in childhood, increase in frequency and worsen in severity during adolescence, increase during menstruation, are markedly reduced during pregnancy, and diminish gradually in the fifth and sixth decades of life. A typical attack lasts 2 to 3 days.[64,279]

The mechanism whereby C1-INH deficiency produces the clinical syndrome of angioedema is incompletely understood. Evidence supports a role for both complement-derived and contact system mediators. Intradermal injection of activated C1s leads to nonpainful, nonpruritic swelling in both humans and guinea pigs. This response does not occur if activated C1 is injected into C2-deficient humans or guinea pigs but is observed after injection into C3-deficient patients.[282] These data provide support for a C2-derived anaphylatoxin in the clinical picture of angioedema. However, in addition to its role as the sole inhibitor of C1 esterase, C1-INH is an important inhibitor, if not the major inhibitor, of factor XII and kallikrein. Plasma from patients with HAE exhibits an impaired ability to inactivate these mediators. Detailed studies of a particular family with dysfunctional C1-INH demonstrated that the molecule was defective in its ability to inhibit C1r and C1s but retained full ability to complex and inhibit kallikrein and factor XIIa. None of the 10 family members whose sera possessed this dysfunctional C1-INH had ever experienced an attack of angioedema.[283] These data support an important role for the contact system in the clinical picture of HAE. Together, these separate lines of inquiry suggest that symptoms probably result from the interaction of several factors within these cascade systems.[279]

As expected from the various C1-INH protein phenotypes, the genetic basis for HAE is heterogeneous. A substantial proportion of type 1 HAE defects are associated with mutations within short, interspersed nucleotide elements called *Alu* clusters. These mutations cause a variety of rearrangements that lead to deletions or duplications within the gene, impaired transcription, and reduced levels of specific mRNA and plasma concentrations of C1-INH. In contrast, type 2 HAE, which is associated with normal concentrations of a dysfunctional protein, is typically caused by point mutations. These mutations usually affect the arginine at the reactive center of the molecule or amino acids in its immediate vicinity. On occasion, mutations affect C1-INH glycosylation. In all instances, the mutation leads to synthesis of a protein with an altered ability to react with its substrates. The resulting altered catabolism is responsible for the normal or elevated concentration of plasma C1-INH.[284,285]

The treatment of HAE, especially of acute life-threatening attacks, is being revolutionized by the availability of purified C1-INH, which, in controlled trials, has initiated the resolution of attacks within 30 to 60 minutes after intravenous administration. Purified C1-INH has also been used prophylactically to successfully reduce the probability of an attack in deficient patients undergoing medical procedures likely to precipitate an acute attack of HAE. Because patients with HAE synthesize some normal C1-INH (see earlier discussion), they do not develop antibodies to purified C1-INH after its administration. Use of purified C1-INH has rapidly replaced the administration of fresh-frozen plasma for treatment of and prophylaxis against acute attacks of HAE; however, it has not yet been approved for use in the United States. Administration of purified C1-INH as long-term therapy for patients with HAE has not yet been reported. Treatment with impeded androgens, despite their significant side effects, to increase the biosynthesis of C1-INH continues to be the mainstay of long-term management of the hereditary form of this disease.[64,278,279,286]

Factor H Deficiency

Factor H is the most abundant complement control protein and the third most abundant component overall (see Table 9.1).[161] As described earlier, factor H is a multifunctional, multiligand-binding protein whose binding specificity is determined by SCRs. SCRs 1 through 4 predominantly mediate binding to fluid-phase C3b and regulation of the alternative-pathway C3 convertase. SCRs 16 through 20 mediate binding to polyanions and C3b on cell surfaces and regulate convertase activity at those sites. Although these properties were determined experimentally, the delineation of their relevance to the pathophysiology of MPGN II, hemolytic-uremic syndrome (HUS), and AMD has underscored the critical importance of controlling C3 convertase formation, both in the fluid phase and on host cells, and the crucial role that factor H plays in this process. Strikingly different clinical consequences result depending on whether factor H is missing (quantitative defect: MPGN II) or exhibits altered function (qualitative defects: atypical HUS [aHUS], AMD).[70–72] The association of different disease states with abnormalities in individual segments of the factor H molecule provides convincing proof of the principle that specific domains within the molecule differentially contribute to its overall function.

Clinical Aspects: Membranoproliferative Glomerulonephritis Type II

Quantitative factor H deficiency is an autosomal recessive disorder characterized by low or absent factor H concentrations and low concentrations of C3. As described earlier, the secondary consumption of C3 that occurs in these individuals is associated with increased risk of infection. A number of these patients also develop MPGN II at an early age. This disorder is characterized by the deposition of substantial quantities of C3 within the glomerular basement membrane, associated membrane thickening, and impaired function. Because MPGN II is not a feature of patients with primary C3 deficiency, C3 deposition appears to be a critical pathologic event. The importance of C3 deposition in this pathophysiologic process is supported by the development of histologically typical MPGN II in factor H–deficient pigs and its prevention by infusion of factor H.[287,288] Most of these patients have mutations that result in truncated factor H molecules, the secretion of which is severely impaired.[72]

Clinical Aspects: Atypical Hemolytic-Uremic Syndrome

HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Most cases occur in young people who present with fever and Shiga toxin–positive diarrhea caused by *E. coli* O157:H7 or other Shiga toxin–producing strains, for instance, O104:H4. A small percentage of HUS cases occur in patients without diarrhea (aHUS), and some of these exhibit familial clustering consistent with an autosomal dominant pattern of inheritance.[72] Investigation of these patients has led to the identification of persons who have normal levels of complement and factor H but whose factor H bears mutations that affect its function. A striking majority of these mutations are clustered in SCR 20, which is exposed in the native molecule and mediates binding to cell-surface polyanions and C3b. Factor H from these individuals is fully capable of regulating C3 convertase formation and expressing cofactor activity in the fluid phase, consistent with the functional integrity of SCRs 1 through 4, but it binds poorly to C3b- or heparin-coated surfaces and to cultured endothelial cells. Consequently, factor H from these individuals is unable to regulate C3 convertases on cell surfaces.[70–72,289,290]

A family of SCR-containing proteins that bear varying degrees of homology to factor H, called factor H–related proteins (FHRs), have been identified over the past 2 decades. The genes coding for these proteins, in the order *CFHR3, CFHR1, CFHR4, CFHR2,* and *CFHR5,* are located 3′ to *CFH* (the gene encoding factor H). The FHRs are believed to modulate complement regulation by factor H through their interactions with surface-bound C3 fragments. CFH-CFHR1 (substitution of the three C-terminal SCRs of factor H with those of CFHR1) and CFH-CFHR3 (substitution of factor H domain 20 with CFHR3) hybrid molecules that arise from recombinational events result in loss of complement regulation at cell surfaces and the development of aHUS. Approximately 10% of all cases of aHUS are associated with function-impairing autoantibodies against factor H, and such individuals commonly harbor deletions of *CFHR1* and/or *CFHR3.* The aforementioned are only select examples of the genetic rearrangements and mutations in the CFH/CFHR locus that lead to the development of aHUS. The molecular basis for complement dysregulation that occurs as a result of these complex defects is only beginning to be unraveled.[291]

Further support for the importance of impaired regulation of the alternative-pathway C3 convertase on cell surfaces in the pathogenesis of the aHUS stems from reports of individuals who have mutations in the gene encoding MCP (also called CD46).[292,293] MCP/CD46 is a membrane homologue of factor H. Its expression is particularly robust on glomerular capillary endothelial cells. aHUS has also been reported in a few individuals with a mutation in factor I. Mutations in factor B and C3 have also been reported in aHUS, but in contrast to the loss-of-function mutations in factor H, MCP, and factor I, these result in gain of function.[70,290]

The renal histopathologic appearance in patients with Shiga toxin–induced HUS is indistinguishable from that in patients with aHUS. From the pathogenetic standpoint, these patients differ only in that the precipitating event is known in "typical" but not in "atypical" HUS. Because only about 15% of patients with Shiga toxin–associated diarrhea develop HUS, these observations suggest that minor variations in the expression levels or functional activity of any of the aforementioned proteins may constitute the substrate for development of HUS in the setting of specific vascular injury (e.g., that induced by Shiga toxin).[132] Renal failure is a significant consequence for patients with factor H–associated MGPN II or HUS. Therapeutic renal transplantation in such individuals is best reserved for those whose pathogenesis of disease is related to membrane complement regulatory proteins. Transplantation corrects the causative defect in such patients. In contrast, in patients whose disease is related to abnormalities in the plasma complement proteins, the defect will persist after transplantation, and renal disease will recur, often rapidly, in the transplanted kidney.

Clinical Aspects: Age-Related Macular Degeneration

Histologic studies and experimental observations have demonstrated that complement is a significant component of the drusen deposits that characterize AMD.[294] Linkage studies have demonstrated a strong association between AMD and polymorphisms in factor H (SCR 7), factor B, and C2.[295–299] For factor H, these studies established either a predisposing (402His) or a protective effect (402Tyr), depending on the specific polymorphism. Together, polymorphisms in factors H and B, and C2, are present in about 75% of patients with AMD, a remarkable association for a complex disease.[300] SCR 7 is an anion-binding site within factor H that exhibits relative binding specificity for heparin, CRP, DNA, and streptococcal M protein.

Factor H 402His showed lower affinity for streptococcal M proteins than factor H bearing Tyr at position 402, which promoted C3b deposition and phagocytosis of group A streptococci. Intriguingly, 402His homozygosity was less frequent in patients with a history of erysipelas or recurrent tonsillitis than in control subjects.[301] As such, it differs from the SCR 16-20 anion-binding site associated with aHUS, which demonstrates relative specificity for GAGs, heparin, and sialic acid.[70,71] Studies in mice have shown that factor H binds to malondialdehyde (MDA), a lipid peroxidation product that accumulates in AMD. Binding of factor H to MDA-modified proteins blocks their uptake by macrophages and reduces proinflammatory responses. In contrast, reduced binding of the 402His (predisposing) polymorphism to MDA-modified proteins results in relatively unimpeded uptake of these proteins by macrophages and a higher inflammatory response, thereby providing a molecular basis for the role of factor H in the pathogenesis of AMD.[302]

CD59 Deficiency: Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon syndrome that typically occurs in adults between the ages of 30 and 50 years. Classically, affected persons present with bouts of intravascular hemolysis that are worse at night and last for several days to weeks. The events precipitating hemolysis are usually inapparent. In contrast, the more common presentation, which occurs in about half of patients, is one of chronic hemolysis. Patients may have back pain, cramping abdominal pain, and headaches. Although the major clinical features of the disease relate to intravascular hemolysis, the full syndrome includes a propensity for venous thrombosis and diminished hematopoiesis. The thrombosis is characteristic but unusual in that it typically involves major intraabdominal and hepatic veins and is often precipitated by surgery.[229]

The basic problem in patients with PNH is an increased susceptibility of their erythrocytes to hemolysis. The peripheral blood of these patients contains varying proportions of three populations of erythrocytes. PNH type 1 red cells are normal, whereas type 2 cells exhibit a 3- to 6-fold increased sensitivity to complement-mediated lysis, and type 3 cells have a 15- to 25-fold increased sensitivity. The severity of the clinical picture correlates best with the proportion of PNH type 3 cells present in the peripheral circulation.[303]

PNH erythrocytes lack more than 20 different surface proteins. The presence of these molecules in normal amounts on endothelial cells in persons with PNH suggests the clonal origin of this disorder within bone marrow precursor cells. The key feature shared by these proteins

is their linkage to the cell membrane through a carboxyl-terminal glycosylphosphatidylinositol (GPI) anchor.[304,305] PNH arises as a result of a defect in the first step of the synthesis of the GPI anchor responsible for linking the missing surface proteins to the cell membrane. This step is catalyzed by an enzyme that transfers activated *N*-acetyl glucose to the phosphatidylinositol acceptor.[305] This enzyme is the product of the *PIGA* gene, and multiple mutations in this gene have been reported in association with PNH. Unlike most defects that affect synthetic pathways, these genetic abnormalities are expressed in a dominant fashion in progeny cells. This unusual characteristic arises because the *PIGA* gene is located on the X chromosome, and the somatic mutations in this gene arise after inactivation of one of the X chromosomes.[304]

Two of the proteins that are missing from the membranes of PNH cells are the complement regulatory proteins DAF and CD59.[306] The inability to regulate complement deposition on PNH cells explains the underlying pathophysiology of this disorder. People who have an inherited defect that affects expression of only DAF on their erythrocyte membranes do not exhibit the PNH phenotype, but those lacking solely CD59 do manifest the phenotype.[307,308] Therefore the absence of the CD59 molecule accounts directly for the increased susceptibility of PNH cells to intravascular hemolysis.

