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Evasion and Interactions of the Humoral Innate Immune Response in Pathogen Invasion, Autoimmune Disease, and Cancer

Trisha A. Rettig^{1,3}, Julie N. Harbin^{2,3}, Adelaide Harrington^{1,3}, Leonie Dohmen^{1,3}, and Sherry D. Fleming^{1,*}

¹Division of Biology, College of Arts and Science, Pathobiology College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506

²Department of Diagnostic Medicine, Pathobiology College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506

Abstract

The humoral innate immune system is composed of three major branches, complement, coagulation, and natural antibodies. To persist in the host, pathogens, such as bacteria, viruses, and cancers must evade parts of the innate humoral immune system. Disruptions in the humoral innate immune system also play a role in the development of autoimmune diseases. This review will examine how gram positive bacteria, viruses, cancer, and the autoimmune conditions Systemic Lupus Erythematosus and Anti-phospholipid syndrome, interact with these immune system components. Through examining evasion techniques it becomes clear that interplay between these three systems exists. By exploring the interplay and the evasion/disruption of the humoral innate immune system, we can develop a better understanding of pathogenic infections, cancer, and autoimmune disease development.

Keywords

complement; coagulation; natural antibodies; B1 B cells; pathogens; cancer; SLE

1.0 The role of complement evasion in disease

The complement system serves as a first line of defense, by directly lysing abnormal cells and pathogens, and recruiting other immune cells such as macrophages and neutrophils. Complement activation occurs by three distinct, but connected pathways: classical, lectin, and alternative. C1q and Mannose Binding Lectin (MBL) initiate the classical and lectin pathways, respectively. Both pathways cleave C4 and C2 to form the C3 convertase

*Corresponding author: Sherry D Fleming, 18 Ackert, Kansas State University, Manhattan, KS 66506, 785-532-6130, sdflemin@ksu.edu.

³Co-first authors

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C2aC4b. The alternative initiation pathway follows constitutive C3 hydrolysis to C3b which, after interaction with Factors B, D and P form the stable C3 convertase, C3bBb [1, 2]. Both C3 convertases cleave C3 into C3a and C3b. C3b binds the C3 convertases to form the C5 convertase to cleave C5. C3a and C5a are anaphylatoxins which stimulate inflammation. C5b initiates the formation of the membrane attack complex (MAC or C5b-9) which leads to cytolysis and cell or pathogen death [3] (Fig. 1). Regulatory factors including CD55, CD46 and complement receptor 1 interact with co-factor, Factor I to control the convertases [4]. In addition, Factor H (FH) with the Factor I (FI) degrades the alternative C3 convertase.

1.1 Complement and Gram positive bacteria

Bacteria use three main methods of evading complement that include binding host inhibitors to the pathogen surface, using bacterial enzymes to cleave active complement components, and degrading surface bound proteins to prevent further activation of the complement cascade.

The ability to bind host inhibitors is prevalent among many different bacterial species, though this paper will focus only on gram positive bacteria. Species such as *Haemophilus influenzae*, *Bordetella pertussis*, and the gram positive *Streptococcus pneumoniae* use a conserved “superevasion” site to bind FH which results in degradation of C3b bound to the bacterial surface [5]. Both the streptococcal family and *Staphylococcus aureus* also use multiple, and sometimes redundant, proteins to bind FH to facilitate the formation of FH:C3b complexes which inhibit complement activation and increase bacterial survival [6–10]. *S. aureus* proteins not only recruit FH to the bacterial surface but also recruit FI which together inactivates C3b to form iC3b [8]. *S. pneumoniae* uses multiple proteins to bind and sequester C1q, as well as modulate complement FH, C4bp, and the C3 convertase [9, 11]. Members of the Microbial Surface Components Recognizing Adhesive Matrix Molecules protein family commonly expressed on *S. aureus*, *Streptococcus equi*, and *Streptococcus mutans* also bind C1q [12]. Finally, bacterial spores utilize similar survival strategies as *Bacillus anthracis* spores recruit FH, Factor H Related Protein 1, C1 inhibitor, and C4bp to their cell surfaces [13]. Thus, both spore forming and non-spore forming, gram positive bacteria evade destruction by recruiting natural host complement inhibitors or sequestering pathway initiators to prevent complement activation.

While recruiting complement pathway inhibitors to the bacterial surface is a common method of immune evasion, bacteria also use their own proteins to cleave active members of the complement pathway, preventing the cascade from proceeding. The most common target of gram positive bacterial enzymes is C3b. For example, *S. aureus* uses at least three proteins to degrade C3b and yet another protein to inhibit the formation of the C3 convertase [8, 14–16]. *S. aureus* also degrades C3 by activating plasminogen, a member of the coagulation pathway [8, 14, 17]. Other bacteria also bind fibronectin (streptococcal family) or interact with plasminogen (*B. anthracis*) to decrease the amount of C3b deposited on the bacteria [7, 13].

Other targets for complement evasion include direct inhibition of the common complement pathway and formation of convertases that lead to this step. Proteins expressed on the *S. aureus* bacterial surface prevent formation of the MAC and bacterial lysis [7, 9]. In addition,

at least four *S. aureus* proteins bind to C3b and prevent the formation of further convertases [8, 14, 16], while an additional *S. aureus* protein prevents formation of the C3 convertase halting the complement cascade [18].

