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Article Type: Review

Title: Role of the Complement Pathway in Inflammatory Skin diseases: A focus on hidradenitis suppurativa (HS)

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Abstract

Although the role of immune dysregulation in hidradenitis suppurativa (HS) has yet to be elucidated, recent studies identified several complement level abnormalities in HS patients. The complement system serves a critical role in the modulation of immune response and regulation of cutaneous commensal bacteria. Complement is implicated in several inflammatory skin diseases including SLE, angioedema, pemphigus, bullous pemphigoid, and HS. A simplified model for HS pathogenesis is proposed, integrating the role of commensal bacteria, cutaneous immune responses, and complement dysregulation. The profound role of complement in disease pathogenesis has led to the development of novel anti-complement agents and clinical trials investigating the efficacy of such treatments in HS.

Introduction

The complement pathway serves an important role in the innate immune response and defense against bacterial infection. Through the opsonization and lysis of pathogens, complement proteins facilitate the removal of immune complexes and damaged cells. Recently, there is a newfound appreciation for the role of complement in neuroinflammation, aging, and cancer (Hajishengallis et al., 2017, Hess and Kemper, 2016, Zhuang and Lyga, 2014). The complement system consists of approximately 50 soluble and membrane-associated proteins, including the classic constituents, C1-C9. While the majority of soluble factors are produced by the liver (Hess and Kemper, 2016), complement proteins are also secreted by a variety of cutaneous cells including mast cells, macrophages, keratinocytes, and fibroblasts (Zhuang and Lyga, 2014). Complement is traditionally considered part of the innate immune system, but its role in B and T-cell activation enhances adaptive immune response as well.

The three core mechanisms of complement activation include the classical, lectin, and alternative pathways (Figure 1). Classical pathway activation occurs by antigen-antibody complexes, lectin pathway by bacterial polysaccharides, and alternative pathway by spontaneous activation at low-levels for background immune surveillance. All three pathways converge to produce C3 convertase, leading to the formation of C5 convertase and eventually the membrane attack complex (MAC). Through the “extrinsic” pathway, certain proteins directly cleave C5, including trypsin, thrombin, elastase, cathepsin D, granzyme B, and proteases produced by specific bacteria (Potempa and Potempa, 2012, Riedemann et al., 2017). Effectors include anaphylatoxins, C3a and C5a, to activate the immune system; and C3b to opsonize cells for phagocytosis. C3a and C5a bind to their respective receptors on a wide range of immune cells to propagate the inflammatory cascade (Frew et al., 2019a, Giang et al., 2018). C5b-9 may

assemble to form the MAC on cell membranes for bacterial lysis. Numerous regulators of this pathway exist to protect self-cells from lysis and overactivation of complement (Clarke and Tenner, 2014).

There is renewed interest regarding the complement pathway as a pathogenic actor in chronic cutaneous inflammatory diseases. Dermatologists need a greater understanding of this complex pathway as potential imbalances may be of critical pathophysiologic significance in inflammatory skin diseases. This review provides an update regarding the role of complement in cutaneous health and disease, integrated into the current pathogenic paradigm for HS. The prospect of complement proteins as novel, rational therapeutic targets is also discussed, with the expectation of significant future developments in this area.

Complement in Cutaneous Health and Disease

Complement appears to play a vital role in the maintenance of cutaneous health. The use of C5a receptor antagonists in mice altered the composition and reduced the diversity of commensal skin flora (Chehoud et al., 2013). Additionally, C5a receptor (C5aR1) deficient mice presented with decreased expression of inflammatory genes and reduced number of infiltrating T-cells and macrophages. It was particularly noteworthy that C3 and C5aR1 deficient mice had accelerated wound healing response time (Rafail et al., 2015). In a murine model of cutaneous inflammation using imiquimod, a TLR7/8 antagonist, C3 was found to exacerbate inflammatory response (Giacomassi et al., 2017, Mihai et al., 2018). Overall, the levels and impact of complement anaphylatoxins appear to be correlated with the extent of inflammation in these murine models.

