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The Central Role of the Alternative Complement Pathway in Human Disease

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BRIEF REVIEWS

The Central Role of the Alternative Complement Pathway in Human Disease¹Joshua M. Thurman* and V. Michael Holers^{2†}

The complement system is increasingly recognized as important in the pathogenesis of tissue injury in vivo following immune, ischemic, or infectious insults. Within the complement system, three pathways are capable of initiating the processes that result in C3 activation: classical, alternative, and lectin. Although the roles that proinflammatory peptides and complexes generated during complement activation play in mediating disease processes have been studied extensively, the relative contributions of the three activating pathways is less well understood. Herein we examine recent evidence that the alternative complement pathway plays a key and, in most instances, obligate role in generating proinflammatory complement activation products in vivo. In addition, we discuss new concepts regarding the mechanisms by which the alternative pathway is activated in vivo, as recent clinical findings and experimental results have provided evidence that continuous active control of this pathway is necessary to prevent unintended targeting and injury to self tissues. The Journal of Immunology, 2006, 176: 1305–1310.

It has been known for several decades that the complement system plays a central role in the inflammatory response and tissue/organ injury that follows direct immune recognition by Abs as well as in processes such as organ ischemia/reperfusion (I/R)³ (reviewed in Ref. 1). However, while we know an increasing amount about the nature of the effector pathways that mediate complement-dependent injury and have a longstanding understanding of the biochemical steps of complement activation in serum in vitro, we know relatively little regarding the mechanisms by which the system is activated during these in vivo inflammatory processes. Recent studies using mice deficient in complement activation pathway proteins such as C4 or factor B of the classical and alternative pathway, respectively, or with inhibitors that are specific for proteins in these pathways, are providing new insights into these important

questions. Herein we review these studies and focus on the essential role shown for the alternative pathway.

Overview of complement and its biologic roles

The complement system is one of the major means by which the body recognizes foreign Ags and pathogens (reviewed in Refs. 1 and 2). The major function of complement traditionally has been thought to be recognition and elimination of pathogens through direct killing (3) and/or stimulation of phagocytosis (4, 5). However, over the last decade the complement system has also been shown to play central immunoregulatory roles such as enhancing humoral immunity (6), modifying T cell immunity (7), shaping the development of the natural Ab repertoire (8), and regulating tolerance to nuclear self Ags such as DNA and chromatin (9, 10). In addition to its immunoregulatory functions, many studies have also elaborated on the pathogenic role of complement during ischemic, inflammatory, and autoimmune diseases (reviewed in Ref. 1).

Brief history of the alternative pathway

It is difficult to review the alternative pathway without at least referring to the complex history underlying its original discovery by Pillemer and colleagues as the “properdin system,” the general dismissal of properdin as an artifact, and the subsequent rediscovery and validation of the original concept of this Ab-independent system. Interested readers should refer to an excellent review by W. A. Ratnoff (11). In addition, in the mid-1980s, when membrane complement regulatory proteins were first identified, the concept of “self vs non-self” recognition by the alternative pathway was developed (reviewed in Ref. 2), whereby foreign pathogens that did not express these human regulatory proteins were recognized and destroyed by the alternative pathway. Thus, in discussing the effects of changes in complement regulatory proteins and activation of the alternative pathway in vivo, it is in the context of this history of the alternative pathway as a major mechanism of recognition and elimination of “foreign” or “dangerous” pathogens. As discussed below, it appears that the same capacity is brought to bear on self-tissues that have become “dangerous” because of biological processes that result in injury or “altered self.”

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³ Abbreviations used in this paper: I/R, ischemia/reperfusion; SLE, systemic lupus erythematosus; aPL, antiphospholipid; HUS, hemolytic-uremic syndrome; aHUS, atypical HUS; MPGN, membranoproliferative glomerulonephritis; AMD, age-related macular degeneration; MAC, membrane attack complex.

Components of the alternative pathway

Components of the complement system that are unique to the alternative pathway are factor B, factor D, and properdin. Factors B and D have been studied *in vivo* using specific inhibitors and gene-targeted mice. Serum from these mice has been used to demonstrate that both proteins are required for efficient alternative pathway activation by zymosan (12, 13).

Mechanisms of alternative pathway activation

The classical and lectin pathways are initiated by the binding of recognition proteins to specific targets. The classical pathway is activated by IgM, complement fixing isotypes of IgG, and several other proteins such as C-reactive protein and serum amyloid P protein (14). The binding of these recognition proteins then allows C1q to be directly bound, initiating the classical pathway activation cascade (reviewed in Ref. 15; Fig. 1). The lectin pathway is initiated by the binding of mannose binding lectin to repeating carbohydrate moieties found primarily on the surface of microbial pathogens (reviewed in Ref. 16), by another family of lectins designated ficolins, which also recognize pathogens (17), or by the protein cytokeratin, which is exposed on ischemic endothelial cells (18).