In the absence of CD59, many MACs are inserted into the platelet membrane, causing vesiculation and providing sites at which prothrombinase is generated, with resultant thrombin formation. Free hemoglobin released during hemolysis scavenges and reduces concentrations of nitric oxide (NO). Low NO concentrations, in turn, increase the susceptibility of platelets to complement-mediated activation.[309] These alterations may contribute to the susceptibility of patients with PNH to thrombosis. In addition, the absence of the GPI-linked urokinase receptor from PNH cells may render clots more resistant to dissolution, although neither of these abnormalities explains the propensity for intraabdominal thrombus formation. Similarly, the absence of the GPI-linked FcγRIII receptor from phagocytic cells may contribute to the slightly increased susceptibility of these patients to infection. Studies on the diminished hematopoiesis that occurs in patients with PNH have suggested that PNH cells do not possess a proliferative advantage within the marrow. However, in an abnormal marrow in which normal cells are at a survival disadvantage, PNH cells appear to be resistant to abnormal influences and emerge as the predominant cell type. The absence of a GPI-linked receptor (e.g., for a growth factor) has been postulated as the basis for this effect. Whether the factors that contribute to the development of an abnormal marrow environment are the same as those giving rise to the somatic mutation responsible for PNH is unknown.[304,305]

The development of a humanized monoclonal antibody to C5 (eculizumab) has provided highly specific, effective, and safe therapy for patients with PNH. A randomized, double-blind, placebo-controlled, multicenter trial clearly demonstrated a reduction in the degree of hemolysis and transfusion requirements for treated patients.[310,311] A subsequent report also documented a decrease in the frequency of thrombotic events.[312]

Patients receiving eculizumab have little or no functional complement activity for the first 2 to 3 weeks after administration of each dose. Like individuals with a deficiency of one of the terminal complement components, they demonstrate increased susceptibility to meningococcal infection (see earlier discussion). Therefore patients with PNH should receive a conjugate, tetravalent meningococcal vaccine several weeks before administration of the first dose of eculizumab. Conversely, intercedent inflammatory diseases may stimulate increased C5 biosynthesis, necessitating a shortening of the monoclonal antibody dosing interval or an increase in the dose at a fixed interval.

COMPLEMENT IN DISEASE STATES

The increasing availability of genetically engineered mice coupled with modern molecular techniques is leading to a progressively sophisticated understanding of the role of complement in various types of inflammation and in the pathogenesis of tissue injury and repair. Mediators released during complement activation may play a role in the development of symptoms or in the outcome of these disorders. Evidence that supports this suggestion includes the observations that (1) the extent of complement activation often parallels disease activity, (2) complement is deposited at the site of tissue injury, and (3) in animal models of these disorders, specific manipulation of complement activation modulates the course of disease. The role of complement has been studied most extensively in infectious diseases, rheumatologic disorders, and glomerulonephritis characterized by obvious inflammation. However, it has become increasingly clear that complement activation and mediator generation play important roles in such diverse entities as atherosclerosis,[313] restenosis, postperfusion injury,[314] demyelination disorders,[315] a variety of dermatoses,[316] and hyperacute graft rejection in xenogeneic transplantation.[317] Damage in these disorders is complement dependent—that is, it can be prevented by complement depletion or by infusion of proteins that regulate complement activation, such as soluble CR1 and CD46.[318] In the case of xenotransplantation, rejection can be prevented through the development of transgenic animals whose organs express the genes for human complement regulatory molecules.[319]

Infectious Diseases

Complement activation during infection is particularly impressive in diseases such as dengue fever, bacterial endocarditis, and sepsis, in which the organisms or their products react with antibodies to form circulating immune complexes and initiate complement consumption. It is especially striking in meningococcal disease and less so in other forms of gram-negative sepsis and septic shock. Studies have convincingly demonstrated a protective role for complement in endotoxic shock. Genetically engineered C3- and C4-deficient mice did not clear endotoxin as efficiently, had higher levels of TNF and IL-1, and had much higher mortality rates than did wild-type mice. C1-INH and fibrinogen levels were also lower in the deficient mice. Reconstitution of C1-INH significantly reduced mortality and restored fibrinogen concentrations to normal, despite the persistent deficiency of C3 or C4. Together, these findings indicate that endotoxin-containing immune complexes initiate complement consumption via the classical pathway and that failure to incorporate C3 into the immune complexes leads to deficient endotoxin clearance, ongoing complement consumption, and C1-INH depletion. The absence of C1-INH permits contact system activation, as manifested by fibrinogen consumption (a potential counterpart of disseminated intravascular coagulation in humans). The fact that C1-INH replacement protected the deficient animals and restored fibrinogen levels to normal strongly implicates the direct involvement of the contact system in endotoxic shock and death.[320] Later experiments demonstrated that the immune complexes initiating this sequence of events were composed of natural IgM antibody with specificity for the endotoxin O antigen.[321] In this respect, these results are reminiscent of those implicating natural antibody and complement consumption via the classical pathway in reperfusion injury.[314] A critical role for LPS in mediating hemodynamic instability and cytokine release was confirmed with use of meningococci in a porcine model of sepsis. In this model, an LPS-deficient mutant also caused "sepsis-like" physiology, although 10- to 20-fold higher challenge inocula were required, suggesting that molecules other than LPS could contribute to morbidity in sepsis in gram-negative bacteremia.[322]

Studies have implicated C5a as a key mediator in the development of septic shock and the acute respiratory distress syndrome in humans,[323] in a monkey model of gram-negative shock,[324] and in the cecal ligation puncture (CLP) model of sepsis in the rat. Of the two animal models, CLP most closely mimics sepsis in humans; affected rats, left untreated, die in 4 to 5 days.[325] Investigators who have used this model have demonstrated substantial C5a bound to neutrophils, which in turn exhibit a markedly suppressed oxidative burst. Treatment of the animals with anti-C5a antibody at the time of CLP reduces rat mortality by 50%, prevents neutrophil dysfunction, and is associated with a marked reduction in bacterial counts in the blood, liver, and spleen.[325] These studies also show that C5aR expression is upregulated in the lung, liver, kidney, and heart early in the course of sepsis and that blockade of this receptor with specific antibody not only improves survival but also reduces the serum concentrations of IL-6 and TNF-α.[326] Immune suppression is a newly recognized hallmark of sepsis in humans, evidenced by the depletion of lymphocytes in the white pulp of the spleen and by peripheral lymphocytopenia that is temporally associated with the multiorgan failure syndrome.[327] In the CLP model, immunodysregulation occurs as a result of caspase-dependent, NF-κB–independent apoptosis

of T lymphocytes in the context of decreased expression of Bcl-X_L. Lymphocyte apoptosis can be blocked by anti-C5a administration.[328] Whether this apoptosis-sparing effect is the result of direct inhibition of events downstream from the ligation of T-cell lymphocyte C5aR or a consequence of decreased levels of cytokines (e.g., IL-6) is unknown.[329]

Complement, in conjunction with the reticuloendothelial system, plays a critical role in the removal of encapsulated bacteria from the bloodstream.[330] Delineation of the contribution of these variables to the clearance process, which was accomplished in an animal model of pneumococcal bacteremia, demonstrated that the more virulent the organism, the greater the role of the spleen in performing this clearance function.[330] Complement depletion led to a significant decrease in the number of pneumococci needed to kill 50% of the animals, demonstrating an important role for complement in the clearance function. Investigations into the role of each of the specific pathways have yielded equivocal results. In one study, clearance of pneumococci was similar in healthy and in C4-deficient animals, indicating that complement activation and fixation to bacteria via the alternative pathway were particularly relevant in this process. Moreover, the presence of immune antibody shifted the burden of clearance from the spleen to the liver, and this effect was absolutely dependent on a functional alternative-complement pathway.[331] Increased mortality in both C1q and factor B knockout mice after intranasal inoculation of *S. pneumoniae* in another study provided evidence for a role of both the classical and alternative pathways in organism clearance.[22] More recently, mice deficient in ficolin, MASP-2, or SAP exhibited increased susceptibility to select pneumococcal isolates.[332–334] Thus the strain of bacteria, challenge dose, and route of inoculation used are all important considerations in drawing conclusions from data in experimental animal models.

Rheumatologic Disorders

Substantial clinical and experimental evidence links complement deficiency syndromes and complement activation to a variety of rheumatologic diseases, most notably SLE (see earlier discussion). Additional support for this relation is the finding that pharmacologic agents (e.g., hydralazine, isoniazid) associated with medication-induced SLE inactivate C4 by nucleophilic attack on its internal thioester and formation of amide bonds.[335] Evidence that complement activation may be associated with manifestations of the disease and with tissue injury includes the demonstration of C3 and immune complex deposition at the dermal-epidermal junction in cutaneous lesions from patients with SLE or discoid lupus erythematosus. Similar immunohistochemical alterations have been demonstrated in biopsy specimens of healthy skin from the same patients. However, the finding of MAC in areas of affected but not unaffected skin from these patients strengthens the hypothesis that complement activation may partially mediate tissue injury in these disorders.[315]

Incorporation of C3 into immune complexes promotes their binding to C3b receptors (CR1) on erythrocytes, as noted earlier. Erythrocyte CR1 is removed along with immune complexes during passage through the liver and spleen. The number of erythrocyte CR1 molecules is reduced in people with disorders such as SLE, which are characterized by circulating immune complexes.[229] The degree of CR1 reduction correlates well with disease activity and with the extent of complement activation. The decrease in CR1, coupled with the inability of circulating erythrocytes to resynthesize them, further exacerbates the defect in immune complex clearance, thereby promoting their deposition in the tissues, with resultant damage to the host. The number of erythrocyte CR1 molecules returns to the genetically prescribed level after the disease is controlled and erythrocyte numbers have returned to normal.

Renal Disorders

Complement deposition in renal diseases that are associated with immune disorders is related to the deposition of immune complexes within the kidney,[336–338] whereas complement deposition in the absence of immune complexes is postulated to occur through activation of the alternative pathway.[339] In a rat model of chronic tubulointerstitial disease, loss of renal mass and function was correlated with increased ammonia production and systemic acidosis. Under these conditions, peritubular deposition of C3 and the MAC was readily demonstrated. Deposition of these components and evidence of tubulointerstitial inflammation were markedly decreased in diseased animals treated with sodium bicarbonate. These and other findings indicate that ammonia attacks the C3 internal thioester to form amidated C3. Amidated C3 serves to activate the alternative-complement pathway in the fluid phase, leads to C3 and C5b-9 deposition in the tissue, and elicits an inflammatory response and tissue injury.[339,340]

Experiments using C6-sufficient and C6-deficient rabbits and the infusion of C8-deficient serum into rats clearly demonstrated that the development of proteinuria in membranous glomerulonephritis depends on the assembly and deposition of a complete MAC on the glomerular epithelial cells. A substantial portion of this injury results from MAC-mediated stimulation of prostaglandin and thromboxane synthesis because development of proteinuria was reduced by treatment with indomethacin, an inhibitor of cyclooxygenase.[341]

Many patients with chronic renal disease ultimately require hemodialysis. Exposure of plasma to first-use filter membranes during dialysis results in complement activation.[342] Anaphylatoxins released during this process (e.g., C5a) have been associated in a concentration-dependent and temporal fashion with the onset of respiratory distress in some dialysis patients.[323,342] This association is believed to relate in part to C5a-dependent neutrophil aggregation and stimulation and the formation of microemboli and their deposition in the lung (see Chapter 8).[323] Strategies to reduce complement activation either at the level of C3 or blockade of C5aR through use of small-molecule inhibitors (e.g., analogues of the C3 inhibitor compstatin) are currently being developed and may represent viable therapeutic strategies to reduce undesired complement activation during hemodialysis.

EVALUATION AND TREATMENT OF COMPLEMENT DISORDERS

Evaluation

Evaluation of the complement system is indicated when the diagnosis of a complement deficiency state is being considered or when specific measures of complement proteins are being used to assess disease activity or response to therapy. As pointed out earlier, several clinical clues should lead the clinician to suspect a complement deficiency state.[208,209] Foremost among these is a medical or family history of recurrent systemic infection caused by encapsulated bacteria, especially meningococci. A family history of fulminant meningococcal disease occurring in males in alternate generations should suggest the possibility of X-linked properdin deficiency. Meningococcal disease occurring in persons older than 10 years, especially when caused by non–group B meningococci, warrants evaluation of the complement system because 5% to 10% of these patients have a complement deficiency state, even in the absence of recurrent disease. Children with a history of recurrent respiratory infections beyond the first few years of life should prompt investigations of the classical and lectin pathways (MBL and ficolins) and an evaluation of IgG subclasses. Likewise, a history of SLE in family members or the occurrence of atypical features of SLE should also suggest the need for evaluation of the complement system. Specific syndromes, including angioedema, partial lipodystrophy, aHUS, AMD, and PNH, are other indications for assessment of complement status.

Any of a number of specific inherited complement deficiencies can produce one of the typical clinical syndromes associated with these disorders; hence, it is important that the initial test be one that measures the function of the *entire* complement cascade. The most common of these tests is the standard CH_{50}, which measures the function of the classical- and terminal-complement pathways. If possible, an initial assessment should also include an analogous test, the AH_{50}, which measures the function of the alternative- and terminal-complement pathways. Many hospital laboratories do not perform the latter test, so it may be necessary to contact a research or commercial laboratory with specific expertise in complement measurement. A negative or extremely low result in either of these two assays warrants further diagnostic evaluation.

The combined results of the tests of classical- and alternative-pathway functions dictate which additional tests need to be performed. If both the classical- and alternative-pathway CH_{50} values are extremely low, the defect must lie in one of the components shared by both pathways:

C3 through C9 (see Fig. 9.1). If the alternative pathway is normal but the classical pathway is not, the deficient component must be C1, C2, or C4. Conversely, a normal classical but defective alternative pathway suggests a defect in factor D, factor B, or properdin. Diagnosis of these specific defects can frequently be accomplished by the use of immunochemical methods to demonstrate an absence of the relevant antigen. However, several complement deficiency states involve absent function in the presence of normal amounts of antigenic protein. In addition, low levels of C3 can be encountered in factor H or factor I deficiency states. Therefore, confirmation of the diagnosis of a specific component deficiency should be documented with specific functional assays for the protein under consideration and by demonstration that replacement of the missing component restores both specific and total complement activity. Such assays require the expertise of a laboratory that specializes in complement function.