Finally, gram positive bacteria prevent complement activation by degrading bound Ab, initiator of the classical pathway. *S. aureus* accomplishes this by using over a half dozen different proteins which work together to degrade the surface bound Ab [19]. This exemplifies an interconnection between the complement pathways and natural antibodies (NAb), discussed below. Gram positive bacteria evolved multiple methods of complement evasion which play a crucial role in bacterial survival during infection.

1.2 Viruses

Viruses have evolved a wide range of novel mechanisms to evade the complement response to host infection. Mechanisms include preventing molecules such as C1q from binding to the antibody-antigen complex on the surface of infected cells, and pathogens. Additionally, viruses can express proteins that mimic complement regulators, thereby allowing the pathogen to enter the cell, and protecting the infected cell from lysis. Still, other viruses incorporate complement regulatory proteins, which are capable of upregulating or downregulating the complement response to the invasion [20, 21].

Specific viruses appear to interfere with the formation of the MAC, crucial to ridding the body of virus infected cells. One example is West Nile virus (WNV), a member of the flavivirus family, which encodes a Nonstructural protein 1 believed to bind to the regulatory protein FH. This binding leads to the inhibition of C3b deposition and to the formation of the MAC [22]. Similarly, Hepatitis C, also of the flavivirus family, prevents the formation of the MAC by interfering with C3, C4 and C9 complement components [23].

Viruses may also express cellular complement regulators on their envelope, which use molecular mimicry to interfere with complement regulation. Human immunodeficiency virus activates the complement system through the classical and lectin pathways, however, the virus appears to incorporate complement regulatory molecules on the virion envelope, preventing complement mediated neutralization [24–26]. One study shows that Nipah virus, of the paramyxoviridae family, can evade the complement pathways using FI to inactivate C3b through cleavage. This evasion mechanism may contribute to the high mortality rate and systemic infection associated with this virus [27]. All of these examples demonstrate mechanisms, which a variety of viruses have evolved, to evade the complement mediated response to invasion by viral pathogens.

1.3 Systemic Lupus Erythematosus (SLE) and anti-phospholipid syndrome (APLS)

Complement plays a major role in the pathogenesis of multiple autoimmune diseases. The mechanisms that cause the development of autoimmune disease are complex and include the dysregulation or depletion of complement cascade components. SLE is a syndrome consisting of a group of systemic, self-reactive, chronic inflammatory responses that may affect the skin (butterfly rash), joints, kidneys (glomerulonephritis), and other organ systems. APLS is an autoimmune disease in which thrombosis occurs in both veins and

arteries due to anti-phospholipid antibodies (aPLs) and often results in spontaneous fetal abortion. As SLE patients may develop APLS, the interconnection appears to include complement playing a significant role in manifestation of both these autoimmune diseases [28, 29].

Deficiencies of the complement proteins C1q, C4 and C3 correlate with development of SLE disease [30, 31]. In 88% of an SLE cohort, a homozygous deficiency in C1q correlated to the development of SLE [32] and lower C1q levels occur during glomerulonephritis flares in SLE patients [33]. C4 deficiency is also correlated to SLE but the risk is higher for patients with C1q deficiency [34, 35]. As a central complement component, a lack of C3 appears to prevent APLS and development of SLE [36]. Similar effects are found with C5 deficiency in APLS and these effects are mirrored in mice treated with anti-C5 Ab [37]. Compared to healthy people, primary APLS patients appear to have unregulated and/or excessive complement activation as demonstrated by low C3 and C4 serum levels and increased levels of anaphylatoxins C3a and C4a [38]. In summary, deficiencies in distinct complement components do not result in a universal phenotype of SLE or APLS disease, though it appears that the further upstream in the complement cascade the deficient protein is located, the higher the risk of disease development.

SLE and APLS both develop significant auto-reactive Ab to initiate complement activation. Specifically, anti-C1q autoantibodies form aggregates with the collagen-like portion of C1q and correlate with SLE development [39]. Low complement levels and another autoreactive Ab, anti-double stranded DNA are also phenotypic of lupus nephritis [40]. The increase in autoreactivity towards both single and double stranded DNA, histones, and chromatin in SLE correlate with defects in the complement receptor 2 (CD21) [41, 42]. Taken together, deficiencies in complement pathways, as well as complement receptors, frequently lead to autoimmune disease development.

1.4 Cancer

Cancer is a disease characterized by uncontrolled cellular growth and possible dissemination of cells from the primary site to distant tissues. Abnormal cells are targets of the complement system, however, some cancer cells take advantage of activation while others inhibit this system. In a phase II clinical trial, pancreatic cancer was shown to inhibit the MBL pathway and treatment with ω -3-rich fatty acids and gemcitabine restored MBL activity, improving patient survival [43]. In contrast, lung cancer cells evade complement-mediated cell lysis by overexpressing the alternative pathway inhibitor FH [44]. Additional studies indicate that cancer cells utilize the classical pathway to their advantage. Ligation of the cell surface receptor for C1q, also known as C1q binding protein, stimulates tumor proliferation and increases the metastatic potential of lung and breast cancer [45–47]. Similarly, C4b, which is cleaved following C1 activation, is overexpressed in cancers such as melanoma [48], pancreatic [49], and breast cancers [50].