In contrast to the specific protein:protein or protein:carbohydrate interactions that characterize classical and lectin pathway activation, the alternative pathway is capable of autoactivation because of a process termed “tickover” of C3 (reviewed in Ref. 19). Tickover occurs spontaneously at a rate of ~1% of total C3 per hour, generating a conformationally altered C3, designated C3(H₂O), that is capable of binding factor B (Fig. 1). Once factor B associates with C3(H₂O), factor B itself changes conformation and can then be cleaved by the constitutively active serum protease factor D, generating Ba and Bb. The Bb fragment remains associated with the complex and can then, through its own serine protease domain, cleave additional C3 molecules, generating a form designated C3b. Once C3b is generated, it associates with factor B to generate more C3-convertase. This overall series of successive proteolytic steps is enhanced by the serum protein properdin, which stabilizes protein:protein interactions during the process. The alternative

pathway can also be initiated as an “amplification loop” when fixed C3b that is generated by classical or lectin pathway activation binds factor B, again resulting in conformational changes in factor B that allow factor D to cleave it similarly to the tickover process.

Continuous active control of alternative pathway activation is necessary

Because of the spontaneous activating capabilities described above, the alternative pathway requires continuous active control. This control can be overwhelmed by specific activators of this pathway, increased concentrations of alternative pathway components (due to local synthesis (20) or the release of these proteins by infiltrating neutrophils (21)), or insufficient function of the complement regulatory proteins (Fig. 2). An “activating” surface is, in large part, one without adequate regulatory protein function to control alternative pathway activation (22) or one that is not favorable to control of the alternative pathway by factor H (23). Congenital or acquired deficiency of the complement regulatory proteins can result from Abs that block endogenous regulatory mechanisms (24) or decreased expression of complement regulatory proteins (25, 26). Diminished function of these regulatory proteins has been identified as a cause of several of the diseases discussed below.

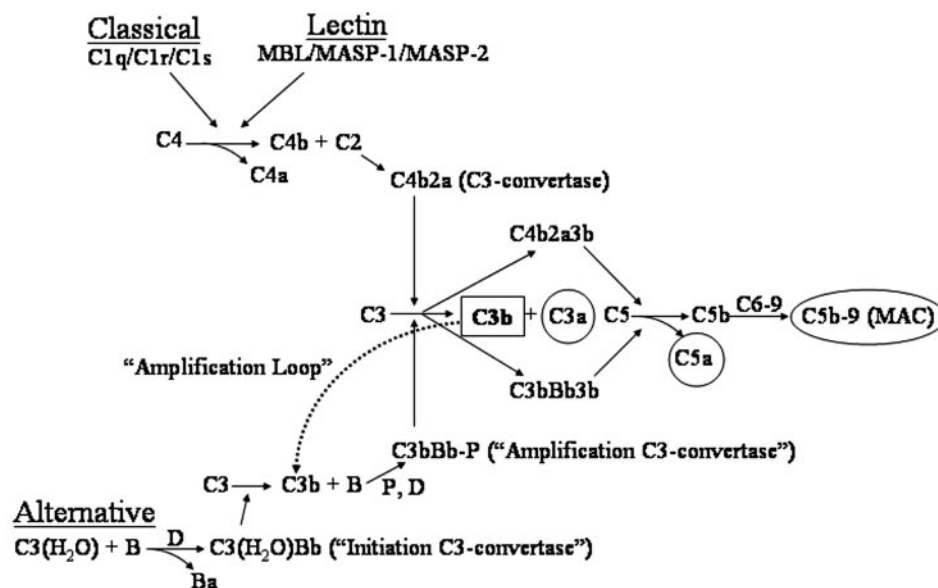
All three activation pathways require engagement of the alternative pathway to cause tissue injury *in vivo*

In the last several years, several investigators have begun to evaluate the question of whether inhibition of the alternative pathway could result in disease amelioration. These studies are reviewed below (see also Table I).