It should be noted that diseases such as aHUS and AMD represent dysregulation of complement and therefore may not be detected by most of the standard complement assays described earlier. Sequencing of one or more target complement genes may need to be carried out to detect mutations (or polymorphisms) associated with the disease. Advances in sequencing technologies, coupled with decreasing costs of sequencing, have prompted efforts to determine the "complotype" of individuals suspected to have disorders characterized by complement dysregulation. Polymorphisms that are associated with either gain or loss of function of individual complement proteins could interact and have a profound effect on overall activity of a pathway.[343] As an example, disease penetrance, severity, or the response to treatment, including the outcome of renal transplantation, depends on the gene(s) affected, as elucidated in a recent study of 745 individuals with aHUS.[344] However, interpretation of such data is not straightforward and requires the expertise of specialized laboratories. A better understanding of the fine balance of complement activation and inhibition may elucidate subtle differences in complement activation among individuals, that is, factors dictating susceptibility to various infections, in addition to the infections classically associated with complete deficiencies of complement components described earlier.

Acquired complement deficiencies can complicate the interpretation of complement disorders. Most acquired complement deficiency states occur in the setting of acute or chronic inflammatory disease and involve activation of the entire complement cascade. As a result, specific component testing typically demonstrates low levels of multiple complement proteins. In contrast, specific component testing in individuals with an inherited complement disorder (other than a defect in one of the complement regulatory proteins) typically demonstrates a low concentration of a single protein.[345] An important corollary of these general principles is that complement assessment can be optimized if testing is performed after the patient has recovered from any acute illness or after the disease has been treated successfully.

Treatment

Therapeutic issues related to specific complement deficiencies have already been described. Two general issues that arise frequently during formulation of a therapeutic approach for patients with complement deficiency states are replacement of the missing protein and prevention of infection.

At the present time, although advances in knowledge of the molecular basis of the various complement deficiency states are beginning to yield alternative means of therapy, replacement of deficient components usually requires the infusion of fresh-frozen plasma. This approach has been successfully used in therapy for acute attacks of angioedema (see earlier discussion),[64,278,279] in restoration of C3 levels toward normal in patients with C3 deficiency, and in treatment of a C2-deficient patient with SLE unresponsive to conventional therapy.[346,347] It has also been recommended as a possible therapeutic option for patients with aHUS or dysfunctional factor H. This approach has several potential limitations, First, the half-life of most complement proteins in vivo is short,[12] although a notable exception occurs in patients with low C3 levels caused by factor I deficiency. In these patients, replacement therapy restores factor I activity, thereby markedly reducing the accelerated breakdown of C3 that is observed in this disorder.[348] Second, replacement of a genetically absent protein may stimulate the production of antibody to the missing component, thereby limiting the value of subsequent therapy. This consideration is of limited concern in persons with autosomally inherited disorders such as hereditary angioedema, whose serum contains some normal protein, and in those with other complement deficiency disorders characterized by the presence of antigenically normal amounts of a dysfunctional protein (e.g., qualitative factor H deficiency). Third, the relative frequency of infection in most of these patients must be balanced against the potential risk of acquisition of any of a variety of bloodborne infections during plasma infusion, especially because alternative modes of therapy are often available. Whether the short-term infusion of fresh-frozen plasma might be beneficial in the treatment of life-threatening infection, especially in properdin-deficient patients, remains untested. Recombinant human MBL is currently being considered for use clinically and may address some of the limitations of fresh-frozen plasma.

The central role of complement activation in the pathogenesis of aHUS prompted the use of eculizumab (a humanized anti-C5 monoclonal antibody) in this condition. Amelioration of symptoms resulted in approval of eculizumab for use in aHUS in Europe and the United States in 2011. Eculizumab may also be useful in the prevention and treatment of recurrences of aHUS after renal transplantation.[349] Individuals with dense deposit disease (MPGN II) who have evidence of increased systemic complement activation (high C5b-9 levels) may also benefit from C5 blockade.[350–352] The clinical course of Shiga toxin–associated HUS was also attenuated after the use of eculizumab in three children.[353]

In an effort to develop a small-molecule inhibitor of complement at the level of C3, a 13-mer cyclic peptide called compstatin, which binds to the C3 and C3b and inhibits the action of C3 convertases on C3, was identified.[354,355] Although the long-term systemic use of C3 convertase–inhibiting compounds could predispose individuals to serious infections, short-term or tissue-specific inhibition of complement could be beneficial. As an example, intraocular instillation of a compstatin analogue has shown promise in the treatment of AMD in preclinical studies.[355,356] Despite strong data linking complement activation with dry AMD, intraocular administration of the Fab fragment of a factor D function-blocking monoclonal antibody (lampalizumab), did not halt progression of geographic atrophy in a phase III clinical trial. These data highlight the complex nature of disease pathology; addressing the predisposing or inciting event at later stages of the disease may not halt or reverse symptoms.

Prevention of infection in complement-deficient patients is best achieved through vaccination. All deficient persons should be vaccinated with a tetravalent conjugate meningococcal vaccine (Menactra [Sanofi Pasteur, Swiftwater, PA] or Menveo [GlaxoSmithKline]). Two recombinant protein vaccines (Bexsero and Trumenba) for group B meningococci are available in the United States and may be considered in complement deficiency, including eculizumab-treated patients. These vaccines are directed against meningococcal membrane proteins and therefore may also protect against nongroupable (unencapsulated) strains that have been associated with invasive disease in eculizumab recipients (see later). Antibody titers wane rapidly during the first year after vaccination, and no recommendations for revaccination are available (see Chapter 211). Those with classical-pathway deficiencies should also receive the polyvalent pneumococcal and conjugated *H. influenzae* vaccines. Given the low cost and high potential benefit of these vaccines, all three should probably be administered to any individual with complement deficiency. Conjugate vaccines, such as that for *H. influenzae,* which initiate a T-cell–dependent response, stimulate the production of higher antibody concentrations and their longer persistence, and induce immunologic memory. The elicitation of T-cell help by such vaccines circumvents the qualitative defect in antibody production observed in these patients[357]; therefore these vaccines are preferred if they are available.

Successful vaccination leads to the production of anticapsular antibodies that promote use of the classical pathway in patients with an alternative-pathway defect and facilitate alternative-pathway use in patients who lack one of the classical-pathway components.[247] In such patients, these antibodies may promote bactericidal activity and microbial elimination by enhancing opsonophagocytosis.

Neither clinical nor in vitro studies have explored the potential for vaccination to help protect C3-deficient persons from infection.

The theoretical basis for this approach lies in the ability of antibody alone to facilitate phagocytic elimination of organisms, albeit at a reduced rate of killing. This property is most relevant to the clearance of organisms from the bloodstream via the reticuloendothelial system, in which the structural architecture and lining of the sinusoids with tissue macrophages contribute greatly to surface phagocytosis. In view of the suboptimal response to protein and polysaccharide antigens in C1-, C2-, C4-, and C3-deficient humans and animals, documentation of the patient's response to vaccination with these antigens seems prudent.

Although anticapsular antibody cannot enhance serum bactericidal activity in persons with a deficiency of one of the terminal complement proteins, it promotes opsonization and killing of these organisms by phagocytic cells.[260] In vitro studies of prevaccination and postvaccination sera have documented the ability of such patients to respond to the tetravalent meningococcal vaccine and the likelihood that their response will facilitate phagocytic killing of the corresponding meningococci.[264,358,359] In addition, clinical investigations have demonstrated that vaccination reduces the frequency of infection from 0.15 to 0.04 episodes per year and prolongs the interval between infections from 3.6 to more than 6 years.[360,361] These studies, like those in other groups of complement-deficient individuals, attest to the usefulness of vaccination as an immunologic means to circumvent the major clinical manifestations of these deficiencies.

Of the terminal complement deficiencies, C5 deficiency presents a unique problem. Whole blood from a C5-deficient patient failed to kill a group B isolate of *Neisseria meningitidis* even in the presence of high titers of specific antibody that supported killing when pure C5 was added back.[362] The rate of meningococcal disease in eculizumab recipients despite meningococcal vaccination was 342 per 100,000 person years,[363] which represents an approximately 2000-fold higher incidence of disease than in the general population. Of the 16 reported cases of invasive meningococcal disease in eculizumab recipients between 2008 and 2016, 11 were caused by nongroupable (unencapsulated) strains, 4 were caused by group Y strains, and the identity of one isolate was not determined. A group B meningococcal vaccine (Bexsero), which targets protein antigens and was expected to provide protection against nongroupable isolates, was administered to 3 individuals. The addition of eculizumab to whole blood from 12 immunized individuals abrogated killing of meningococci in every instance, illustrating again the importance of C5 for opsonophagocytic efficacy of antimeningococcal vaccine antibody.[364] In contrast, C7 blockade permitted killing of meningococci by whole blood in a strain-dependent manner.[364] Lack of C5a generation differentiates C5 deficiency (and pharmacologic inhibition of C5 with eculizumab) from deficiencies of C6 through C9. Despite loss of C5a and the resulting impairment of neutrophil recruitment and function, there is no evidence to suggest that the clinical course of invasive meningococcal disease in C5-deficient persons differs from disease severity in other late complement component deficiencies. As discussed earlier, lack of MAC formation, a common feature of all terminal complement deficiencies, limits endotoxin release and may be the overriding factor that contributes to milder disease in these individuals. In light of these observations, meningococcal vaccines may not confer the expected protection in individuals on long-term treatment with C5 inhibitors such as eculizumab, necessitating constant and careful evaluation for invasive disease.

An alternative strategy for the prevention of meningococcal disease is the use of prophylactic antibiotics[365] or the administration of antibiotics for preemptive treatment at the onset of symptoms. The use of prophylactic antibiotics significantly reduces the frequency of infection in C6-deficient persons and has its greatest use in populations in which group B disease is highly prevalent.[366] Such a strategy may merit consideration in persons with C3 or C5 deficiency (either congenital or acquired), in whom vaccination may not provide maximal protection for reasons discussed earlier. It is unclear whether antibiotic prophylaxis should be lifelong or whether the development of antibiotic resistance will limit its efficacy.

ACKNOWLEDGMENT

The author and editors recognize and thank Dr. Peter Densen, who prepared this chapter and its antecedents for eight editions over 40 years, before turning it over completely to the next generation. We appreciate his career as a physician and scientist, and his work educating his colleagues all over the world about innate immunity and complement. Such dedication is at the bedrock of civilization, allowing knowledge to be transferred forward for posterity.

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10 Human Genetics and Infection

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SHORT VIEW SUMMARY

- This chapter summarizes the evidence for host genetic susceptibility to infection.
- Rapid technologic advances have brought about increasing use of high-throughput genome-wide approaches including microarray genotyping of 0.5 to 1 million polymorphisms, "whole-exome" sequencing of the 30 million bases of the protein-coding genome, and "whole-genome" sequencing of the 3 billion bases of the entire human genome.
- Genome-wide association studies using microarray genotyping technology have become increasingly dominant and continue to provide new insight into genetic susceptibility to infection. These studies use very stringent statistical thresholds to declare associations, meaning that the reported signals are substantially more robust and reproducible than in the candidate gene era.
- Substantial evidence has now accumulated to link variation in HbS, *ABO,* and *G6PD* to susceptibility to *Plasmodium falciparum* malaria. However, robust reappraisal of variants linked to malaria susceptibility in the candidate gene era has brought numerous previously reported associations into question, emphasizing the need for cautious interpretation of candidate gene literature more broadly.
- The HLA locus plays an important role in a number of infectious diseases, ranging from viral infection, including human immunodeficiency virus (HIV) and hepatitis B virus; bacterial diseases, including enteric fever, tuberculosis, and leprosy; and protozoal diseases, including visceral leishmaniasis.
- Evidence is also accumulating that host variation elsewhere in the genome affects susceptibility to a variety of infectious diseases. The growing list of robust non-HLA loci now includes, for example, *CFB* and *CD40* for persistence of hepatitis B virus, and *WT1* and *ASAP1* for tuberculosis, in addition to emerging findings including, for example, *STAT4* for nontyphoidal *Salmonella* bacteremia.
- The importance of robust study design is underscored by the recent finding that beyond the 32-bp deletion in *CCR5,* most of the previously reported associations with susceptibility to, as opposed to control of, HIV infection can be attributed to frailty bias because cohorts comprising prevalent cases of HIV are enriched for variants associated with prolonged disease-free survival.
- Increasing sample sizes and improved technologies have brought into question many other previously reported susceptibility signals, including the widely known associations between variation in the *MBL* locus and susceptibility to a variety of traits including sepsis.
- Overall studies investigating host genetic susceptibility continue to tell us much about the molecular basis of the host response to infection, providing important insights that can guide drug and vaccine development.

Genetic variation in the host has a substantial influence on the course of infectious diseases caused by many microorganisms. Such interactions have been well studied in humans because the pathogens and the host genome are well characterized. In recent years, the methods and techniques available for analyzing human genetic variation have advanced rapidly, leading to the identification of a large number of genes associated with altered susceptibility to infectious pathogens. Entire human genome sequences and millions of mapped single nucleotide polymorphisms (SNPs) provide a powerful resource for molecular analysis of differential susceptibility. Although many genes have already been associated with susceptibility to various diseases, it is likely that these represent only a small fraction of all the relevant genes. Indeed, susceptibility to most infectious diseases in humans is likely to be highly polygenic, and the identification of susceptibility and resistance genes is providing new insights into disease pathogenesis and resistance mechanisms. Conversely, it appears increasingly likely that a substantial proportion of the functional variation in the human genome has evolved to facilitate defense against infectious pathogens, leading to the observed polygenic variation in susceptibility among individuals and different human populations.