Tumor cells also circumvent MAC formation preventing cell lysis by overexpressing CD59, a MAC inhibitor [51–54]. Gastric cancer cells overexpress C9 suggesting it plays a role independent of MAC formation in cancer [55]. These findings could explain the failure of some monoclonal antibody (mAb) therapies requiring complement mediated cell lysis. Ab

activation of complement-induced inflammation may be beneficial for multiple cancer types and inhibition of MAC formation could reduce the therapeutic activity of the mAb. If the Ab targets mucins away from the cell membrane, the MAC will not form [56], causing inflammation without cytotoxicity. Consequently, targeting the complement dysregulation [43, 55] in association with mAb may be a promising chemotherapy [57].

Activation of any complement pathway produces the anaphylatoxins, C3a and C5a [58]. Overproduction of C5a and/or C3a stimulates tumor progression in lymphoma [59], ovarian [60], lung [61, 62], and breast cancer [63]. Metastasis also involves anaphylatoxins as C3a is required for cellular cohesion during cell migration, [64] and C5a contributes to immune regulation [65, 66]. In summary, cancer cells may down regulate the lectin and alternative pathways to escape the immune response but may utilize the classical pathway, to induce inflammation for growth and metastasis. Cancer cell expression of complement inhibitors prevent MAC formation and cell lysis.

2.0 The role of coagulation evasion in disease

The coagulation pathways function to maintain vascular integrity, protecting the vascular system from injury and invading pathogens. Composed of proteolytic cascades, cleavage of inactive plasma enzymes releases an active factor, which acts on the next enzyme in the cascade leading to clot formation. There are two coagulation pathways, intrinsic and extrinsic, which merge into the common pathway, resulting in the formation of a fibrin clot (Fig. 2) [67]. Damage to endothelial cell surfaces activates the intrinsic pathway through Factor XII. This activation allows binding of kallikrein (KLK), and high molecular weight kininogen (HK) to the cell surface which is crucial for the propagation of cleavage events producing additional Factor XIIa as a product. Factor XIIa then cleaves Factor XI which prompts the cleavage of subsequent Factors IX, VIII and X in the intrinsic cascade. Trauma may induce exposure of Tissue Factor (TF) and initiate the extrinsic pathway with subsequent cleavage of Factor VII into VIIa, or direct activation of TF resulting in the cleavage of Factor IX and Factor X. Activation of Factor X initiates the common pathway and when complexed with Factor Va activates thrombin. Thrombin, the primary regulator of coagulation, cleaves fibrinogen in the formation of a fibrin clot. Pathogens persist in the host by interfering with the function of the coagulation cascade. Dysregulation within these pathways occasionally leads to autoimmune disease and is implicated in some cancers.

2.1 Gram positive bacteria

During bacterial infections, coagulation is often used to contain bacteria, making them easier for the host to destroy [68]. Some bacterial species disrupt the coagulation pathway by breaking up fibrin clots, while others cause a fatal overactivation of the coagulation pathways leading to disseminated clots [68]. In the case of *B. anthracis*, the peptidoglycan layer activates platelets and causes coagulation dysfunction [69, 70]. *Clostridium perfringens* can occasionally induce intravascular coagulation by secreting an extracellular alpha toxin that forms complexes composed of activated platelets, fibrin, and leukocytes [71].

S. pyogenes infections significantly impact host coagulation as shown by decreased fibrinogen levels in the plasma, increased levels of fibrin degradation products, and up to a 50% reduction in platelet count [71, 72]. Killed bacteria, washed to remove any non-*S. pyogenes* proteins, increase the production of TF from cells, as shown *in vitro* [71]. The M protein complexes with up to four fibrinogen molecules and platelets already activated by anti-*S. pyogenes* Ab [72, 73]. These activated platelets then interact with neutrophils and monocytes to increase the release of TF, increasing clotting and releasing heparin-binding protein. [72–74]. The bacteria also produce the enzyme streptokinase, which cleaves fibrinogen [75]. Additional redundancy includes, *S. pyogenes* upregulates the host's coagulation system by sequestering protein S [71].

Sequestering coagulation pathway components is an additional mechanism by which *S. pyogenes* inhibits clotting. The M proteins recruit HK to the bacterial surface exposing it to a secreted protein that cleaves HK releasing bradykinin, leading to vascular permeability [75, 76]. Additional *S. pneumoniae* surface proteins adhere to host plasminogen, bringing it into contact with the urokinase-type plasminogen activator, leading to the activation of plasmin [74, 77].

S. aureus has a variety of other mechanisms for altering the coagulation pathway. Two secreted homeostasis factors activate prothrombin without enzymatic cleavage and prevent clot formation [15, 73, 78, 79]. As another important survival mechanism, *S. aureus* forms a pseudo-capsule of fibrin and fibrinogen [73, 79]. Extracellular Binding Protein complexes with fibrinogen and surface bound C3b to create a shield. This shield hides immune factors, such as Ab and C3b, that are bound to the bacterial surface preventing phagocytosis [15]. Other *S. aureus* surface proteins associate directly with multiple members of the coagulation pathway. For example, coagulase binds to thrombin, fibrinogen, and fibronectin, while Von Willebrand Factor Binding Protein recognizes thrombin and factor XII [79]. Multiple additional *S. aureus* proteins are substrates of members of the coagulation cascade including factors VII, IX, X, Xa, XIIIa and prothrombin [79, 80]. Studies with *S. aureus* suggest that clot formation prevention is required for bacterial survival, as coagulase mutants cannot survive in the skin of infected mice, and the host clears bacteria immobilized in a clot [73, 79]. Redundancy in *S. aureus* coagulation evasion is critical and the loss of key bacterial proteins can lead to bacterial clearing. By avoiding immobilization within a clot, gram positive bacteria escape detection and destruction. Given this important role, it is easy to see why a variety of evasion mechanisms have evolved.

