IgA nephropathy (IgAN), initially described by Jean Berger in 1968, is the most frequent primary glomerulopathy worldwide, leading to ESRD in up to 40% of patients within 20 years after diagnostic biopsy. The diagnosis is on the basis of finding IgA as the dominant or codominant immunoglobulin in the glomerular immunodeposits. The IgA is exclusively of the IgA1 subclass. Complement component C3 is usually present in the same distribution as IgA, and the immunodeposits may contain IgG, IgM, or both. Recent studies have confirmed an autoimmune nature of IgAN. The pathophysiology of the disease is considered to be a four-hit mechanism. The first hit is characterized by increased levels of circulating polymeric IgA1 with aberrant O-glycosylation of the hinge region. These molecules lack galactose on some O-glycans (galactose-deficient IgA1 [Gd-IgA1]), thus exposing N-acetylgalactosamine. The second hit is the presence of circulating Gd-IgA1–binding proteins that are considered to be mainly glycan-specific IgG or IgA1 autoantibodies targeting terminal N-acetylgalactosamine in the hinge region of Gd-IgA1. The third hit is the formation of Gd-IgA1–containing circulating immune complexes, some of which may deposit in the glomeruli and incite injury (hit four), potentially leading to chronic kidney damage. Other proteins, such as the soluble form of Fcα receptor, can bind Gd-IgA1 to create complexes, although it is not clear whether such circulating protein complexes would activate complement.

Complement activation can generally occur through three different pathways (Figure 1). The first one, the classical pathway, is activated by IgG (IgG1, IgG2, and IgG3 but not IgG4) or IgM containing immune complexes through binding by C1q. C1qrs is then assembled and cleaves complement components C2 and C4 to form the C4b2a enzyme complex, a C3 convertase.

The alternative pathway is initiated constantly by spontaneous hydrolysis of C3 (tickover), leading to the formation of C3(H2O)Bb, which cleaves C3 into C3a and C3b. The alternative pathway is also activated when the classical or lectin pathway is blocked or when other pathways are induced. This initiates the formation of C3 convertase, which cleaves C3 into C3a and C3b. C3a is a potent inflammatory mediator, while C3b is a C3 convertase.
Figure 1. Three pathways of complement activation. The classical pathway is triggered by IgG- and/or IgM-containing immune complexes. The alternative pathway is constantly initiated by spontaneous hydrolysis of C3 [C3b(H2O)] that is efficiently powered by the covalent attachment of C3b on an activating surface. The lectin pathway requires a particular sugar moiety pattern (N-acetylglucosamine [GlcNAc]) to be recognized and bound by MBL, leading to a classical pathway-like activation cascade. Each pathway leads to formation of a C3 convertase. The addition of C3b to the C3 convertase creates a C5 convertase that, in turn, triggers the assembly of the membrane attack complex (C5b-9), which is also known as the terminal pathway complete complex. Regulatory factors are in red. CR1, complement receptor 1; FD, factor D; MAC, membrane attack complex; MCP, membrane cofactor protein; P, properdin.

The activation of the lectin pathway is on the basis of the covalent binding of C3b to activating surfaces (e.g., bacterial surface) followed by cleavage of factor B (FB). In the presence of factor D and properdin, this process leads to formation of the alternative pathway C3 convertase (C3bBb). Properdin promotes association between C3b and FB, thus stabilizing the alternative pathway C3 convertase (C3bBb). Factor H (FH) and factor I (FI) tightly negatively regulate the alternative pathway in solution and on self-cellular membranes, whereas decay accelerating factor (DAF; CD55), membrane cofactor protein (CD46), and CD59 regulate the alternative pathway on only self-cellular membranes.

The activation of the lectin pathway is on the basis of recognition of microbial cell surface carbohydrates by mannan-binding lectin (MBL) or ficolin. This process leads to cleavage of C2 and C4 to form the classical pathway C3 convertase C4bC2a.

Activation of each of three complement pathways produces a C3 convertase that accounts for the cleavage of C3 into C3a (an anaphylatoxin) and C3b; the addition of C3b then turns C3 convertases into C5 convertases. This last product ultimately leads to formation of the terminal pathway complete complex consisting of C5b, C6, C7, C8, and C9 (C5b-9) that inserts into the lipid bilayer of cellular membranes. For nucleated cells, the amount of C5b-9 is rarely sufficient to induce the lysis of the cells, but these sublytic quantities are nevertheless deleterious. Sublytic C5b-9 on podocytes can increase release of various proteases, oxidants, cytokines, and components of extracellular matrix that disrupt the function of the glomerular basement membrane and induce apoptosis and glomerular scarring. Mesangial cells are also affected by C5b-9, which is shown by fibronectin synthesis, production of TGFβ and IL-6, or cellular apoptosis in a rat model of mesangioproliferative nephritis.