MAGNITUDE OF THE HOST GENETIC EFFECT

There are various types of evidence that demonstrate a significant role for host genetics in variable susceptibility to infectious disease. For some infections, such as leprosy, disease has long been known to cluster in families,[1] but it is often difficult to assess the relative importance of proximity to an index case and shared genes. Nonetheless, the increased risk of disease in a sibling compared with the general population is a useful measure of the extent of the genetic component in a multifactorial disease and appears to be of the order of 1.5 to 5 for several infectious diseases.[2]

Apparent interpopulation and racial differences in susceptibility have also been noted.[3,4] Particularly striking is the apparently increased susceptibility to viral infections and tuberculosis (TB) in some previously unexposed populations.[5] In the Qu'Appelle Indians of Saskatchewan, after some decades of exposure to TB and resulting high mortality, the incidence of disease dropped 50-fold, likely reflecting a very strong selective pressure against TB susceptibility loci.[6] The use of malaria therapy for syphilis in nonimmune individuals[7] and the accidental vaccination of some children in Lübeck, Germany, with *Mycobacterium tuberculosis* rather than the Calmette-Guérin bacillus provided direct evidence of variable susceptibility to these pathogens.[7a]

In addition to these observational data, studies of adoptees and of twins have provided more direct quantification of the importance of host genetics in susceptibility to infectious diseases. In a study of more than 900 Scandinavian adoptees, the early death of a biologic, but not an adoptive, parent from an infectious disease was associated with an almost sixfold increase in the risk of an infectious cause of death in the adoptee, consistent with a substantial role for host genetics.[8] Also, several twin studies have found higher concordance rates among monozygotic than among dizygotic twin pairs, who share, respectively, 100% and, on average, 50% of their genes. The diseases studied include TB, poliomyelitis, leprosy, persistent hepatitis B virus (HBV) infection, *Helicobacter pylori* infection, and malaria (Table 10.1).[9–14] In general, it has been easier to demonstrate a significant role for host genetic factors in infections in which only a proportion of those infected develop disease, in chronic rather than in acute infections, and in the severity of infectious disease rather than susceptibility to infection itself.

TABLE 10.1 Twin Studies of Selected Infectious Disease Phenotypes

PHENOTYPE	POPULATION	CONCORDANCE MZ	CONCORDANCE DZ
Tuberculosis	Germany United States United Kingdom	65 62 32	25 18 14
Poliomyelitis	United States	36	6
Leprosy	India	52	22
Hepatitis B	Taiwan	35	4
Helicobacter pylori infection	Sweden	81	63

[a]All showed significantly higher concordance in monozygotic (MZ) than in dizygotic (DZ) twin pairs.

TABLE 10.2 Examples of Genes That Confer Resistance to Malaria

GENE NAME (SYMBOL)	VARIANT	*PLASMODIUM* SPECIES
β-Globin *(HBB)*	Sickle, thalassemias	*P. falciparum*
α-Globin *(HBA)*	Thalassemias	*P. falciparum*
Erythrocyte band 3 *(SLC4A1)*	27-bp deletion	*P. falciparum, P. vivax*
Duffy chemokine receptor *(DARC)*	Promoter variant	*P. vivax*
Glycophorin C *(GYPC)*	Exon 3 deletion	*P. falciparum*
Glucose-6-phosphate dehydrogenase *(G6PD)*	Deficiency variants	*P. falciparum*
ABO glycosyltransferases *(ABO)*	Blood group O	*P. falciparum*

Approaches

Several different and usually complementary approaches have been taken to identifying genes involved in susceptibility to infectious diseases. Historically, a widely adopted strategy was the assessment of candidate genes in case-control association studies. Here, the frequencies of common variants (with a frequency >1%, defined as polymorphisms) of a gene with a suspected role in resistance are compared in individuals with and without the disease. However, the degree of replication between reported association studies of candidate genes is often disappointingly low, in part reflecting limitations in study design. In particular, such studies often have inadequate sample sizes and fail to perform statistical correction for multiple testing of different polymorphisms. Despite these shortcomings, a number of robust genetic associations with infectious disease susceptibility have been successfully identified with the candidate gene approach.

Another largely historical approach was to search for genetic linkage to, rather than association with, an infectious disease in family studies. Identification of a chromosomal region genetically linked to susceptibility indicates that there is a susceptibility gene (or genes) somewhere in that region. Limitations of the linkage approach have been the difficulty in recruiting multicase families and a lack of study power. Such family-based linkage approaches were successfully used, however, to map and identify genes underlying rare monogenic susceptibility phenotypes such as susceptibility to weakly pathogenic mycobacteria.[15,16]

More recently, the availability of millions of SNPs mapped onto the human genome sequence has allowed the development of microarrays of typically 0.5 to 1 million SNPs, allowing markers across the entire human genome to be studied for possible disease association in a single experiment. This approach, known as a genome-wide association study (GWAS), uses tagging SNPs to identify markers that may be causally associated with susceptibility. A major advantage of the genome-wide approach is that, unlike the use of candidate genes, it is not dependent on existing biologic hypotheses and so has the potential to uncover novel, previously unsuspected susceptibility genes and pathways. A drawback is that very large sample sizes (typically comprising thousands of individuals) are needed to provide statistical power to detect disease associations after correction for the large number of polymorphisms tested. Collecting such large sample sizes poses a major challenge in many infectious diseases, particularly diseases with acute presentations in resource-poor countries. Despite this, the application of GWAS to selected infectious diseases has been successful in both replicating genes implicated from candidate and linkage approaches and identifying novel susceptibility genes.[17]

Finally, recent advances in sequencing technology have permitted the selective sequencing of complete coding regions of the genome (the "whole exome"), and indeed the entire genome, in individual humans. Because coding regions comprise only 1% of the human genome, whole-exome sequencing is an efficient strategy for the identification of rare variants underlying disease. Although this approach will not reveal causal variants in noncoding regions, it is widely presumed that the majority of large-effect variants will be located in coding regions. Whole-exome sequencing has been successfully used to identify rare single-gene variants underlying extreme phenotypes of infectious disease, primarily monogenic primary immunodeficiencies. Rare mutations in genes leading to primary immunodeficiency states are described in Chapter 12.

DISEASES

Malaria

Historically, more genes have been implicated in the differential susceptibility to malaria than to any other infectious disease of humans or other animals (Table 10.2). This in part reflects the very strong selection of malaria on pre–reproductive-age human mortality, the early success in identifying the relevance of the sickle hemoglobin (HbS) polymorphism, and the geographic distribution of some malaria resistance alleles.[18] Early evidence of differential susceptibility to malaria in nonimmune subjects came from studies of the use of malaria therapy in the management of syphilis.[7] Marked differences in susceptibility to the same dose and strain of malaria parasite were observed among individuals. Numerous studies of HbS heterozygotes and glucose-6-phosphate dehydrogenase (G6PD) deficiency provided evidence of their protective relevance against *Plasmodium falciparum* malaria.[19,20]

Based on the distribution of thalassemia in the Mediterranean, Haldane[21] proposed that certain hemoglobin gene variants might have reached high frequencies in malarious regions by providing resistance to this disease. The protective efficacy of HbS in heterozygotes against *P. falciparum* malaria was discovered a few years later.[18] The greatest protection is afforded against death and severe life-threatening malaria, with somewhat less protection against uncomplicated disease and least protection against becoming infected. This pattern of greater protection against severe disease than infection appears to apply for many resistance genes.

The α- and β-thalassemias are extremely common disorders of hemoglobin synthesis that lead to imbalanced globin chain production. The mild forms of thalassemia are among the most prevalent single-gene disorders. Both α- and β-thalassemias have been shown to afford some protection against *P. falciparum* malaria, in keeping with their geographic distribution, but the mechanism of protection remains unknown.[22–24] There is no detectable impairment of parasite growth in vitro, and the protection afforded appears less marked than for HbS. Hemoglobin C (HbC),[25,26] which is common in parts of West Africa, and HbE,[27,28] which is widely distributed in Southeast Asia, are also protective. Interestingly, there appears to be an epistatic interaction between the protective HbAS and α-thalassemia genotypes such that they cancel out each other's protective effect against malaria; this might explain why very mild forms of α-thalassemia have not reached higher frequencies in sub-Saharan Africa.[29]

Early work on the human leukocyte antigen (HLA) locus found both class I and class II alleles associated with malaria susceptibility in Africa in large case-control studies.[30,31] However, there is evidence that the particular alleles showing such associations differ among populations. Interpopulation heterogeneity can result from many causes, but a

prominent one in malaria is likely to be the marked polymorphism of immunodominant malaria antigens. Indeed, HLA has been found to influence the strain of malaria parasite associated with clinical malaria, and complex interactions among parasite strains may lead to further variability in HLA associations.[32] Overall it seems likely that the antigenic specificities of predominant immune protective mechanisms against malaria vary geographically with transmission patterns.

Malaria parasites vary in their capacity to form rosettes with uninfected erythrocytes and to sequester in capillary beds, and both of these phenotypes have been implicated in increased malaria severity.[33,34] Later work therefore investigated the relationship between candidate genes and malaria susceptibility, including the polymorphic host receptors involved in parasite sequestration such as *CD36* and *ICAM1* (intercellular adhesion molecule 1).[35–37] However, a reappraisal of reported malaria resistance loci by the Malaria Genomic Epidemiology Network (MalariaGEN) found only a weak protective signal for *CD36* and no relationship between *ICAM1* variants and susceptibility.[38] Moreover, this study, based on data from 11,890 cases and 17,441 controls from 12 locations in Africa, Asia, and Oceania, found that 22 of the 27 previously reported associations were not replicated, underscoring the importance of interpreting the candidate gene literature with caution.

One important locus that did replicate was the *ABO* gene, which encodes the glycosyltransferase enzyme that determines ABO blood groups. Individuals with blood group O had a reduced risk of both cerebral malaria and severe malarial anemia.[38] Consistency across these two distinct clinical phenotypes was also observed for HbS and HbC, which implies a general pathogenic mechanism, contrasting with the strong heterogeneity observed at other malaria susceptibility loci including the *G6PD* gene.[38] A number of variants around *G6PD* cause deficiency of the G6PD enzyme in an X-linked fashion, which in certain situations predisposes to hemolytic anemia.[39] Acute hemolysis can be triggered by a variety of drugs, some infections, and ingestion of fava beans; male G6PD-deficient infants may have neonatal jaundice. More than 100 mutations of *G6PD* have been described through molecular analysis, and a small group of uncommon variants is associated with chronic hemolytic anemia in the absence of any environmental agents. Most *G6PD* variants are associated with lesser degrees of enzyme deficiency and are found at higher frequency. As with the hemoglobinopathies, the geographic distribution of G6PD deficiency in the "malaria belt" suggests its selective advantage.[6] In several locations, populations with historical exposure to malaria had significantly higher frequencies of G6PD deficiency than related populations that had not been exposed to malarial selection. In the MalariaGEN study, the major form of G6PD deficiency in Africa (G6PD+202T) appeared to have independent effects on cerebral malaria and severe malarial anemia, with a strikingly neutral effect on severe malaria overall.[38] Moreover, amalgamating the effects of multiple *G6PD* variants by using a World Health Organization (WHO) classification system revealed that other variants at the locus also contribute to malaria susceptibility such that increasing G6PD deficiency is associated with decreased risk of cerebral malaria and increased risk of severe malaria anemia.[40] Such results raise the possibility that the high rates of G6PD polymorphism seen in human populations may in part be due to an evolutionary trade-off between different outcomes of *P. falciparum* infection.[40]

Four large GWASs of severe malaria have been reported to date. The first replicated the association at HbS but only after sequencing the region in a subset of samples using a technique called genetic imputation to infer the HbS genotypes in the remainder of the collection.[41] Undertaken in The Gambia, this important study illustrated some of the challenges of studying genetic susceptibility in non-European populations, including greater population structure due to differences in the ancestry of cases and controls, and smaller haplotype blocks—especially in African populations—which mean causal variants are less likely to be tagged by the SNPs included on genotyping arrays. A second GWAS again confirmed the protective effects of HbS and of blood group O but also identified two novel associations including the *ATP2B4* (ATPase plasma membrane Ca^{2+} transporting 4) gene encoding the primary calcium pump of erythrocytes,[42] a finding that has since been replicated.[40,43] Two subsequent publications from the MalariaGEN Consortium have now brought the sample size up to 25,000 individuals and identified a novel resistance locus close to a cluster of genes encoding glycophorins, which are the receptors for erythrocyte invasion by *P. falciparum*.[43,44] Fascinating to note, this signal overlaps a region of genome in which genetic variation has been maintained by balancing selection identified by analysis of haplotype sharing between humans and chimpanzees.[44] Later, sequencing of African individuals from 10 ethnic groups revealed that a previously unknown and complex copy number variant that underlies a distinct blood group antigen known as Dantu is associated with protection against severe malaria.[45]