Lupus nephritis

The complement system is integrally involved in the pathogenesis of tissue injury in systemic lupus erythematosus (SLE), and complement components appear to mediate tissue damage initiated by autoantibodies. Direct support of this hypothesis was first provided by the findings that an inhibitory anti-C5 mAb blocks the development of glomerulonephritis in the (NZB × NZW)F₁ model of SLE (27). Subsequently, direct support for

FIGURE 1. Complement activation pathways. The complement system can be activated by the classical, lectin, or alternative pathways, resulting in the formation of C3-convertases. Activation by all of these pathways engages the alternative pathway through the “amplification loop.”



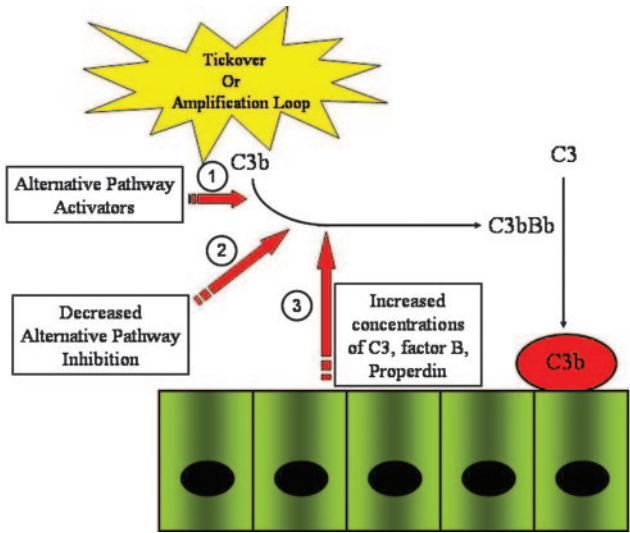


FIGURE 2. Alternative pathway mediated injury. The alternative pathway is continuously activated through a process called “tickover.” Ordinarily, inhibitory proteins expressed by host cells prevent autologous injury. This protection may be overwhelmed by 1) local factors that favor activation, 2) impaired function of cell surface inhibitors, or 3) increased activation due to high local concentrations of alternative pathway components.

a key role of the alternative pathway was provided by the finding that $fB^{-/-}$ MRL/ lpr mice are protected from renal disease (28), as are $fD^{-/-}$ mice (29), both to a similar extent found in mice in which C3 activation is blocked using transgenic expression of soluble Crry (30), a C3 convertase inhibitor, or recombinant Crry as an Fc fusion protein designated Crry-Ig (31). Of interest, eliminating expression of the classical pathway protein C4 in models of SLE leads to an increase in autoimmunity to self-Ags and increased target tissue injury rather than protection from autoantibody-induced tissue injury (9). This is perhaps the most clear-cut demonstration of the very distinct roles that the classical and alternative pathways can play in vivo.

Rheumatoid arthritis

Another demonstration of the differences in the pathogenic roles of the classical and alternative pathways is afforded by the K/BxN-derived anti-GPI serum transfer model of rheumatoid arthritis. In the present study, inflammatory joint disease was found to be ameliorated in $fB^{-/-}$ C57BL/6 mice receiving anti-

GPI serum but interestingly not in $C4^{-/-}$ mice (32). With regard to collagen-induced arthritis, backcrossing of the $fB^{-/-}$ genotype into the DBA1/j strain resulted in substantial disease amelioration, although in that report the authors pointed out that introduction of the H-2^b MHC into DBA1/j, which is H-2^a, did not allow them to make a formal conclusion regarding the role of fB itself as compared with other H-2 genes (33).

Antiphospholipid (aPL) Ab syndrome

The aPL syndrome is an autoimmune disease characterized by recurrent fetal loss, vascular thrombosis, and thrombocytopenia in the presence of aPL Abs. Although the pathogenesis of this syndrome had been attributed to the ability of these Abs to directly modify clotting-related activities or cell activation (34), using a murine model of aPL syndrome in which pregnant mice are injected with human aPL Ab-containing IgG Abs, it was found that inhibition of the complement cascade in vivo using the C3 convertase inhibitor Crry-Ig, or using mice deficient in complement C3, resulted in the absence of fetal loss and growth retardation (35). In a subsequent study, the authors demonstrated that several factors are necessary for the development of disease in this model, including C4, factor B, C5a, and neutrophils (21). Interestingly, the depletion of neutrophils prevented C3 deposition in the deciduas, perhaps because infiltrating neutrophils (initially recruited by classical pathway activation) fuel complement activation by releasing alternative pathway components. The critical role of alternative pathway activation was later confirmed using an inhibitory mAb to factor B (36).