The role of complement in the pathogenesis of IgAN has been suspected since the discovery of the disease, because the components of complement activation have been commonly detected in the renal biopsy specimens. Here, we will review the understanding of the mechanisms of complement activation in IgAN and its role in development of the disease.

COMPLEMENT PATHWAYS IN IgAN

Alternative Pathway

C3 mesangial codeposition is a hallmark of IgAN, being present in >90% of patients. Properdin is codeposited with IgA and C3 in 75%–100% of patients and FH in 30%–90% of patients. Complement activation through the alternative pathway leads to accumulation of FI–, FH–, and complement receptor 1–induced C3 proteolytic fragments (e.g., iC3b and C3d) (Figure 2). Several centers have described increased plasma levels of these fragments in patients with IgAN, which were associated with severity of the histologic lesions in one study and progression of the disease in another study. Circulating levels of C3 breakdown products are also increased in 70% of pediatric patients with IgAN.

IgA has been shown to activate the alternative pathway in vitro. The Fab fragment of immobilized human IgA can activate C3 in alternative pathway–specific conditions. Notably, the hinge region of IgA1 was not critical in this process, but the polymeric form of IgA was necessary. Another study has reported that, although IgA1 and its Fab fragments reduced complement activation through the classical pathway mediated by IgG antibodies, surface-bound IgA1 activated the alternative pathway. The mechanism of IgA–mediated alternative pathway activation remains poorly understood but is thought to require stabilization of the C3 convertase.
Lectin Pathway
Recent data revealed the ability of polymeric IgA to activate the lectin pathway. Polymeric IgA can bind MBL in vitro in a Ca²⁺-dependent manner, probably through the N-linked glycans, because IgA1 and IgA2 share this property. MBL is codeposited with IgA in 17%–25% of IgAN biopsies; studies showed correlation of MBL codeposits with severity of the disease. Mesangial deposition of C4 (especially C4d) and C4-binding protein is not rare in IgAN and was initially considered to be a consequence of classical pathway activation. However, these C4 deposits are probably due to activation through the lectin pathway, because they are associated with codeposition of MBL–associated serine proteases (MASPs; MASP-1 and MASP-2) and t-ficolin.

Classical Pathway
Evidence of classical pathway activation, such as C1q deposits, is usually lacking in kidney biopsy specimens of patients with IgAN. Components of this pathway are rarely observed in the glomeruli (<10% of biopsy specimens), and their deposition is limited to patients with poor clinical outcomes. In this setting, activation of the classical pathway may not be specific for the disease but rather occurs in highly damaged kidneys. C4 (and C4d) and C4-binding protein, present in the mesangial area in approximately 30% of patients' biopsies and initially thought to be markers of classical pathway activation, are more likely products of activation of the lectin pathway.

TERMINAL PATHWAY
Regardless of which pathway of complement activation is in play, generation of C5b triggers the terminal sequence that culminates in formation of C5b-9. Mesangial deposits of this terminal pathway complete complex, also called membrane attack complex, are commonly observed in IgAN on the basis of detection of C9 neoantigen corresponding to the C5b-9 complex. Urinary excretion of the soluble form of the complex is elevated in patients with IgAN, likely because of complement activation in urinary space. The blockade of C5 could, in this respect, represent a promising therapy for some patients. The first case of a child with rapidly progressive IgAN who benefitted from treatment with a monoclonal anti-C5 antibody, eculizumab, was recently published.

INHERITED PARTIAL DEFICIENCIES OF ALTERNATIVE PATHWAY PROTEINS
Inherited partial deficiencies of several complement component or regulatory proteins have been found in some patients with IgAN. Three instances are related to regulators of the alternative pathway: properdin, FH, and FI. These findings may suggest that lower than normal serum levels of alternative pathway proteins are sometimes related to development or clinical expression of IgAN. A recent sequencing study of 46 patients with IgAN did not find mutations of the FH gene although the conclusions are limited by a small cohort size. Another small study identified no mutation of CFH, CFI, and Membrane Cofactor Protein genes in 11 patients with IgAN presenting with severe thrombotic microangiopathy. Clearly, larger-scale sequencing efforts will

Figure 2. C3 proteolytic cascade. The hydrolysis of C3 leads to the release of activation products C3a, a potent anaphylatoxin, and C3b, which contains a highly reactive thioester bond that can covalently bind activating surfaces, such as a bacterial wall. This attachment of C3b initiates a powerful amplification system from the formation of the C3 convertase to the subsequent proteolysis of new molecules of C3, allowing more C3b to bind to the surface. This amplification is controlled by regulator molecules, such as FI, FH, and complement receptor 1 (CR1), that degrade C3b into products that cannot contribute to the formation of the C5 convertase. Detection of these inactive breakdown products (iC3b, C3c, C3dg, and C3d) is considered evidence of activation of C3. The numbers, in kilodaltons, represent the molecular masses of the corresponding polypeptides. MCP, membrane cofactor protein.
be needed to systematically assess the contribution of any rare variants in these genes to the risk of IgAN.