Finally, although most genes that influence susceptibility to infectious disease probably have relatively small effects, it is noteworthy that a few malaria resistance genes are exceptions to this rule. In particular, heterozygosity for HbS provides about 90% reduction in the risk of severe malaria.[30] In addition, the protection from *Plasmodium vivax* afforded by the Duffy-negative (CD234, chemokine receptor) blood group is essentially complete because this parasite is almost always unable to invade Duffy-negative erythrocytes.[46]

Mycobacterial Diseases

Genetic susceptibility studies of mycobacterial diseases have been relatively common for several reasons. Familial clustering of leprosy and TB has long been recognized, and leprosy was regarded by some as a genetic disorder before *Mycobacterium leprae* was identified.[1] An accident in Lübeck, Germany, in which children immunized with *M. tuberculosis* rather than Calmette-Guérin bacillus experienced a range of clinical manifestations from asymptomatic to severe infection and death, provided early evidence for variable susceptibility to TB.[47] This was substantiated by several large twin studies that found higher concordance rates among monozygotic compared with dizygotic twin pairs (see Table 10.1),[9] although a reanalysis of the most recent of these studies found less clear evidence of a genetic effect.[48] A large twin study of leprosy in India also reported higher concordance rates in monozygotic twins but was inconclusive on the question of genetic susceptibility to leprosy type.[11] Observations on the introduction of TB to some populations previously free of the infection suggested that the decline in frequency of the disease over time might in part reflect some natural selection for resistance genes.[6] Candidate gene approaches to TB and leprosy susceptibility implicated a number of genes (Table 10.3), including the HLA region, *SLC11A1* (solute carrier family 11 member 1; previously known as natural resistance–associated macrophage protein-1 gene [*NRAMP1*]), the signaling adaptor protein *TIRAP* (Toll–interleukin-1 [IL-1] receptor [TIR] domain containing adaptor protein; as known as MyD88 adaptor-like [Mal]) and the C-type lectin *CD209,* although the level of replication between studies has been generally low.[48–55] Despite the availability of large multicase families, genome-wide linkage studies in TB had only limited success,[56,57] whereas such studies in leprosy provided a number of promising findings including a signal overlapping the *MRC1* (mannose receptor C-type 1; previously known as the human mannose receptor) gene.[58] In addition, a gene-centric approach identified a coding variant in the *TLR1* (Toll-like receptor 1) gene that was associated with a reduced risk of leprosy, a finding that is notable because of the substantial differences in frequency among populations, perhaps due to natural selection.[59]

More recently, there have been several GWASs of TB and leprosy of varying sample size. The first GWAS of TB found a susceptibility locus within a gene-poor region on chromosome 18q11.2. The signal was driven by a variant that is common in Africa but rarer in other regions, and although the causative variant remains unknown, a regulatory effect on a gene elsewhere on the chromosome is likely.[60] A second GWAS identified a TB resistance locus on chromosome 11p13 in an intergenic region downstream of the *WT1* (Wilms tumor 1) gene.[61] This protein has been shown to interact with the vitamin D receptor, which was previously implicated in TB pathogenesis. A further GWAS of TB in over 10,000 individuals of Russian ancestry not only replicated the *WT1* locus but also identified a further signal overlapping the *ASAP1* (ArfGAP with SH3 domain, ankyrin repeat and PH domain 1) gene, for which there was signal in the earlier African datasets.[62] These variants are believed to alter expression of *ASAP1* in dendritic cells, which may impair their migration to lymph nodes during the early stages of infection. Two additional GWASs undertaken in Europeans and Han Chinese

TABLE 10.3 Examples of Susceptibility and Resistance Genes Implicated in Bacterial Diseases and Related Phenotypes

GENE SYMBOL	DISEASE	METHOD	EFFECT
HLA-DR/HLA-DQ	Leprosy	GWAS/candidate	Susceptibility
TLR1	Leprosy	Gene-centric	Susceptibility
NOD2	Leprosy	GWAS	Susceptibility
TNFSF15	Leprosy	GWAS	Susceptibility
RIPK2	Leprosy	GWAS	Susceptibility
HLA-DR/HLA-DQ	Tuberculosis	GWAS/candidate	Susceptibility
ASAP1	Tuberculosis	GWAS	Susceptibility
HLA-DR	Enteric fever	GWAS	Resistance
ABO glycosyltransferases	Cholera	Candidate	Susceptibility
MBL	Pneumococcal disease	Candidate	Susceptibility
PTPN22	Pneumococcal disease	Candidate	Susceptibility
Mal/TIRAP	Pneumococcal disease, bacteremia, tuberculosis	Candidate	Resistance
CFH-CFHR3	Meningococcal disease	GWAS/candidate	Resistance

ASAP1, ArfGAP with SH3 domain, ankyrin repeat and PH domain 1*; CFH,* complement factor H; *CFHR3,* CFH-related protein 3; *GWAS,* genome-wide association study; *HLA,* human leukocyte antigen; *IL23R,* interleukin-23 receptor; *Mal,* myeloid differentiation primary response gene 88 (MyD88) adaptor-like protein; *MBL,* mannose-binding lectin; *NOD2,* nucleotide-binding oligomerization domain containing 2; *PTPN22,* protein tyrosine phosphatase nonreceptor, type 22; *TIRAP,* Toll-interleukin-1 receptor (TIR) domain adaptor protein; *TLR1,* Toll-like receptor 1 gene; *TNFSF15,* tumor necrosis factor (ligand) superfamily member 15.

TABLE 10.4 Examples of Susceptibility and Resistance Genes Implicated in Viral Diseases

GENE SYMBOL	DISEASE	METHOD	EFFECT
CCR5	HIV infection/progression	GWAS/candidate	Resistance
CCR2	HIV viral control	GWAS/candidate	Resistance
HLA-B, HLA-C	HIV progression	GWAS/candidate	Resistance
HLA-DP	HBV persistence	GWAS/candidate	Resistance
IL28B	HCV persistence	GWAS/candidate	Resistance
MICB	Dengue shock syndrome	GWAS	Susceptibility
PLCE1	Dengue shock syndrome	GWAS	Susceptibility
IFITM3	Influenza A	Candidate	Susceptibility
FUT2	Norovirus disease	Candidate	Resistance

CCR, Chemokine receptor; *FUT2,* fucosyltransferase 2; GWAS, genome-wide association study; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HLA,* human leukocyte antigen; *IFITM3*, interferon induced transmembrane protein 3; *IL28B,* interleukin-28B; *MICB,* major histocompatibility complex class I polypeptide–related sequence B; *PLCE1,* phospholipase C, epsilon 1.

confirmed the association between TB and the HLA class II region,[63,64] and introduced additional putative susceptibility loci including a region on chromosome 1 where genes of potential relevance to TB pathogenesis included *MFN2* (mitofusin 2) and *TNFRSF8* (TNF receptor superfamily member 8).[64] Finally, given the challenges involved in diagnosing TB, an alternative approach to investigating susceptibility to TB is to study high-risk individuals who do not develop disease. Illustrating this, a GWAS of human immunodeficiency virus (HIV)–positive individuals in Uganda and Tanzania identified two SNP haplotypes including a missense *IL9* (interleukin-9) variant that protected against tuberculin skin test positivity during follow-up.[65]

The first GWAS of leprosy susceptibility reported robust associations with polymorphisms in the loci *HLA-DR/HLA-DQ, RIPK2* (receptor-interacting serine-threonine kinase 2), *TNFSF15* (tumor necrosis factor [TNF] superfamily member 15), *LACC1* (laccase domain containing 1; previously known as *C13orf3*), *CCDC122* (coiled-coil domain containing 122), and *NOD2* (nucleotide-binding oligomerization domain containing 2), and a weak association with *LRRK2* (leucine-rich repeat kinase 2).[66] Current understanding of the functions of *C13orf31/LACC1* and *CCDC122* remains incomplete, but, interesting to note, the other associated loci *RIPK2, TNFSF15,* and *NOD2* and *TLR1* all encode proteins known to be involved in the innate immune response. A second GWAS of leprosy identified two more susceptibility genes, *IL23R* (interleukin-23 receptor) and *RAB32* (RAB-32, member *RAS* oncogene family).[67] Two further expansions of these analyses also set in Chinese Han populations have increased the total to 22 susceptibility loci.[68,69] The latest of these studies both fine-mapped the signal in HLA to the *DRB1**15:01 allele and estimated the heritability from the GWAS data at 0.199, such that genome-wide significant associations explain approximately 13.5% of leprosy risk.[69]

Genome-wide approaches have been highly successful in the study of leprosy, perhaps reflecting the relatively conserved nature of this pathogen, and these findings may further understanding of the biology of other diseases in addition to leprosy. For example, there is a striking overlap between genes underlying susceptibility to leprosy and Crohn's disease: *NOD2, TNFSF15, LRRK2, C13orf31/LACC1, CCDC122,* and *IL23R* are all associated with Crohn's disease,[68] raising the intriguing possibility of a shared immunologic basis for these two conditions.

Viral Diseases

Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome

The role of human genetic variation in susceptibility to HIV infection and progression of acquired immunodeficiency syndrome (AIDS) has been extensively investigated. This work followed the early observation that a small minority of individuals remain HIV seronegative despite repeated exposure from infected sexual partners.[70] Some of these resistant sex workers have immunologic evidence of exposure to the virus. There also is clear evidence that individuals vary in the rate of disease progression to AIDS once infected, and a small number of genes have now been found to influence this rate (Table 10.4).

A large number of studies of HLA type in relation to rate of disease progression have been reported. Although there are marked differences among studies, some alleles have now been associated with susceptibility or resistance in more than one population. Variation in the HLA class I region appears consistently more important than in the HLA class II region, with *HLA-B* being the most important gene in the class I locus.[71] *HLA-B**35 and the HLA-A1-B8-DR3 haplotype have been associated with more rapid disease progression in several studies.[72–74] Similarly, *HLA-B**27 and *HLA-B**57 are associated with a lower rate of progression.[75,76] Particular combinations of HLA class I and II alleles and variants of the transporter associated with antigen-processing genes have also been implicated.[77] Evidence of linkage of the major histocompatibility complex (MHC) locus to the rate of decline of CD4-positive T cells in patients with AIDS provides further support for the relevance of polymorphism in this region.[78]

It is possible to cluster HLA class I types into so-called supertypes based on the types of peptides bound by particular molecules; analysis of supertypes also shows convincing association with rate of disease progression.[79] The HLA type of the host appears to influence the diversity of HIV sequences that emerges during an infection, indicating that HLA variation can directly influence virus evolution.[80] Genome-wide association studies found evidence that loci other than *HLA* genes within the MHC may affect the rate of disease progression.[81,82] A further large GWAS of untreated HIV-1 controllers and progressors identified more than 300 significantly associated polymorphisms, all located in the MHC.[83] This study extended previous HLA associations to identify *HLA-B**57:01, *HLA-B**27:05, and *HLA-B**14 as protective and *HLA-C**57

as associated with progression to AIDS. Genes encoding the killer cell immunoglobulin-like receptors (KIR), which modulate natural killer cell activity and interact physically with HLA class I molecules, may also interact genetically or epistatically so that KIR gene variants modulate the risk associated with an HLA type.[84]

The discovery of the role of chemokine receptors as coreceptors with CD4 for viral entry into macrophages and lymphocytes has given rise to numerous studies of genetic variants of these receptors and their ligands. The *CCR5* (C-C motif chemokine receptor 5) gene associations with resistance to infection and slower disease progression are well established,[85] and variants in the *CCR2* (C-C motif chemokine receptor 2) gene have also been associated with altered disease progression.[86] A 32-bp deletion in the CCR5 chemokine receptor (*CCR5*Δ32) is found at allele frequencies of up to 10% in European and derived populations.[87] This variant is rare or absent in other populations.[88] CCR5 is the coreceptor for macrophage-tropic strains of HIV-1 involved in viral transmission. Heterozygotes for *CCR5*Δ32 progress more slowly to AIDS once infected but are not at reduced risk of HIV infection.[85] In contrast, homozygotes for this variant have very substantial resistance to HIV infection, and only a few infected homozygotes have been identified. Important to note, the effects of these genetic variants helped support successful pharmaceutical programs to develop a new class of anti-HIV drug, entry inhibitors, that block the interaction of HIV with these coreceptors. Finally, the high prevalence of *CCR5*Δ32 among northern Europeans is intriguing. Initial analysis of flanking molecular markers suggested that this deletion is found on a rare background haplotype and suggested that it arose less than 3000 years ago.[89] Because this implied that the variant allele had probably been subjected to positive selection, there was much speculation about the possibility that this process had been driven by an infectious disease such as bubonic plague or smallpox; however, subsequent work suggests that the allele may in fact be older than previously thought, with substantially weaker evidence of selection such that the deletion may have been a neutral variant present in European populations prior to arrival of HIV.[90]

Following much interest in identifying additional HIV susceptibility loci, a large collaboration compiled data from 25 cohorts, studies, and institutions including matched HIV-uninfected controls.[91] Crucially, this GWAS found the previous links between HLA and susceptibility can be attributed to frailty bias because cohorts comprising prevalent cases of HIV are enriched for variants associated with prolonged disease-free survival (i.e., long-term nonprogressors). Remarkably, beyond *CCR5*Δ32 homozygosity, this study found no convincing evidence to support earlier associations with HIV susceptibility; the investigators concluded that genetic influences on HIV acquisition either are rare or have small effect sizes.[91]