Intestinal and renal I/R injury

Studies using $fB^{-/-}$ mice have shown that an intact alternative pathway is necessary for the full development of injury after renal I/R (37). In contrast, neither $C4^{-/-}$ mice (38) nor recombination-activation gene-1-deficient mice (39) were protected from renal I/R injury, and Ig deposition was not seen in post-ischemic kidneys (39). Importantly, renal biopsies of patients with acute tubular necrosis, the human clinical correlate of experimental I/R injury, show a similar pattern of C3 deposition on the tubular basement membrane as is seen in rodents. They do not demonstrate concomitant C4d deposition (40), indicating that complement activation after ischemia of human kidneys is also mediated by the alternative pathway.

Selective activation of the alternative pathway after renal I/R may be due to the acquired loss of complement regulation by

Table I. Human diseases or disease models in which the alternative pathways is required or centrally involved

Human Disease	Model(s)	Pathway Requirements	
		Classical	Alternative
1. Rheumatoid arthritis	K/BxN serum transfer	N ^a	Y
	CIA	?	Y
2. Anti-phospholipid syndrome	aPL Ab transfer	Y	Y
3. Lupus nephritis	MRL/ lpr strain	?	Y
	$C4^{-/-}$ B6. lpr strain	N	?
4. Asthma	Ova, ragweed immunization/inhalation	N	Y
5. I/R	Intestinal	Y	Y
	Renal	N	Y
6. Atypical hemolytic-uremic syndrome	NA	?	Likely
7. Type II membranoproliferative syndrome	$fH^{-/-}$ strain	N	Y
8. Macular degeneration	NA	?	Likely
9. Spontaneous fetal loss	$Crry^{-/-}$ strain	N	Y

^a N, no; Y, yes; ?, unknown; NA, not available.

the proximal tubular epithelial cells. The only cell-bound complement inhibitor expressed by the proximal tubules of the mouse kidney is Crry, which is ordinarily expressed along the basolateral aspect of the cells. After renal I/R, the polarity of Crry is rapidly disrupted, and within hours, many tubules display reduced expression of Crry (unpublished results). These changes appear to permit alternative pathway activation. Furthermore, the injection of rats with an inhibitor of Crry causes tubular injury (41), and *Crry*^{+/-} mice (which express approximately half the Crry in their kidneys as wild-type controls) are more sensitive to mild ischemic injury than wild-type animals (unpublished results). Clearly, continuous alternative pathway inhibition by the tubular cells is necessary to prevent autologous injury.

In contrast to the kidney, complement activation after intestinal ischemia requires an intact classical pathway and appears to be initiated by natural Ab. However, injury also depends upon an intact alternative pathway, and *fD*^{-/-} mice are protected from intestinal I/R injury (42). The dependence of this intestinal I/R model on the presence of natural Ab as well as intact classical and alternative pathways may indicate a similar sequence of events to that seen in the aPL model discussed above, although this has not yet been determined.

Asthma

Asthma typically is thought to be a hypersensitivity syndrome whose pathogenesis is primarily due to IgE production, cellular immune abnormalities, and generation of Th2 cytokines (43). Using *C3aR*^{-/-} mice, though, the C3aR has been shown to play an unexpectedly important role in experimental models of asthma (44). Furthermore, levels of C3a and C5a are elevated in bronchoalveolar lavage fluid from patients with asthma (45). The role of the alternative pathway has been studied recently in a mouse model of airway hyperresponsiveness (46). In this model, *fB*^{-/-} mice and wild-type mice treated with a mAb to factor B demonstrated significantly lower airway responsiveness to methacholine and less airway inflammation, compared with wild-type controls. However, *C4*^{-/-} mice were not protected compared with wild-type controls. B cells and IgG also do not appear critical for the development of injury in this model (47). Thus, pathogenic complement activation in this model appears to require the alternative pathway but does not require an intact classical or lectin pathway.

Atypical hemolytic-uremic syndrome (HUS)

The HUS is a thrombotic microangiopathy usually associated with diarrheal illness caused by Shiga-toxin-producing bacteria (48). Non-Shiga-toxin-associated HUS, or atypical HUS (aHUS), has been associated with mutations of the fluid phase alternative pathway inhibitor factor H (49–51), the membrane-bound inhibitor membrane cofactor protein (CD46) (52, 53), factor I (54), and autoantibodies to factor H (55). Alternative pathway activation during the disease has been detected by decreased levels of C3 and factor B as well as increased levels of C3 activation fragments (56–58), whereas C4 levels are unaffected (56). C3 but not C4 is also deposited in the glomeruli and arterioles of patients with aHUS (59). Interestingly, the regulatory protein mutations associated with aHUS are generally heterozygous. The mutations of factor H associated with aHUS reduce the ability of the protein to bind polyanion-rich surfaces (such as the glomerular basement membrane) (60, 61),

perhaps allowing uncontrolled alternative pathway activation after insults such as certain infections or drugs (48).