**COMPLEMENT FACTOR H–RELATED GENES 1 AND 3 GENE DELETION: A ROLE OF COMPLEMENT FACTOR H–RELATED GENES 1 AND 3 PROTEINS IN REGULATION OF COMPLEMENT ACTIVATION**

Large international genome–wide association studies have identified several genomic regions associated with the risk of IgAN.51–54 Apart from loci in the HLA region, these studies also associated the disease with SNP rs6677604 (Chr. 1q32), which represents a proxy for the deletion of complement factor H–related genes 1 and 3 (CFHR1/3). In the latest meta-analysis of 20,612 individuals, this variant was confirmed to have a log-additive protective effect; inheritance of a single allele reduces the disease risk by 26% (odds ratio=0.74), while inheritance of two alleles reduces the disease risk by 45% (odds ratio=0.55).54 Another analysis of this protective effect reveals that this deletion correlates with a higher plasma FH level and a lower plasma C3a level as well as less mesangial C3 deposition.55 The allelic frequency of the deletion exhibits marked differences across populations worldwide. The deletion has the highest frequency in individuals of African ancestry (e.g., 55% in DNA samples from Nigeria) and the lowest frequency in samples from South America and East Asia (0%–5%). The allelic frequency of the CFHR1/CFHR3 gene deletion in the United Kingdom and other European populations ranges from 18% to 24%.56,57

CFHR genes are located downstream of the gene encoding FH and consist of five genes (CFHR1–CFHR5). The CFHR proteins share a high sequence homology with FH, with a short consensus repeat (SCR) repetition frame.58 The number of SCRs varies from five for CFHR1 and four for CFHR2 to nine for CFHR4 and CFHR5. CFHR proteins are smaller than FH, which contains 20 SCRs.59 CFHR1, CFHR2, and CFHR5 but not CFHR3 and CFHR4 contain N–terminal dimerization domains that allow homo- and heterodimers to form (e.g., a CFHR1 molecule can bind CFHR1, CFHR2, or CFHR5).60 CFHR1 circulates exclusively as dimers (homo- and heterodimers) formed because of the interactions of the two N-terminal domains.60 The C-terminal SCRs sequences are highly conserved in CFHR proteins (36%–100%) and correspond to SCR19 and SCR20 of FH, which account for the recognition of C3b and cell surfaces (Figure 3). FH presents C3b-binding sites at each end of the molecule. The N–terminal C3b–binding site mediates the accelerated decay of the alternative pathway C3 convertase (C3bBb) and the cofactor activity for the FI–dependent proteolytic inactivation of C3b. The C-terminal region binds C3b and polyanions normally present on cell surfaces (e.g., heparan sulfates and glycosaminoglycans). This region is essential for the complement–regulatory activity of FH on surfaces and to discriminate between self and pathogens. Most pathogens lack these polyanions on their surfaces. Each of five CFHR proteins binds to C3b and C3d and discriminates between cell and noncell surfaces.59,60

CFHR1 lacks cofactor activity for FI for the cleavage of C3b and lacks decay activity for the dissociation of the C3 convertase, C3bBb.61 In contrast, CFHR3 shows low cofactor activity for FI. However, CFHR3 and CFHR1 compete with FH for binding to C3b.62 Only CFHR1 may have the ability to block C5b-9 formation, a function mediated through the binding of the SCR1–SCR2 domains of CFHR1 to C5 and C5b6 (Figure 4).64

Deletion of CFHR1/3 protects against development of not only IgAN but also age–related macular degeneration.63–65 The hypothetical mechanism of this protection is on the basis of (1) competition between FH and CFHR1 that increases the functional activity of FH on surfaces in the absence of CFHR159,60 and (2) higher FH concentrations associated with the deletion conferring an increased protection.67 Interestingly, homozygosity for the deletion has been associated with a particular form of atypical hemolytic uremic syndrome (aHUS), where anti-FH antibodies frequently occur. In this disease, absence of CFHR1 and CFHR3 in the circulation is thought to favor the emergence of FH–specific antibodies that target mostly the region of C3 and surface recognition.68–72 These sites are