Persistent Hepatitis

The ability or inability to clear HBV is one of the most striking immunogenetic dichotomies in medicine, with 1% to 12% of infected individuals becoming chronic carriers. Factors such as age of acquisition significantly affect the likelihood of developing chronic carriage, although evidence suggests that host genetic influences also play a role. One relatively small twin study in Taiwan provided evidence that susceptibility to HBV chronic carriage, but not to HBV infection itself, is genetically determined.[12] After early candidate gene studies implicated HLA class I and II genes in HBV clearance and chronic infection, the role for variants in both *HLA-DP* and *HLA-DQ* in the HLA class II region in protection against chronic HBV infection was clearly established by GWASs.[92,93] The precise mechanism remains unclear, although it may reflect a specific effect on viral clearance after antigen presentation. Subsequent GWASs have expanded the list of associated genes to include *CFB* (complement factor B), which lies within the HLA region, in addition to *CD40* located elsewhere.[94–96] Important to note, several other associations reported in these studies were replicated in a further GWAS with a more stringent design in which cases were compared with controls shown serologically to have cleared the virus (based on negative hepatitis B surface antigen (HBsAg) and positive anti-HBs and anti-HBc) rather than unselected members of the general population.[97] In addition to linking the HLA signal to specific alleles by imputation, this study also identified a novel susceptibility locus that appears to function as an expression quantitative trait locus for the *INTS10* (integrator complex subunit 10) gene. Although this gene had not previously been implicated in viral disease, functional studies provided support for a role in HBV pathogenesis including viral clearance through interferon regulatory factor 3 (IRF3).[97]

Spontaneous clearance of hepatitis C virus (HCV) has also been studied with both candidate gene and GWAS approaches, and clear associations have been described with polymorphisms close to the *IFNL3* (interferon lambda 3; also known as *IL28B*) gene in European and African populations.[98,99] Of interest, the same *IFNL3* polymorphism is also associated with response to IFN-α treatment of HCV.[98,100–103] *IFNL3* encodes IFN-λ3, which binds to a different receptor complex than IFN-α but activates the same Janus kinase (JAK) signal transducer and activator of transcription (STAT) antiviral pathway.[104]

Finally, studies involving combined analysis of host and pathogen genome are scarce. However, a study of HCV integrated GWAS data from the host with whole-genome sequencing of the virus and demonstrated that polymorphisms in both *ILNL4* and *HLA* are associated with sequence polymorphisms in the HCV genome. Moreover, remarkably, this study demonstrated that interaction between *ILNL4* genotypes and a specific amino-acid residue in the HCV NS5A protein determined viral load.[105]

Other Viral Diseases

Host genetic variants underlying other viral diseases have also been identified. Dengue has been subject to study by GWAS; polymorphisms within the MHC region and at *PLCE1* (phospholipase C, epsilon 1) on chromosome 10 were identified in association with susceptibility to dengue shock syndrome in a study of Vietnamese children.[106] The gene responsible for the MHC association signal has not yet been fully defined, although the strongest signal appears to originate from the gene *MICB* (MHC class I polypeptide-related sequence B), which encodes an inducible activating ligand that is upregulated in human dengue infection[107] and activates antiviral immunity by natural killer and $CD8^+$ T cells.[108] Further study is required to robustly identify the exact gene underlying this disease association and to explore its role in the pathogenesis of dengue shock syndrome.

Studies of the development of severe influenza A in humans have found association with polymorphism in the gene encoding IFITM3 (interferon-inducible transmembrane protein), an antiviral protein that restricts viral entry into host cells.[109,110] Two different *IFITM3* polymorphisms have been described in association with risk of severe influenza, although these findings have not been replicated across all cohorts.[111] Use of whole-exome sequencing has identified genetic deficiency of *IRF7* (interferon regulatory factor 7) with impaired amplification of type I and III interferon in a single, previously healthy child who developed severe influenza.[112] In a similar approach, mutations in genes encoding RNA polymerase III subunits underlying acute severe varicella zoster infection in four otherwise healthy children have been reported.[113] However, the contribution of such individually rare mutations to influenza and varicella susceptibility at the population level is currently unknown.

Other Infectious Disease Phenotypes

Host susceptibility to a variety of other infectious disease phenotypes have been studied. These range from disease caused by specific pathogens to composite traits such as bacteremia or sepsis, which may be caused be a range of pathogens. However, although an increasing number of susceptibility loci that confer susceptibility to specific pathogens have been identified, it would appear that variants affecting such composite traits either are rare or have small effect sizes, which is perhaps a consequence of their diverse etiologies. For example, a GWAS of susceptibility to bacteremia among African children identified a novel susceptibility locus overlapping a lincRNA on chromosome 7 that was associated with susceptibility specifically to pneumococcal bacteremia[114] and a *STAT4* (signal transducer and activator of transcription 4) variant in association with nontyphoidal salmonella,[115] whereas only the previously reported HbS polymorphism was associated with susceptibility to bacteremia overall.[114] Along similar lines, there are no current GWAS reports of susceptibility loci for sepsis despite substantive efforts to understand this major cause of premature death. Two studies of survival

among patients with sepsis[116,117] reported promising findings including intronic variants in the *FER* (FER tyrosine kinase) gene, encoding a non–receptor tyrosine kinase, that almost halved the risk of death in sepsis due to pneumonia.[116] Independent replication of these findings is still needed, but the *FER* pathway represents a potential therapeutic target.

Various other forms of invasive bacterial infection have been studied, including meningococcal disease. On the basis of this, a key role for complement in immune defense against *Neisseria meningitides* is now well established, polymorphisms in the *CFH* (complement factor H) gene having been robustly associated with susceptibility to meningococcal disease through candidate gene approaches and GWASs.[118,119] Rare mutations in complement components are also well described in association with recurrent meningococcal disease.[120] In addition, a GWAS of susceptibility to enteric fever set in Vietnam and Nepal found the *HLA-DRB1**04:05 allele conferred a nearly fivefold greater resistance against disease than the minor allele.[121] This result is important because it represents one of relatively few links between HLA and bacterial disease in the GWAS era.

Other work has focused on identifying susceptibility genes for neglected tropical diseases including schistosomiasis, visceral leishmaniasis, and rheumatic heart disease. For example, a family linkage study of *Schistosoma mansoni* worm burden in Brazil found evidence of linkage to a region of the long arm of chromosome 5.[122] This region may also be relevant in Senegalese families[123] and encodes genes for numerous cytokines, including *IL4, IL9,* and *IL13*. The same region has been genetically linked to various manifestations of atopy and asthma, consistent with the speculation that a gene selected for resistance to helminthic infections might predispose to asthma or atopy. Furthermore, a GWAS for susceptibility to visceral leishmaniasis in patients from India and Brazil found a robust association with polymorphisms in the *HLA-DRB1*/*HLA-DQA1* class II region.[124] The functional polymorphism within *HLA* has not yet been defined, and the extensive linkage disequilibrium across this region makes precise identification of causative variants challenging.

There has also been interest in delineating susceptibility to rheumatic heart disease, which is triggered by group A *Streptococcus*. The first GWAS, set in the South Pacific, identified a susceptibility locus in the immunoglobulin heavy chain locus fine-mapped to a haplotype of coding variants overlapping the *IGHV4-61* (immunoglobulin heavy variable 4-61) gene.[125] This finding is of interest because it represents the first time the immunoglobulin heavy chain locus, which recombines during B-cell maturation, has been linked to susceptibility to disease in the GWAS era. Another GWAS in Aboriginal Australians confirmed the association with the HLA class II region identified in the candidate gene era.[126] Once again, independent replication and larger studies are needed to confirm these findings.

Finally, studies of new-variant Creutzfeldt-Jakob disease (CJD) attributed to infection with the bovine spongiform encephalopathy agent,[127,128] sporadic CJD,[129] iatrogenic CJD,[130] and kuru[131,132] have all shown strong associations with variation in the *PRNP* (human prion protein) gene. Common variation in *PRNP* genotype appears to have a striking effect on susceptibility to disease; homozygotes for either of the amino acids methionine or valine, commonly found at position 129 in *PRNP,* were markedly more susceptible to disease than heterozygotes.[130,133] The maintenance of this genetic variant over long periods of human evolution has been interpreted as evidence for widespread cannibalism in some early human populations.

SPECIFIC SUSCEPTIBILITY AND RESISTANCE GENES

For several human genes, there is compelling evidence that one or more genetic variants affect susceptibility to infectious pathogens (see Tables 10.2 to 10.4). Specific genes and pathways of interest are discussed in this section.

Blood Groups

ABO blood group associations were investigated in a large number of infectious diseases in early studies. The most striking blood group association is of the Duffy blood group with susceptibility to *P. vivax* malaria. This parasite uses the Duffy blood group antigen as the receptor to invade erythrocytes.[134] The Duffy blood group antigen is a promiscuous chemokine receptor (CD234). Most sub-Saharan Africans are Duffy blood group negative because of homozygosity for a mutation in the promoter of this gene and are completely resistant to almost all *P. vivax* infections. Such individuals also express the Duffy antigen on some other tissues because the promoter mutation that is in the recognition site for an erythroid-specific enhancer is tissue specific.[135] It is unclear whether the Duffy genotypes prevented *P. vivax* from ever entering Africa or whether an earlier and more virulent form of this parasitic infection might have selected the variant in Africa. In addition, as described earlier, a large study of Africans provided compelling evidence that blood group O associates with reduced risk of severe *P. falciparum* malaria.[40] Moreover, a complex copy number variant, involving the replacement of the *GYPB* (glycophorin B) gene with two copies of a novel *GYPA-B* hybrid, corresponding to a serologically distinct blood group antigen known as Dantu, has been associated with a 40% reduction of risk of severe malaria.[45]

Other traits linked to blood groups include cholera; blood group O has been associated with increased severity of cholera symptoms in several studies.[136,137] Moreover, the ability to secrete blood group substances (such as secretor histo-blood group antigens [HBGAs]) into saliva and at other mucosal surfaces is genetically determined. Most individuals are secretors, but about 20% of most populations are nonsecretors because of mutation in the *FUT2* (fucosyltransferase 2) gene.[138] In relatively small studies, nonsecretion was suggested to be associated with susceptibility to some bacterial and fungal infections and with resistance to certain common viral infections.[139,140] Nonsecretor status is clearly associated with susceptibility to recurrent urinary tract infection,[141] and a possible mechanism for this has been proposed.[142] Nonsecretor status was found to protect completely against infection with Norwalk virus in volunteer challenge studies[143] and is associated with substantially reduced risk in the general population.[144] More recent studies have reported symptomatic norovirus infection in nonsecretors, and in vitro binding studies have demonstrated that binding of noroviruses to HBGA is strain specific, with some norovirus strains evolving different HBGA-binding targets in an attempt to evade genetically determined host resistance.[145] However, overall, apart from associations with norovirus and urinary tract infections, there is a need for larger studies to show compelling evidence of association.

Human Leukocyte Antigens

The pivotal position of HLA in the initiation and regulation of immune responses, together with their well-documented polymorphism, has led to numerous studies of their influence on infectious disease susceptibility.

After early evidence from candidate gene and linkage approaches, GWAS of infectious disease have robustly confirmed the key role for HLA in susceptibility to many of these conditions.[17] Evidence that particular HLA types are associated with altered susceptibility to infectious disease supports the view that the remarkable diversity of HLA types has been generated and maintained through natural selection by infectious pathogens. The relatively modest magnitude of the reported associations, compared with some HLA associations with autoimmune disease, is in keeping with this possibility. Small selective effects can, over time, markedly change allele frequencies. The observation that cellular immune responses are restricted by HLA molecules suggested an attractive mechanism whereby heterozygosity for HLA type might be evolutionarily advantageous.[146] Heterozygotes should be able to recognize more peptide epitopes in a foreign pathogen than homozygotes, permitting a more protective immune response. For example, a protective effect of heterozygosity has been observed for HLA class II antigens with regard to clearance of HBV,[147] for HLA class I antigens in HIV disease progression,[74] and in human T-cell lymphotropic virus type 1 (HTLV-1) proviral load.[148]

Another feature of HLA associations with infectious disease is that associations often vary geographically. In some cases, this may result from geographic strain variation in the infectious pathogen, and an association between HLA type and the strain of parasite causing infection has been reported in malaria.[32] More detailed analysis of the mechanisms

of identified associations should further explain this population diversity and provide insights into the immune mechanisms of protection and pathogenesis.

Mannose-Binding Lectin

Mannose-binding lectin (MBL) is a serum protein that plays a role in innate immunity. It is a collagenous lectin with at least two important roles in host defense.[149] It binds to sugars, particularly *N*-acetylglucosamine and mannose, on the surface of microorganisms and facilitates their opsonization by macrophages. It also activates complement by means of two MBL-associated serine proteases. Inactivating mutations of the *MBL2* gene are quite prevalent, with frequencies of up to 40% in various populations. Three single amino-acid changes are found at codons 52, 54, and 57, each of which leads to a substantial reduction in MBL concentration in heterozygotes. Homozygotes and compound heterozygotes for these variants have absent or extremely low MBL levels in serum; variation in the promoter of the gene has less marked functional effects.[150]

The potential role of MBL deficiency in susceptibility to human infection has been extensively investigated. Early case reports and small-scale studies suggested that MBL deficiency might predispose to a wide variety of infectious phenotypes,[151] but most subsequent studies of individual diseases, including meningococcal disease, malaria, TB, persistent HBV infection, and sepsis have failed to show clear associations.[152–154] An exception is that homozygotes for *MBL* codon changes appear to be more susceptible to invasive pneumococcal disease (IPD), with a 2.5-fold increase in risk.[155,156] A large, population-based longitudinal study of adults failed to show an association between MBL deficiency and excess mortality or morbidity,[157] and the degree to which MBL deficiency influences susceptibility to infectious disease remains controversial.