2.2 Viruses

Viruses which cause hemorrhagic fever (HF), are in principle distinct, because these viruses specifically interfere with the coagulation pathways, among other essential defense mechanisms. Ebola virus, a filovirus responsible for over 10,000 death in 2014 alone, causes hemorrhagic disease in infected patients. Over-expression of TF leads to excessive activation of the TF activated coagulation pathway and fibrin accumulation in tissue associated with viral Ag, as well as a loss of endothelial barrier function, which are also characteristic of Ebola HF [81]. Dengue HF, caused by Dengue virus, a flavivirus, activates fibrinolysis through the direct degradation of fibrinogen, initiating a secondary activation of

pro-coagulants such as TF. Dengue virus expresses molecules, which mimic coagulation factors, contribute to the inhibition of thrombin activity, and enhance fibrinolysis [82].

Enveloped viruses acquire envelopes from host cells, thus expressing and utilizing host cell and cell membrane properties. Herpes Simplex Virus 1 uses host components to activate thrombin and enhance infection and encodes glycoprotein C which contributes to the enhancement of factor X activation [83]. Adenovirus type 5 also uses a host coagulation factor to overcome a complement-mediated blockade and infect the liver. Additionally, Adenovirus type 5 associates with factor X which protects this virus from complement-mediated and Ab neutralization [84].

Viruses interfere with the regulation of the coagulation pathways in multiple ways. Some viruses cause the overexpression of coagulation activation molecules. Others utilize host molecules to interfere with important regulatory components, while some associate with coagulation factors which decrease the immune response.

2.3 Systemic Lupus Erythematosus and anti-phospholipid syndrome

SLE and APLS may be characterized by hypercoagulability. SLE and APLS patients have increased TF expression in monocytes and aPL Ab found in these patients increase TF expression [85, 86]. Endothelial cell activation by aPL leads to production of TF and upregulation of adhesion molecules [87]. In APLS and SLE this leads to pathogenesis and hypercoagulability. In addition, compared to healthy people, SLE patient plasma contains significantly reduced levels of TF pathway inhibitor [88].

A reduction of annexin A5 anticoagulant activity is correlated with SLE pathogenesis in children [89]. Annexin A5 binds anionic phospholipids and blocks coagulation enzymatic reactions [90]. In addition, aPL Ab can interfere with annexin A5 binding to phospholipids and promote thrombosis events in APLS patients. This is influenced by an increased level of anti-domain 1 Ab to beta-2-glycoprotein I, which are found in children and adolescents with SLE [89] and significantly increase the risk of thrombosis [91].

Activation of the intrinsic pathway in coagulation occurs through activation of factor XII specifically by preformed fibrin clots [92]. High levels of factor XIIa-antithrombin (AT) complex are found in APLS and SLE patients with previous venous and arterial thrombosis [92, 93] and SLE patients have a higher risk of developing thrombosis compared to APLS [94]. Thus, multiple mechanisms alter the coagulation cascade in SLE and APLS patients.

2.4 Cancer

The association between thromboembolic events and cancer was reported over a century ago by Armand Trousseau who called them *phlegmasia alba dolens* (Latin for painful white inflammation). He later gave his name to the vessel inflammations caused by blood clots [95]. The incidence of thromboembolic events in cancer patients is extremely variable between studies (3–40%) but is always higher in cancer patients [96, 97]. Three main risk factors have been reported: patient-related (such as age, race, or comorbid conditions), site of primary tumor and treatment (surgery and chemotherapy increasing the risk of developing a thromboembolic event) [96–98].

Human kallikreins (KLK), a group of 15 serine proteases, initiate the intrinsic pathway and play a role in carcinogenesis; however, not all KLK play the same role in carcinogenesis or the same role between cancer types. Upregulated KLK13 is associated with a good prognosis in non-small cell lung cancer [99], whereas KLK5 is downregulated in some breast cancer subtypes leading to a misregulation of miRNAs and expression of genes involved in metastasis [100]. KLK6 activates the MAPK pathway through the receptor PAR2 in non-small cell lung cancer and increases tumor survival [101]. In addition, increased expression of both KLK6 and KLK10 are potential prognostic marker in gastric cancer and correlate with poor survival. Indeed, there is a positive correlation between high expression and nodal involvement as well as shorter survival (KLK6) or advanced-stage disease (KLK10) [102]. KLK7 regulation varies in different cancer types: it is overexpressed in colon [103] and cervical cancer [104] whereas it is down regulated in prostate cancer [105]. Factor XII, which is activated by the KLKs, seems to increase the metastatic potential of ovarian cancer by transforming monocytes/macrophages into tumor-associated macrophages [106].

In the extrinsic pathway, the androgen receptor in breast cancer [107], or hypoxia-inducible factors in ovarian cancer [108], may upregulate factor VII. The role of TF is complex with full length TF cooperating with PAR2 and FVIIa in stimulating the angiogenic response, and the alternatively spliced isoform of TF in cancer being independent of PAR2. The alternatively spliced variant of TF activates the PI3K, MAPK pathway. This leads to increased migration and angiogenesis, though the newly formed vessels are leaky [109]. The overexpression of TF is associated with high incidence of thrombotic events and poor survival, making it a useful diagnostic tool [110].