Type II membranoproliferative glomerulonephritis (MPGN)

MPGN is a glomerular disease defined by its histologic appearance by light microscopy and is further categorized as type I, II, or III based on the appearance of the glomeruli by electron microscopy. All three types of MPGN are associated with perturbations in complement levels and deposition of C3 within the glomeruli (62, 63). However, type II MPGN does not demonstrate immune complex or C4 deposition and shows preserved levels of C4 (62). Greater than 80% of the patients with type II MPGN have circulating autoantibodies, termed nephritic factors, that bind the alternative pathway convertase C3bBb (62–64). These nephritic factors stabilize the C3bBb complex and prolong its half-life. In addition, type II MPGN is associated with homozygous deficiency of factor H (63), as well as inhibitory serum Abs to factor H (65). Homozygous deficiency of factor H in both pigs (66) and mice (67) also leads to the development of MPGN. However, crossing the factor H-deficient mice with factor B-deficient mice rescues the offspring from this phenotype (67), confirming that activation of the alternative pathway is critical to the pathogenesis of disease in these mice.

Spontaneous fetal loss

Studies have suggested that appropriate complement regulation may be important for normal pregnancy and that recurrent immune-mediated fetal loss may in part be mediated by inappropriate alternative pathway activation (68). A significant percentage of patients with recurrent spontaneous abortions demonstrate a decline in hemolytic activity and C3 and factor B levels (69), indicating possible alternative pathway activation. Perhaps most compelling is the finding that homozygous *Crry* deficiency is lethal in utero in mice (26), and the placentae of mice carrying *Crry*^{-/-} fetuses demonstrate C3 deposition. This phenotype is rescued when the *Crry*^{-/-} mice are crossed with either *C3*^{-/-} mice (26) or *fB*^{-/-} mice (70), but crossing them with *C4*^{-/-} mice or B cell-deficient mice does not promote survival (70). Thus, spontaneous fetal loss in *Crry*^{-/-} mice is due to activation of the alternative pathway and does not require the classical pathway.

Macular degeneration

Age-related macular degeneration (AMD) is an important cause of age-related visual loss in the elderly. Three separate groups simultaneously reported that a tyrosine-histidine polymorphism at aa 402 of factor H is associated with the development of AMD (71–73). This polymorphism is located in the binding region for heparin (74) and C-reactive protein (75), the same region of factor H of a mutation identified in a patient with MPGN (55). The retinal lesions of AMD, called drusen, contain deposited components of the membrane attack complex (MAC), as well as serum amyloid P (76), and are structurally similar to those seen in patients with type II MPGN (77). These findings suggest that impaired alternative pathway inhibition by the associated factor H allele either causes or contributes significantly to the development of AMD.

Unanswered questions and future directions

Why is alternative pathway activation so important in vivo even when initiation appears to depend upon one of the other pathways? It may be that the initial insult induces deficiencies of regulatory proteins in necrotic cells or regions of injury, setting the stage for amplification of injury by the alternative pathway. Alternatively, infiltrating cells such as neutrophils bring in C3 and properdin that increase activation specifically at that site by providing additional substrate for the alternative pathway (21). It may also be that the amplification loop is much more robust in vivo than previously appreciated. Indeed, a recent in vitro serum study indicates that under certain experimental conditions amplification by the alternative pathway may account for 80% of the C5a and MAC generated by classical pathway activation, much more than has been thought traditionally (78).

Selective inhibition of this pathway may offer therapeutic benefit without the side effects seen with inhibitors that work at the level of the C3 and C5 convertases (79). Another exciting possibility is that alternative pathway complement inhibitors could be targeted to specific anatomic sites using Fab or single-chain variable fragments (80) or that linking inhibitors to the CR2 complement receptor could target them specifically to sites of complement activation (81). Such an approach may be advantageous to the dosing of such agents and reduce the side effect profile. For patients with single gene mutations in complement regulatory proteins, replacement of the dysfunctional or deficient protein may be of benefit. Patients with aHUS and membrane cofactor protein mutations have successfully received kidney transplants without disease recurrence, as might be predicted since the membrane bound protein is expressed by the graft itself (63). Unfortunately, even though factor H is synthesized primarily in the liver, attempts at liver transplantation in patients with factor H mutations have not been successful (63). As we gain ability to perform gene therapy safely, this too may become a viable therapeutic option for patients with factor H deficiency or dysfunction.

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