Toll-Like Receptor Pathway Genes

With increasing definition of the importance of the TLRs, NOD-like receptors (NLRs), and retinoic acid–inducible gene (RIG)-like helicases (RLHs) and their signaling pathways in detecting pathogens and triggering and amplifying innate immune responses,[158,159] analyses of genetic variation in these pathways have increased. Both rare mutations and common polymorphisms have been reported in genes encoding TLR pathway members in association with different infectious disease phenotypes (Fig. 10.1). Rare mutations within four genes in the TLR pathway have been described in individuals with primary immunodeficiencies characterized by episodes of IPD: *IKBKG* (inhibitor of nuclear factor kappa B [NF-κB] kinase subunit gamma; also known as NF-κB essential modulator [*NEMO*]), *NFKBIA* (NF-κB inhibitor alpha), *IRAK4* (IL-1 receptor associated kinase 4), and *MYD88* (myeloid differentiation primary response 88).[160] Mutations in *NEMO* and *NFKBIA* interrupt not only the TLR pathway but also multiple other pathways that converge on NF-κB, and the resulting immunodeficiency conditions are characteristically severe and with a broad spectrum, including encapsulated bacteria, atypical mycobacteria, fungi, and viruses.[160] In contrast, mutations in *IRAK4* and *MYD88* interrupt only TLR and IL-1 receptor signaling and lead to a narrow-spectrum immunodeficiency characterized by childhood susceptibility to pyogenic encapsulated bacteria, particularly recurrent IPD.[160] Such rare mutations appear to have little impact on susceptibility to pneumococcal disease at the population level in adults, however.[161] Other rare TLR pathway mutations have been reported in association with susceptibility to severe viral disease in otherwise immunocompetent children—for example, mutations in TLR3 signaling genes and herpes simplex virus 1 (HSV-1) encephalitis (HSE), and IRF7 deficiency and severe influenza.[112,162]

Coding polymorphisms are observed relatively frequently in some of the TLRs, with functional consequences. A TLR1 coding variant has been implicated in leprosy[59,163]; coding changes in TLR4 in malaria[164]; and a change in the flagellin receptor TLR5 in *Legionella* pneumonia.[165] All of these implicated changes have functional consequences for receptor signaling. Polymorphisms in *NFKBIA* and the related genes *NFKBIZ* (NF-κB inhibitor zeta) and *TONSL* (Tonsoku-like, DNA repair protein; also known as *NFKBIL2*), which encode IκBs, have also been found to be associated with risk of IPD.[166–168] A common polymorphism in the Mal/TIRAP adaptor protein that binds to TLR2 and TLR4 appears to render the variant nonfunctional, and heterozygotes were found to be less frequent in series of patients with bacteremia, IPD, TB, and malaria.[54] Healthy volunteer individuals with the protective heterozygote Mal/TIRAP genotype generate intermediate levels of inflammatory cytokine signaling after intravenous challenge with lipopolysaccharide, a gram-negative bacterial cell wall component that is recognized by TLR4.[169] Taken together, these findings suggest that both common and rare genetic variation in this key signaling pathway may affect the risk of multiple major infectious diseases and, furthermore, that extremes of TLR signaling may be detrimental.

EVOLUTIONARY PERSPECTIVE

From an overview of the information currently available, it seems likely that susceptibility to most infectious diseases will prove to be highly polygenic. The contrary view—that there may be a few major single genes for many infectious diseases—has been suggested by complex segregation analysis of multicase families[170] and may be incorrect,[171] despite the occurrence of well-documented, diverse, but very rare monogenic phenotypes.[172] The existence of multiple genes affecting infectious disease susceptibility probably simply reflects the major role that infectious pathogens have played in shaping variation in the human genome through natural selection. Indeed, it has been found that genes playing a role in host defense against infectious pathogens evolve at a higher rate than any other class of genes.[173] Also, initial analysis of the distribution of variants associated with infectious diseases between exons and regulatory regions of genes indicated that relevant variants are more often found in exons for infectious than for noninfectious disease.[174] This likely reflects the impact of selection for resistance amplifying the frequencies of many exonic mutations and has led to increasing use of whole-exome sequencing in the field. Natural selection for resistance to infectious pathogens may also explain why the observed effects of most individual genes are relatively modest in magnitude. In the absence of a counterbalancing selective force, alleles that markedly increased or decreased the risk of a major infectious disease would have been quickly eliminated or selected to very high frequency, abolishing polymorphism. Polygenic susceptibility has also been found in extensive analyses of the genetic basis of susceptibility to autoimmune diseases in both humans and mice.[175]

Given the pressure for fixation of selectively advantageous variants, one of the major questions in evolutionary biology has related to the mechanisms that maintain substantial genetic diversity in populations. Some aspects of this question are particularly well addressed in human populations, in which the host genome and the infectious pathogens have been characterized in most detail. Heterozygote advantage is an attractive mechanism by which two alleles may be maintained in a population, and it is classically exemplified by the HbS polymorphism and resistance to malaria. However, this appears to be a relatively unusual means of maintaining genetic diversity, and other mechanisms, such as frequency-dependent selection and fluctuations in selection, may be more generally important.

Another relevant factor is likely to be variation in the genome of infectious pathogens and in the microbiome. Increasing attention is being paid to specific interactions between variants of the host and the pathogen. Particular HLA types have been associated with disease caused by specific serotypes of human papillomavirus,[176] HLA type may also influence the strain of *P. falciparum* causing malaria,[32] and HIV may evolve away from prevalent HLA class I types.[80] Furthermore, immunologic mechanisms have been identified that may underlie interaction between competing strains of microorganisms.[32] There may be exquisite specificity in some of these host-pathogen interactions, leading to coevolution of genetic variation in host and pathogen. This implies that individual susceptibility to disease is the result of a variety of host and pathogen genetic factors that have been tempered by a constellation of environmental variables. This dynamic evolutionary perspective implies that the genes affecting susceptibility to an infectious disease may show significant interpopulation heterogeneity resulting from geographic variation in the pathogen genome, in the environment, and in the frequencies of interacting genes in the host, a prediction well supported by available data on malaria susceptibility.

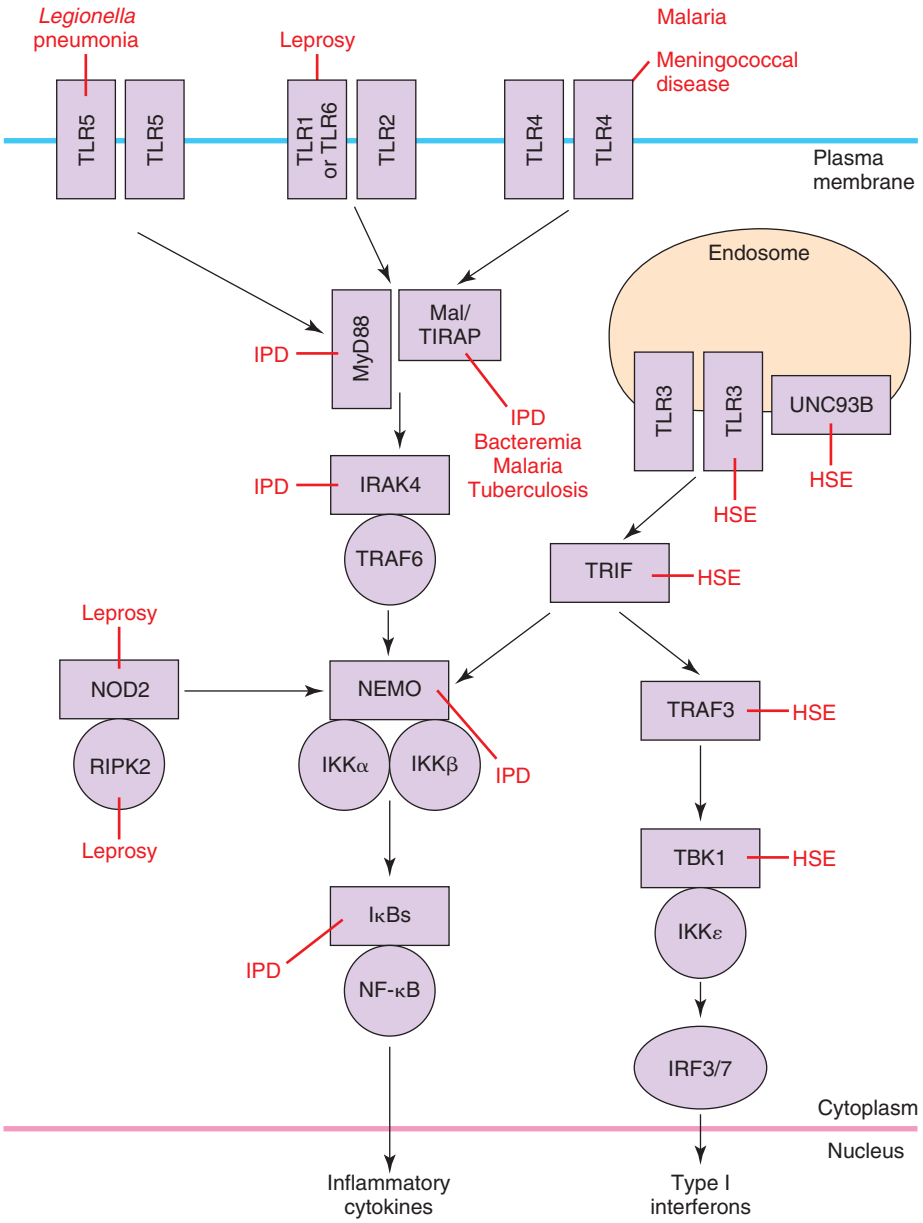

FIG. 10.1 Toll-like receptor signaling and infectious disease. Examples of pathway components for which genetic variation is associated with specific human infectious diseases are highlighted. *HSE,* Herpes simplex virus encephalitis; *IκBs,* inhibitors of NF-κB activation; *IKKα, β, ε,* inhibitory kappa (alpha, beta, epsilon) kinase; *IPD,* invasive pneumococcal disease; *IRAK4,* interleukin-1 receptor-associated kinase 4; *IRF3/7,* interferon regulatory factor 3/7; *Mal,* myeloid differentiation primary response gene 88 *(MyD88)* adaptor-like protein; *NEMO,* NF-κB essential modulator; *NF-κB,* nuclear factor kappa B; *NOD2,* nucleotide-binding oligomerization domain containing 2; *RIPK2,* receptor-interacting serine-threonine (protein) kinase 2; *TBK1,* TANK (TRAF-associated NF-κB activator)–binding kinase 1; *TIRAP,* Toll–interleukin-1 receptor (TIR) domain adaptor protein; *TLR,* Toll-like receptor; *TRAF,* tumor necrosis factor receptor-associated factor; *TRIF,* Tir-domain adaptor inducing interferon-β; *UNC93B, unc-93* homologue. *(Modified from Chapman SJ, Hill AV. Human genetic susceptibility to infectious disease.* Nat Rev Genet. *2012;13:175–188.)*

APPLICATIONS

Most of the apparent genetic component to any infectious disease cannot be accounted for by the polymorphisms and associations identified to date. The increased use of GWAS and more recently whole-exome sequencing has, however, greatly enhanced the power of genomic analyses. There are several potential advantages for the use of modern molecular genetics to understand genetic susceptibility more fully. An obvious application is in risk prediction, which might influence behavior, the use of prophylactic antimicrobials, or immunization or travel patterns. In the future, it may be possible to offer a genetic profiling test to estimate individual susceptibility to particular pathogens. This may be particularly relevant to individually rare or even unique, highly penetrant genetic variants that may be important contributors to risk of developing severe infectious diseases.[177]

Another application is in the understanding of particular pathways used in host resistance to infection. For example, the HLA-B53 association with resistance to malaria[30] supported a protective role for $CD8^+$ T cells in this disease, encouraging efforts to develop vaccines that elicit this immune response.[178]

A third application will be in the identification of molecules and pathways as targets for pharmacologic intervention. Demonstration of the almost complete resistance to HIV infection of homozygotes for a deletion in the *CCR5* gene underpinned successful attempts to develop pharmacologic blockers of this viral coreceptor. In another example, a whole-exome sequencing approach to patients with recurrent respiratory infection and bronchiectasis has identified underlying mutations that increase phosphoinositide 3-kinase delta (PI3Kδ) activity[179]; isoform-selective PI3Kδ inhibitors have been developed for the treatment of

hematologic malignancy and may also prove effective in this immunodeficiency.[180]

Finally, an increased understanding of the genetic basis of infectious disease may uncover genetically distinct subgroups of patients that are clinically indistinguishable but may respond differentially to specific therapies. The potential of genomics to identify "molecular subphenotypes" may have particular application in the use of immunomodulatory agents to treat excessive inflammation in the setting of infectious diseases—for example, in sepsis or severe forms of community-acquired pneumonia or meningitis. Clinical trials in these areas have been largely disappointing, which may reflect the grouping together of patients with unidentified, distinct inflammatory response profiles; tailoring specific immunomodulatory therapy to individual patients on the basis of their genetically determined inflammatory phenotype may add a new level of precision to clinical trials and pave the way for personalized medicine.