In the common pathway, thrombin is associated with cancer proliferation, invasion and angiogenesis [111, 112]. Thrombin stimulates inflammation at the tumor site in breast cancer patients [113] and plays a role in tumorigenesis of colitis-associated colon cancer [114]. Fibrinogen levels may provide a prognostic parameter, since a high level of fibrinogen is associated with advanced stage and poor survival in multiple types of cancer including cervical [115], ovarian [116, 117], endometrial, [118] or vulvar cancers [119]. A strong correlation also exists between coagulation and cancer progression and as such, anticoagulants including low molecular weight heparin, constitute a treatment of choice against cancer. This treatment is even more prevalent when patients present with co-morbidity factors [96, 97, 105, 120]. Other treatments include drugs targeting KLK [121], or novel oral anticoagulants which directly target Factor X and thrombin [122].

In conclusion, cancer cells activate almost every step of the coagulation cascade leading to clot formation. In turn, the clot may serve as a scaffold for cancer cells to metastasize. This explains the high incidence of thromboembolic events in cancer patients compared to controls. When the clots detach from the endothelial cells small capillaries may be blocked.

3.0 The role of natural antibodies in disease

Natural antibodies (NAb) are polyreactive Ab, composed of mostly IgM, but also IgG and IgA [123–125]. The Ab originate from the germ line and can be found in newborns and

germ-free animals [123–125]. Ab are produced by recombination of multiple gene segments, including the variable region alleles. This rearrangement provides for Ab diversity and specificity. However, NAb utilize only a limited number of variable region alleles as many alleles are inaccessible during Ab rearrangement [126]. NAb are structurally highly flexible, which enables low affinity binding to a broad range of microbial and self Ag [124, 125, 127] (Fig. 3). NAb interact with serum lectins and the complement cascade similar to Ab from the adaptive response [124, 127, 128]. As such, these Ab protect the host with a basal level of immunity to pathogens but as NAb recognize self antigens, an excessive response may result in pathology.

B1 B-cells, a subpopulation of B-cells, are the main producers of NAb (Fig. 3). Unlike B2 B-cells, B1 B-cells do not require Ag presentation by T-cells to produce Ab [129]. T-cell independent spontaneous Ab responses, such as lipopolysaccharides, can develop more quickly than T-cell dependent Ab, helping prevent infection in the earliest stages and play a critical role in protection of the vital organs [129, 130]. B1 B-cells are divided into two groups, B1a B-cells (CD5+, CD11b-) which secrete high levels of IgM and have low T-cell interaction [131] and B1b B-cells (CD5-, CD11b+) which have lower levels of IgM and do not express CD5, but higher levels of CD86 and are more likely to interact with T-cells [131].

3.1 Gram positive bacteria

Among other functions, NAb protect the host from Ag that do not develop monoreactive Ab, such as capsular polysaccharides [128]. Pathogens will encounter these Ab almost immediately as they are constantly present in normal serum, though they degrade more quickly than monoreactive Ab [123, 127, 132]. Many pathogens attempt to circumvent the host immune system by using antigenic variation, but NAb's ability to bind to a number of Ag helps the host fight infections that they have never seen before or with antigenic variation [130].

Some of the specific targets of NAb are found on *S. pneumoniae* and *B. anthracis* [126]. A specific idiotype, T15, recognizes phosphocholine on the surfaces of some bacteria, including *S. pneumoniae* [100, 124, 133]. NAb against the *S. pyogenes* capsule have been found in rabbits and in humans, NAb target cell wall polysaccharides [134, 135]. Mice injected with the polysaccharides found on *S. pneumoniae* developed low affinity, high avidity, polyreactive germline derived Ab, suggesting that NAb are an important line of defense against the bacteria [136]. These Ab also selectively used the VH1 and IGHJ6 genes, which encodes for a tyrosine rich CDR3, indicative of polyreactive Ab [133, 136]. Mice who fail to develop these Ab are more susceptible to *S. pneumoniae* infections, but NAb alone are not sufficient to clear the infection. [126, 132].

While B1a B-cells are long lived (longevity and replacement rates for B1b-B-cells are still unknown), their rate of replacement is very slow and their loss may be irreversible. One *S. aureus* protein, *Staphylococcal aureus* Protein A, is a B-cell superantigen causing cross linking of B-cell receptors, leading to cell death and a hole in the Ab repertoire. This depletion can still be detected over one year after exposure to the protein [137]. Protein L of

Peptostreptococcus magnus also functions similarly as a B-cell superantigen leading to cell death and producing an irreparable immune system gap [138].

3.2 Viruses

B1 B-cells are unique due to a poor response to receptor-mediated activation but a rapid and strong response to cytokines and pathogen-encoded signals. This enhances the ability of B1 B-cells to attenuate early viral replication and greatly decreases the chance of autoimmunity [139]. Studies show that influenza virus infection may be neutralized by natural IgM mediated activation of the complement system. Through the binding of C1q to a complement-fixing immunoglobulin such as IgM, the complement cascade is activated resulting in not only the neutralization of influenza, but the prevention of viral pneumonia [140]. Based on this mechanism, NAb mediated activation of the complement system is crucial in attenuation of the early influenza virus infection.

Although the role of IgM in WNV infection remains poorly understood, a mouse model study of WNV infection demonstrated that induction of natural IgM plays a role in early protection, but does not appear to neutralize or attenuate the infection [141]. The authors of this study hypothesized that natural IgM helps link the innate and adaptive immune response during infection. In contrast, vesicular stomatitis virus (VSV) infection was attenuated by NAb when μ MT (B-cell deficient) mice are passively immunized with NAb following infection. NAb appear to influence the initial distribution of VSV, preventing viral dissemination to vital organs, and directing dissemination to secondary lymphatic organs accelerating and enhancing the immune response [142].