Figure 3. Comparison of the structures of FH, CFHR1, and CFHR3. The structure of FH contains 20 SCRs. SCR1–SCR4 possess the regulatory activity (FI cofactor activity and decay acceleration of the C3 convertase) as well as a weak C3b-binding capacity. SCR19 and SCR20 contain the most powerful C3b-binding zone that is critical for binding to cell surfaces. This latter area is the hotspot of aHUS–associated mutations, likely explained by the loss of ability of an abnormal FH to bind to endothelial cells. CFHR1 and CFHR3 resemble FH by the presence of structurally similar SCRs. These molecules possess the corresponding C3b-cell surface–binding zone (SCR4 and SCR5 corresponding to SCR19 and SCR20, respectively, of FH) but lack the regulatory portion (having no region equivalent to SCR1–SCR4). In contrast to FH and CFHR3, CFHR1 contains a unique pair of highly conserved SCR1 and SCR2 (also shared with CFHR2 and CFHR5). These domains enable formation of homo- and heterodimers involving CFHR1, CFHR2, and CFHR5.
The activation of classical pathway on IgG– or IgM–containing circulating immune complexes is a common feature in several autoimmune disorders (e.g., systemic lupus erythematosus) and can lead to discordant results: tissue codeposition of immune complexes with attached complement with ensuing local inflammation or clearance of the complexes from the circulation. In IgAN, it is unclear whether IgA1–containing circulating immune complexes have complement elements. Nevertheless, elevated serum concentrations of C3–derived products in patients with IgAN suggest a soluble-phase activation of the alternative pathway. Proteomic analyses of patients’ circulating immune complexes and complexes formed in vitro from Gd-IgA1 and antiglycan IgG revealed the presence of C3. In this study, cleavage products (iC3b, C3c, C3dg, and C3d) were detected in high-molecular-mass fractions, suggesting that the activation and regulation of the alternative pathway occurred directly on the immune complexes. This finding could mean that these complexes have an activating surface and carry C3bBb, a C3 convertase.

Complement alternative and lectin pathways can also be activated in situ in renal immunodeposits. Notably, C3 GN displays mesangial proliferation associated with C3 glomerular deposits in the absence of immunoglobulin in most patients. The pathogenesis is likely driven by either an inherited defect in the regulation of the alternative pathway (e.g., internal duplication of the CFHR5 gene) or an acquired excess of alternative pathway activation (presence of a C3 nephritic factor). The data from studies of this disease indicate that (1) glomerular C3 deposition is favored by a defect in the regulation of the alternative pathway that is possibly triggered by immune complexes and (2) C3 alone can induce glomerular lesions similar to those in IgAN.

Mesangial cells have been shown to be a player in local complement–driven glomerular inflammation. Mesangial cells produce FH and, in an inflammatory environment (IL-1 or TNFα), they produce C3. Furthermore, the autoantigen in IgAN, polymeric Gd-IgA1, can itself stimulate mesangial cells to produce and secrete C3 and C5a. Activation products C3a and C5a can induce cultured human mesangial cells to produce DAF, a potent membrane–bound regulator of the alternative pathway. The expression of DAF and C3 mRNA by mesangial cells in IgAN was confirmed by in situ hybridization as relatively specific for the disease. C3a, the anaphylatoxin produced by cleavage of C3, induces a secretory phenotype of cultured mesangial cells characterized by an increase in expression of genes encoding components of the extracellular matrix (collagen IV, osteopontin, and matrix Gla protein). This transition may explain the in vivo expansion of the mesangial matrix commonly observed in renal biopsies of patients with IgAN. The effect has been shown to be dependent on C3a receptor, although another study using in situ hybridization failed to find C3a receptor expression on normal human mesangial cells.

### WHERE COMPLEMENT IS ACTIVATED: FROM SOLUBLE CIRCULATING IMMUNE COMPLEXES TO GLOMERULI

Theoretically, in patients with immune–mediated mesangioproliferative GN, complement can be activated directly on immune complexes in a soluble phase, in the mesangial deposits, or at both locations. In patients with IgAN, the setting where complement activation takes place remains to be determined.

The activation of classical pathway on IgG– or IgM–containing circulating immune complexes is a common feature in several autoimmune disorders (e.g., systemic lupus erythematosus) and can lead to discordant results: tissue codeposition of immune complexes with attached complement with ensuing local inflammation or clearance of the complexes from the circulation. In IgAN, it is unclear whether IgA1–containing circulating immune complexes have complement elements. Nevertheless, elevated serum concentrations of C3–derived products in patients with IgAN suggest a soluble-phase activation of the alternative pathway.