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The complete reference list is available online at Expert Consult.

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11 Nutrition, Immunity, and Infection

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Although the effects of malnutrition have been recorded for centuries, the mechanistic links between nutrition, immunity, and resistance to infection have been defined only over the past several decades.[1,2,3] The relationship between nutrition and immunity is most striking in the developing world, where protein-energy malnutrition (PEM) occurs. In both developed and developing countries, however, specific micronutrient deficiencies are relatively common, even where PEM syndromes are rare. Both PEM and micronutrient deficiencies are associated with immune dysfunction and infection risk. Malnutrition not only affects immune function but also virulence of infectious agents, progression of chronic infections such as mycobacteria infections and human immunodeficiency virus (HIV), and transcriptional regulation of inflammatory genes that may determine the outcome of infections.[1,2]

Overnutrition—obesity—has emerged as a major public health threat, particularly in the United States in the past 2 decades as noted in reports from the Centers for Disease Control and Prevention (www.cdc.gov/obesity/data/index.html). According to the World Health Organization (WHO), obesity and overweight are evolving issues in developing countries, where some populations, usually in urban areas, are experiencing rapidly increasing numbers of overweight people, while other populations, usually in rural areas, continue to experience widespread undernutrition.[4] Obesity, typically defined as a body mass index (BMI) of 30 or higher, has a complex association with infection risk and outcome, including increasing risk for developing diabetes. More recent developments are highlighted in the following sections.

This chapter explores the relationships among nutritional factors, host immunity, and the virulence of pathogens and their ties to the management of infectious diseases.

EPIDEMIOLOGY OF MALNUTRITION

The WHO estimates that 462 million people were underweight and more than 1.9 billion adults were overweight or obese in 2015.[5] More than 30% of residents in developing nations are affected by hunger and malnutrition, with PEM cited as the primary cause of immunodeficiency worldwide. Among children younger than 5 years of age, five infectious diseases—acute respiratory infections, diarrhea, malaria, measles, and HIV/acquired immunodeficiency syndrome (AIDS)—account for more than 50% of all deaths, and about half of those deaths are due to malnutrition.[2,6,7] In developed nations, at-risk populations for malnutrition, primarily micronutrient malnutrition rather than PEM, include children, elderly adults, pregnant women, people experiencing homelessness, people who abuse alcohol, patients affected by acute and chronic illnesses including HIV/AIDS and end-stage renal disease, and people with dietary restrictions such as patients with anorexia nervosa or bulimia and consumers of vegan diets.

Malnutrition can be broadly defined as a decrease in nutrient reserve; under this definition, undernutrition is prevalent even in the United States, affecting up to 15% of ambulatory outpatients, 25% to 60% of patients in long-term care, and 35% to 65% of hospitalized patients.[8] A major reason for the wide range of rates cited is that many definitions for malnutrition exist. Several biochemical markers of nutritional status can provide a general indication of nutritional reserve[9] including visceral proteins (albumin, prealbumin, and transferrin), but such low levels of visceral protein are not specific and may have other causes including hepatic or renal disease and increased capillary permeability. Global indicators of cell-mediated immune function are commonly used to assess malnutrition and can be estimated by total lymphocyte count and delayed-type hypersensitivity testing with a series of common antigens. Compromise of cell-mediated immunity due to malnutrition is suggested by a total lymphocyte count less than 1000/mm^3 or by lack of skin induration greater than 5 mm above glycerin control at 48 hours to recall antigens such as mumps, unless another cause of lymphocyte dysfunction is present.[9] However, these tests should be interpreted cautiously during an acute illness because cell-mediated immunity may be depressed in the absence of malnutrition.

The Institute of Medicine of the National Academy of Sciences and the Food and Nutrition Board provide dietary reference intakes including recommended dietary allowances for overall protein, calories, and specific nutrients including age-specific, sex-specific, and condition-specific recommendations.[10] The dietary reference intakes and recommended dietary allowances should guide clinicians in their assessments of patients' nutritional status and in their plans to address macronutrient malnutrition as well as micronutrient deficiencies.

MALNUTRITION AND IMMUNE FUNCTION

PEM describes nutritional macrodeficiency syndromes including marasmus (deficiency of calories), kwashiorkor (deficiency of protein), nutritional dwarfism in children, and wasting syndromes in adults. Primary PEM, caused by inadequate nutrient intake, typically affects children and elderly adults, and effects of brief PEM are typically reversible with nutritional therapy. However, primary PEM can result in serious and irreversible changes in organ function and growth. Secondary PEM results from illnesses, injuries, or treatments that alter appetite, digestion, absorption, or metabolism and has similar effects as primary PEM. Although the nutritional deficits of patients with PEM due to gastrointestinal tract dysfunction often can be restored to normal if adequate nutritional support is provided by dietary supplements, enteral tube feeding, or parenteral nutrition, wasting disorders such as cancer or AIDS are characterized by involuntary weight loss, often despite increased caloric intake. In wasting diseases, alterations in metabolism result in greater loss of muscle tissue than would be expected from reductions of caloric intake alone, and muscle mass is not restored by nutritional supplementation unless the underlying inflammatory disease is treated. Weight gain that occurs as a result of nutritional support in patients with these syndromes usually represents increases in fat mass and body water without significant change in lean tissue.

PEM has been associated with a number of impairments in immune responses.[1,2,11,12] Documented abnormalities of innate immunity include reduced production of cytokines, reduced phagocytosis, interruptions in the integrity of physical barriers and diminished quality of mucus, and reductions in complement components (especially C3 and C5). Alterations in adaptive immunity include reduced or delayed cutaneous hypersensitivity to recall and new antigens and reductions in $CD4^+$ and $CD8^+$ T-cell subsets, $CD4^+/CD8^+$ ratio, lymphocyte proliferative capacity, immunoglobulin (Ig) G (IgG), and secretory IgA. In addition, malnourished children and elderly adults demonstrate elevated baseline biomarkers of inflammation such as interleukin (IL)-6.

SPECIFIC NUTRIENTS AND THEIR ROLES IN IMMUNITY

A complete review of nutrients and their roles in immune function is beyond the scope of this chapter. Several outstanding reviews are available, and the reader is referred to these publications for additional

information.[11–13,14,15] A very brief review of major micronutrients and their roles in immunity follows.

Fat-Soluble Vitamins

Vitamin A

Vitamin A is a subclass of the retinoic acids, a family of lipid-soluble compounds that includes retinols, β-carotenes, and other carotenoids. Retinol, or preformed vitamin A, is the most biologically active form; it is found primarily in animal food sources or can be synthesized from carotenoids. The important role of vitamin A in immune system function is well established.[16] Vitamin A deficiency can affect host immunity through direct actions on immune cell function and through indirect effects on epithelial cell differentiation and, consequently, host barrier defenses. Vitamin A deficiency results in reduced mitogen-stimulated T-cell proliferation and antigen-specific IgA and IgG production, impairs the ability of $CD4^+$ T lymphocytes to stimulate Th2 responses (B-cell antigen-specific IgG_1 responses), and limits the ability of neutrophils to phagocytose bacterial pathogens.[16,17]

Vitamin A supplementation has been examined in a number of randomized, double-blind, placebo-controlled trials of undernourished and malnourished children in developing nations. Antibody-mediated responses are impaired in individuals with vitamin A deficiency; in some (but not all) studies, supplementation has improved antibody titer responses to measles vaccine, sustained gut integrity, and lowered the incidence of respiratory tract infections.[18] A Cochrane Review found that oral vitamin A supplementation in children in developing countries reduced overall mortality (relative risk [RR], 0.76; 95% confidence interval [CI], 0.69–0.83) and diarrheal-associated mortality (RR, 0.72; 95% CI, 0.57–0.91). Furthermore, supplementation reduced the incidence of diarrhea (RR, 0.85; 95% CI, 0.82–0.87) and measles (RR, 0.50; 95% CI, 0.37–0.67) and reduced the prevalence of vision problems in children 6 months to 5 years of age.[19]

In light of these trials and others demonstrating beneficial effects of vitamin A supplementation on immune function and its efficacy in preventing infection, the WHO recommends that vitamin A supplementation be provided to young children and mothers residing in developing nations even in the absence of signs or symptoms of deficiency. Implementation of this program has been one of the great WHO success stories.

Although studies suggest an association between vitamin A deficiency and increased mortality, disease progression, and vertical transmission of HIV, vitamin A supplementation has not been shown to improve outcomes in HIV-infected populations.[20] Similarly, vitamin A supplementation does not improve outcomes in patients with active tuberculosis. Therefore, caution should be exercised in applying recommendations to other populations, especially populations at much lower risk for vitamin A deficiency. Excessive intake of vitamin A can produce acute toxic manifestations (headache, vomiting, stupor, and papilledema). Chronic toxicity is associated with weight loss, nausea, and vomiting; dryness of the mucosa of the lips; bone and joint pain; hyperostosis; and hepatomegaly with parenchymal damage and fibrosis. In the 1990s two large trials evaluating the role of β-carotene in lung cancer prevention observed an increased risk for lung cancer in subjects (male smokers) receiving β-carotene.[21,22] The reasons for this finding of increased risk remain unclear, emphasizing the need for further study on the role of retinoids in human health and immunity before widespread supplementation with retinoid precursors can be recommended in well-nourished populations.

Vitamin D

Vitamin D deficiency is remarkably common even in developed nations, particularly in areas with reduced exposure to sunlight or reduced dairy intake or both. However, as with other vitamins, the prevalence depends on which metabolite is measured (25-hydroxyvitamin D or 1,25-dihydroxyvitamin D) and the cutoff value used to identify deficiency. Most population surveys use serum 25-hydroxyvitamin D levels because they are easy to measure and widely available, but the definition of deficiency depends on the outcome measured, and no standard is uniformly accepted.[23] Regardless of the standard used, women more than men, older adults more than young adults and children, and people of races other than white are more likely to be deficient.[24] Older institutionalized adults and people with comorbidities that reduce their sunlight exposure or oral intake of fortified dairy products are also at greatly increased risk.

The role of vitamin D (1,25-dihydroxycholecalciferol) in immune response has been recognized for decades but has received increased attention more recently as the importance and ubiquity of vitamin D receptor expression have been shown and its role in host defenses against mycobacterial disease has been better defined. Vitamin D suppresses many adaptive immune responses (T-cell proliferation, antibody production) and some innate immune responses (dendritic cell costimulation and cytokine secretion). In contrast, vitamin D is essential for proper function of other aspects of the innate immune system, particularly in macrophage-mediated defenses.[15] This appears to be principally important in Toll-like receptor–mediated defenses against intracellular pathogens such as *Mycobacterium tuberculosis*.[25–27]

Individuals with low 25-hydroxyvitamin D levels appear to be more susceptible to tuberculosis and at higher risk for progression from infection to disease.[28,29] However, vitamin D supplementation trials have not demonstrated an improvement in tuberculosis-related mortality or sputum smear conversion rates.[30–32] Additional data suggest that vitamin D deficiency is associated with impaired wound healing and predisposes to periodontal disease[15,26] and upper respiratory tract infection,[33] although no trials to date document benefits of supplementation. Ongoing studies of vitamin D supplementation for the reduction of infection-related, cancer-related, and cardiovascular disease–related morbidity and mortality should provide additional insight into the complex role of vitamin D in human disease.

Vitamin E

Eight closely related fat-soluble compounds (four tocopherols and four tocotrienols) all exhibit vitamin E biologic activity, but α-tocopherol is the most active form, is abundant in many foods, and is most widely available as a supplement. Vitamin E is one of a group of antioxidants that scavenge free radicals formed in redox reactions throughout the body.[34] Vitamin E activity is complemented by that of selenium, which, as a constituent of glutathione peroxidase, also metabolizes peroxides before they cause membrane damage.

Hypovitaminosis E resulting from a deficient diet is uncommon in the developed world and occurs almost exclusively in association with severe fat malabsorption, in low-birth-weight infants, and in patients with rare genetic disorders such as abetalipoproteinemia. Vitamin E supplementation has multiple immunologic effects including enhanced T-cell proliferation, perhaps mediated by suppressed production of prostaglandin E_2, a T-cell–suppressive compound, and enhanced delayed-type hypersensitivity responses.[13,14,34] Clinical effects of vitamin E supplementation have been studied mainly in elderly subjects (see "Older Adults"). There has been extensive research on the biology of immune senescence and the effects of vitamin E on age-related immune dysfunction.[34] Although beneficial effects of vitamin E supplementation have been suggested in a variety of noninfectious diseases such as macular degeneration and hepatic steatosis, the findings of increased risk for prostate cancer in healthy men receiving vitamin E supplementation will impact future intervention trials with vitamin E.[35]

Water-Soluble Vitamins

Vitamin C

Vitamin C (ascorbic acid) can augment a number of biochemical reduction reactions involving iron and copper and acts as an enzymatic cofactor and antioxidant in physiologically important processes including fatty acid transport, collagen synthesis, and neurotransmitter formation.[13,14] In humans, vitamin C deficiency is manifested as scurvy, which is due to impaired collagen synthesis, with signs including capillary fragility, bleeding gums, delayed wound healing, and impaired bone formation. Animal studies and a limited number of human studies have suggested an immunomodulatory role for ascorbic acid, with increased resistance to viral illness and some anticarcinogenic effects, perhaps via reduced T-cell apoptosis.[13,14]

Many trials have been performed to determine whether vitamin C may be useful to prevent or treat the common cold. A comprehensive review