These contrasting results suggest that although NAb are important in the early attenuation of viral infections, and the protection from invading pathogens, NAb are not consistently sufficient to neutralize infection. This variability in effectivity is likely the result of differences in viral evolution and adaptation to host immune responses.

3.3 Systemic Lupus Erythematosus and anti-phospholipid syndrome

The highly variable clinical manifestations make SLE a challenge to accurately diagnose but, a major component includes auto-reactive Ab, composed primarily of IgG isotypes. The absence of IgM in lupus prone mice significantly increased IgG autoantibody levels [143]. Recent studies demonstrated that auto-reactive, NAb may decrease the risk of SLE development [144–146]. Initial studies showed that patients with higher IgM, compared to IgG, had a lower risk of developing lupus nephritis [144]. Importantly, subsequent studies showed that anti-apoptotic cell, natural IgM Ab decreased the inflammatory response and blocked in vitro Toll-like receptor activation of dendritic cells [146, 147]. Together these studies suggest that NAb may down regulate the inflammatory response in lupus and that the IgM to IgG ratio may be crucial in autoimmune disease development.

Specific autoreactive IgM includes anti-lipid Ab, anti-nuclear Ab, and Ab that recognize proteins bound to lipids. In SLE, anti-phosphatidylcholine IgM levels decrease while anti-phosphatidylserine IgM levels increase possibly due to increased apoptosis levels leading to increased phosphatidylserine on the cell membrane [145]. Studies also showed that Ab

which recognize oxidative epitopes on apoptotic cells, and both single and double stranded DNA are prevalent in patients with SLE [144]. These patients specifically develop anti-nuclear factor and anti-native DNA IgM [148]. Compared to healthy controls, APLS patients have elevated levels of IgG to the serum protein, β 2-GPI, which binds phosphatidylserine. In addition, APLS patients produce low affinity IgM and IgA anti- β 2-GPI Ab [149], suggesting that these Ab are NAb. Elevated levels of IgG aPL may increase thrombotic events and abortion in APLS and SLE patients [149, 150].

The excess Ab produced in SLE lead to formation of immune complexes that lower the threshold for platelet activation [151]. SLE patients with aPLs Ab show increased complement activation on platelets compared to healthy individuals [30, 152, 153]. The platelet bound aPL initiate the classical pathway resulting in complement deposition and platelet activation selectively via p38 MAPK [154–156]. A new approach to treat and prevent thrombotic events in APLS patients utilizes inhibitor of p38 MAPK [157].

B1 B-cells may directly contribute to SLE as lupus patients appear to have an increased number of B1b B-cells [131]. SLE patients, but not their healthy counterparts, express increased levels of CXCL13 which recruits B1 B-cells to the kidney and results in increased renal damage [158]. However, the latter study did not determine the specific B1 B-cell subtype in the kidney. Similarly, a mouse model for lupus expressed an increased B1 B-cell population compared to wild type mice [159]. In addition, Sialic acid-binding Ig-like lectin (Siglec)-G protein, an inhibitory receptor that downregulates the B1 B-cell response [160], contributes to increased autoantibody production and kidney damage in a mouse model of lupus [161].

3.4 Cancer

Many studies report that NAb in cancer patients may result in apoptosis of cancer cells [162–165]. In particular, the NAb SAM-6 exhibits an anti-cancer effect on myeloma by targeting the protein GRP 78. This heat-shock protein localizes to the lumen of the reticulum under physiological conditions, but translocates to the surface of myeloma cells [166]. SAM-6 NAb also induce lipoptosis, an excess lipid accumulation in cancer cells but not in normal cells, that leads to cell death [167, 168] and reviewed in [169]. However, the role of IgM NAb in tumorigenesis is not always clear. For example, the natural NAb isoagglutinins cross-react with the tumor cell Ag T and Tn but it is unclear if these Ab are beneficial or detrimental. Also, the level of isoagglutinins is reduced in pancreatic cancer but there is no known correlation with outcome [170].

Independent of NAb, B1a-B-cells play an important role in cancer development. The tumor microenvironment contains lymphocytes which express a decreased level of CD5. Because CD5 inhibits T-cell receptor and B-cell receptor signaling, it may be hypothesized that decreasing CD5 may be a strategy to maintain the T-cell anti-tumor effect [171]. However, some cancer cells seem to escape this downregulation of CD5, as B1a B-cells are present in the melanoma cells microenvironment and enhance metastasis [172, 173]. B1a B-cells, like any other cell type, may also accumulate mutations, transform, and become the primary tumor. Many chronic lymphocytic leukemia derive from NAb producing cells in which

natural IgM stimulates proliferation [174–176]. Binding of IgM activates the MAPK cascade, which in turn enhance the oncogene MYC expression [177].

NAb are utilized in the treatment of tumors. For example, intra-tumor injection of the anti-gal NAb increased survival of some renal or pancreatic cancer patients [178]. This required a complement-dependent cytotoxicity, which illustrated the important interconnections that exist between the different systems involved in innate immunity. Contrary to what was expected, NAb activated not only the classical complement pathway, but also the lectin pathway through an interaction with H-ficolin [179].