Figure 4. Proposed mechanism to explain the protective effect of CFHR1,3 deletion on the development of IgAN. CFHR1 and CFHR3 proteins can bind to C3b in competition with FH. The regulatory activities of CFHR1 and CFHR3 are less efficient than those of FH. CFHR1,3 deletion, thus, allows FH to bind C3b effectively and thereby to strongly inhibit the initiation and amplification of the alternative pathway cascade.
IgAN: not altered in one study but increased in another study. In the latter report, serum levels of F1, FB, and properdin were also increased in a cohort of 50 patients with IgAN versus 50 healthy controls.

Excretion of complement components has also been suggested as a biomarker of the activity of IgAN. Urinary levels of FH and soluble C5b-9 correlated positively with proteinuria, serum creatinine increase, interstitial fibrosis, and percentage of global glomerular sclerosis, whereas urinary properdin was associated with only proteinuria. The urinary excretion of these biomarkers was higher in patients with IgAN than in healthy controls. Another study described increased excretion of FH in patients with more severe histologic lesions. These analyses, however, have not included disease controls with proteinuria to assess a non-specific excretion caused by a damaged glomerular filtration barrier.

Biopsy–based complement immunostaining is another potential prognostic biomarker. This evaluation was excluded from the Oxford classification system. It is notable that C3 was absent in an autopsy series designed to estimate IgAN prevalence, meaning that C3 is usually absent in asymptomatic mesangial deposition of IgA. In a recent study of Iranian patients, mesangial C3 was associated with increased serum creatinine level, higher frequency of crescent formation, and more endocapillary hypercellularity, mesangial cellularity, and segmental sclerosis. Interestingly, in a study of Korean patients, a low plasma concentration of C3 correlated with the intensity of mesangial C3 deposition, and each finding predicted a higher risk of progression to ESRD.

The predominance of C3c deposition versus that of C3d was associated with a more severe clinical expression, which was manifested as a more rapid decline in eGFR. The glomerular activation of the lectin pathway has been also shown as a biomarker for disease severity. Mesangial staining for MBL has been associated with worse renal clearance function and greater proteinuria. C4d mesangial deposition is a potential biomarker of growing interest. The assessment is widespread in renal pathology laboratories and can be easily performed routinely. In a study of Spanish patients, the 20-year renal survival was strikingly worse when C4d was detected in the mesangium (C4d+, 28% versus C4d−, 85%). This differential effect was independent of eGFR and proteinuria at time of biopsy. A study in Asia showed a similar effect.

**CONCLUSION**

Activation of complement plays a key role in the pathogenesis and clinical expression of IgAN (Figure 5). This process is mediated through the alternative and lectin pathways and likely occurs systemically on IgA–containing circulating immune complexes as well as locally in glomeruli. As with aHUS and C3 nephropathy, details about disease-expression influence by alternative pathway regulatory genes are emerging and indicate the importance of regulation of the complement alternative pathway in the development of IgAN. Activation of C3, assessed in plasma by its decreased levels and increased levels of its breakdown fragments and in biopsy specimens by its glomerular deposition, represents a biomarker of activity. Mesangial deposition of C4d needs additional evaluation to determine its efficacy as a clinically useful tool. Approaches that target complement activation (such as the recently available anti-C5 and anti-C5aR antibodies) may represent a promising option for treatment of some patients with IgAN.

**Figure 5.** Integrative view of the role of complement activation in the four-hit model of the pathogenesis of IgAN. C3 can be activated directly by IgA1–containing immune complexes formed from Gd-IgA1 and anti-glycan antibodies and increase the pathogenic potential of these complexes. Other proteins can bind Gd-IgA1, such as the soluble form of Fcα receptor (sCD89), to generate complexes with Gd-IgA1. An association between the levels of sCD89-IgA complexes in serum and the severity of IgAN has been observed. Specifically, patients with IgAN without disease progression had high levels of sCD89 in contrast to low levels of sCD89 in the disease progression group, suggesting that sCD89-IgA complexes may be protective. In contrast, an animal model suggested that interaction between four entities—Gd-IgA1, sCD89, transferrin receptor, and transglutaminase 2 in mesangial cells—is needed for disease development. The lectin and alternative pathways can each contribute to the glomerular damage induced by immune complexes in the mesangium. Mesangial cells can also play an active role, arising from their capacity to be stimulated by C3a as well as produce C3 in response to an inflammatory stimulus.
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DISCLOSURES

None.

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