In conclusion, B1 B-cells and NAb play a role as gatekeeper in cancer progression. Efficacy is limited since we observe cancer formation; however, an overexpression of NAb through direct injection demonstrates promising results in patients [178, 180]. Unfortunately, this strong interrelation between the different systems can also be deleterious as IgM elimination of cancer cells depends in part on complement activation which is dysregulated. Additionally, NAb activate coagulation, which may be beneficial for metastasis. Finally, mutation of B1 B-cells biases the entire system by producing IgM which provides a positive feedback to the B1 B-cells, especially in CD38-positive cells [181].

4.0 Interactions, Redundancies, and Conclusions

While the complement system, coagulation cascades, and NAb are three distinct entities, these cascades are highly interconnected in multiple clinical conditions (Fig. 4). Evasion of these immune responses is important for bacterial and viral survival. This is illustrated by the failure of the coagulase mutants in *S. aureus* to survive in mouse models [73, 79]. Cancer cells are less sensitive to the immune response than some bacteria and viruses, as these cells derive from the host itself. Cancer cells continually accumulate mutations which allow escape from the host's regulatory signals. Finally, either over-stimulation or suppression of the immune system is detrimental to the host and may result in disease pathogenesis. In autoimmune diseases, symptoms of dysregulation are comparable in severity to those caused by pathogens or cancer.

All three pathways of complement activation lead to the formation of C3 convertase. NAb and Factor XII interact with the classical pathway also leading to proteolytic cleavage of C3. Another coagulation cascade member, plasminogen, also leads to activation of C3 and this further activates C5 and leads to TF release. This release leads to the cleavage of prothrombin into thrombin, and initiates clot formation further downstream. Thrombin also interacts with the complement system activating both C3 and C5 resulting in formation of the MAC.

The interaction between complement and coagulation systems is further exemplified in the generation of the anaphylatoxin, C5a, by KLK and thrombin [112, 182]. These complex relations are evident in patients with autoimmune diseases. SLE patients develop venous thrombosis and present with an increased deposition of C1q, C3, and C4 on platelets. It is still unclear why there is no increased deposition of complement factors in SLE patient with arterial thrombosis [30, 155].

NAb and coagulation also interconnect as demonstrated in two specific studies: a case of disseminated intravascular coagulation caused by IgM-mediated hemolysis in an 11-month old baby, and a xenograft rejection leading to DIC in baboons [183, 184]. NAb initiate complement activation not only through the classical pathway but also through the lectin pathway. In an animal model of myocardial infarction, for example, damage to the heart requires both natural IgM and the lectin pathway [185].

Increased understanding of the humoral innate immune system components and their interplay can lead to the development of novel or improved therapeutics for these specific clinical condition. In a recent African green monkey model study of avian influenza, treatment with anti-C5a mAb improved survival and decreased acute lung injuries in monkeys infected with H7N9, normally associated with high morbidity and mortality rates in humans [186]. As the first line of immune defense, advances in the understanding of the role of humoral innate immunity in the pathogenesis of pathogenic infection, cancer, and autoimmune disease provides a new approach to treatment.

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Abbreviations

Ab	antibody/antibodies
MBL	Mannose Binding Lectin/s
Ag	Antigen/s
FH	Factor H
FI	Factor I
MAC	Membrane Attack Complex
mAb	monoclonal antibody/ies
TF	Tissue Factor
HF	Hemorrhagic Fever
APLS	Antiphospholipid syndrome
aPL	antiphospholipid
SLE	Systemic Lupus Erythematosus
WNV	West Nile Virus
NAb	natural antibody

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Highlights

- Pathogens evade the immune response with multiple, frequently redundant proteins.
- Cancer may evade or utilize the innate response for growth and metastasis.
- A dysregulated humoral innate response may result in autoimmunity.
- Complement, coagulation and natural antibodies interact to prevent disease.

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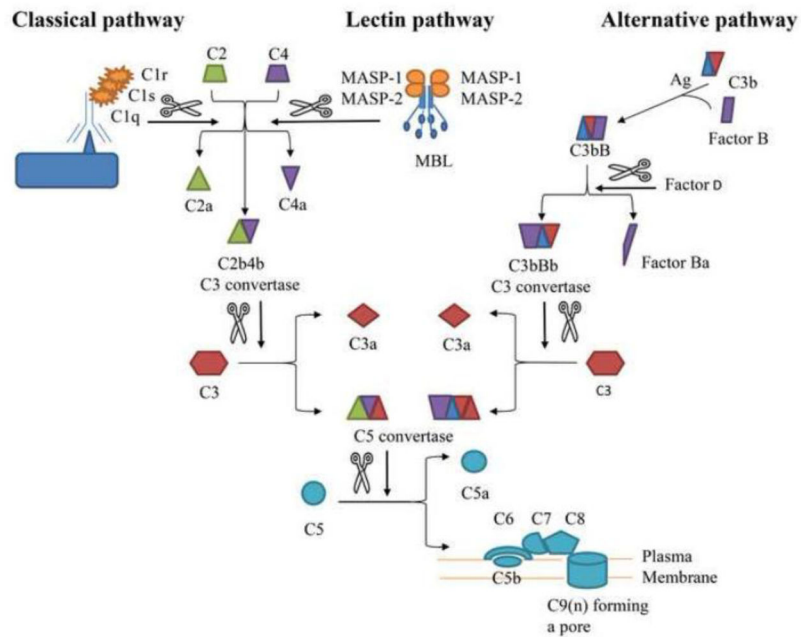


Figure 1. Schematic representation of the complement cascade
 Upon recognition of a foreign or damage cell surface, one or more complement pathway is activated. The classical and lectin pathways differ in their initiation. The alternative pathway is distinct from the other two pathways but all three pathways converge toward the formation of a C5 convertase, which is necessary for the MAC formation. The ✂ indicate a cleavage. Ag, antigen; MAC, Membrane Attack Complex.

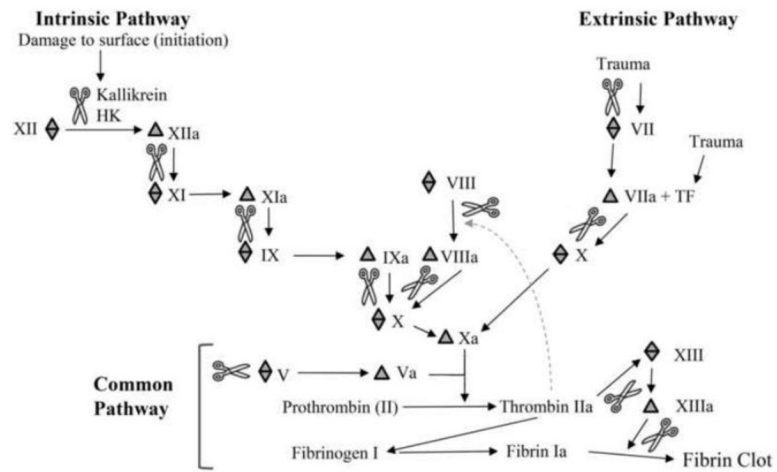


Figure 2. Diagram of the coagulation cascades

There are two coagulation cascades, intrinsic and extrinsic, which converge into the common pathway. These cascades are advanced through proteolytic cleavage of coagulation factors. ◊ represents factor prior to cleavage, ▲ represents the cleavage product and ✂ represents a proteolytic cleavage. HK, High molecular weight kininogen.

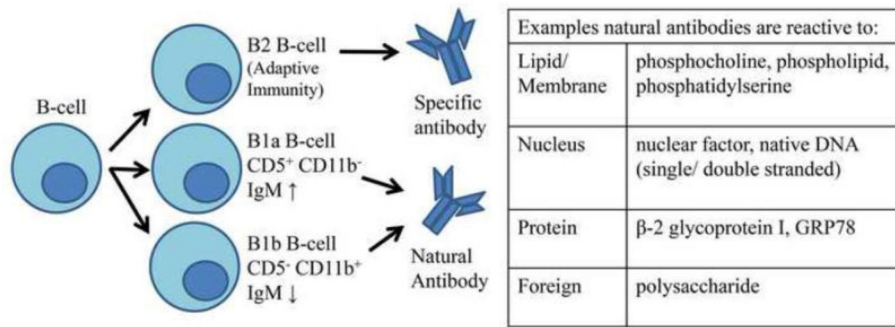


Figure 3. B1 B cells produce self-reactive NAb

B-cells include two distinct populations, B1 and B2 B-cells. B2 cells participate in the adaptive immune response while B1 cells participate in the humoral innate immune response. Based on surface marker expression, B1 B-cells are further divided into two subpopulations B1a and B1b. The NAb produced by the B1 B-cell subpopulations are reactive to “self” proteins, membrane, and nucleus components as well as foreign molecules.

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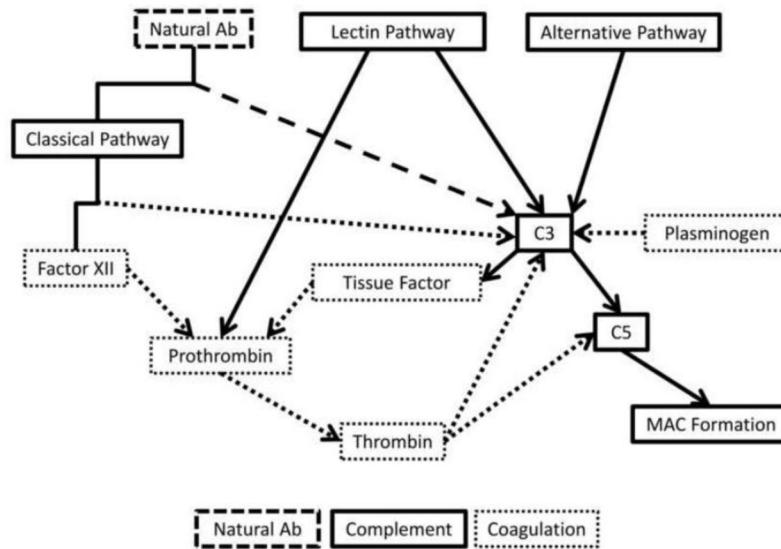


Figure 4. The interplay between complement, coagulation, and natural antibodies

The three pathways for activation of the complement pathway (solid lines) are the classical, lectin, and alternative pathways. The classical pathway interacts with Factor XII from the coagulation pathways (dotted lines) also leading to C3 activation. Natural antibodies (dashed lines) also interact with the classical pathway to activate C3. Plasminogen, from the coagulation pathway, also activates C3 directly. C3 activation leads to two outcomes, both a continuation of activation in the complement cascade to C5, but also leading to the release of tissue factor, which activates prothrombin, leading to the activation of thrombin, both members of the coagulation pathway. Thrombin is also able to lead to both C3 and C5 activation, causing formation of the MAC.