Complement has been directly implicated in a number of human skin diseases, including HS, angioedema, SLE, pemphigus and bullous pemphigoid among others described in Table S1.

Coates et al recently reviewed the role of innate antimicrobial immunity in the skin (Coates et al., 2018). Complement evasion strategies have been developed by specific microbes (Panelius and Meri, 2015). *Borrelia* spirochetes bind soluble complement regulatory proteins to avoid attack. The capsule and thick peptidoglycan layer of Group A Streptococcus (*Streptococcus pyogenes*) decreases the efficacy of MAC and protects the bacteria from phagocytosis. *Staphylococcus aureus* secretes small proteins that interfere with C3 function to inhibit opsonization and anaphylatoxin production (Panelius and Meri, 2015). Herpes simplex virus type 1 may also evade the effects of complement through a complement-interacting glycoprotein that can interfere with C1q, C3, C5, and properdin (Lubinski et al., 2002). Hence, the complement pathway serves an important role in the maintenance of skin health, pathogenesis of cutaneous disease, and in host-microbial interactions.

Pathogenic pathways in Hidradenitis Suppurativa

HS is a chronic skin disease characterized by recurrent painful nodules, abscesses, and suppurative dermal tunnels in intertriginous body regions. Recent epidemiologic data suggests HS may be relatively common, with an estimated prevalence of 1.19% (Ingram et al., 2018). Several updated guidelines for the management of HS have been recently published (Alikhan et al., 2019a, 2019b, Ingram et al., 2019).

Although the specific pathogenic pathways and cytokine signatures of HS remain unclear, a proposed model is presented in Figure 2, incorporating recently published data from a systematic review of the literature (Hoffman et al., 2017, Vossen et al., 2018). As dilated follicular units in intertriginous areas in predisposed individuals rupture, hair follicle contents, including commensal microbiota and keratin may initiate innate immune response. Release of IL-1 from activated inflammasomes drives the production of pro-inflammatory cytokines, including TNF,

IL-6, and IFN- γ . These inflammatory mediators lead to dendritic cell activation, IL-23 production and Th17 polarization. Keratinocyte response results in the increased production of TNF and antimicrobial peptides (AMPs). HS keratinocytes show a distinct pattern of AMP production in response to microbial products compared to control keratinocytes (Hotz et al., 2016). Several AMPs have been measured in the serum of HS patients, such as S100A8 and S100A9 (Wieland et al., 2013) and lipocalin (LCN2) (Wolk et al., 2017). Complement activation of inflammasomes directly may amplify these cutaneous inflammatory circuits (Arbore and Kemper, 2016).

The role of sweat glands in this disease also remains unclear. Although the intertriginous areas are rich in apocrine glands (Hoffman et al., 2017, Vossen et al., 2018), eccrine gland transcriptomic signatures are also dysregulated in HS (Coates et al., 2018). Additional dermal factors, such as aberrant fibroblast responses, may contribute to the inflammatory sequelae of HS (Frew et al., 2019b), including dermal tunnels, persistent suppuration, fibrosis, and systemic inflammation. The development of biofilms on epithelialized tunnels may explain HS flares. Although skin and serum TNF levels from HS translational studies have shown inconsistent results (Frew et al., 2018), the anti-TNF agent, adalimumab (Humira, Abbvie), is currently the only FDA-approved biologic for HS. Ongoing clinical trials are investigating the efficacy of other immunomodulator anti-cytokine therapies, including anti-IL-1, anti-IL-17, (Theut Riis et al., 2018, Wlodarek et al., 2019), and anti-IL-23 (clinicaltrials.gov).

Complement Pathway in Hidradenitis Suppurativa

Kanni et al. recently reported elevated C5a and soluble C5b-9 in the serum of 54 HS patients compared to 14 healthy controls, although neither correlated with disease severity (Kanni et al., 2018). A previous study conducted by this group found that the peripheral blood mononuclear

cells (PBMCs) of HS patients produced less cytokines in response to bacterial stimuli relative to controls (Kanni et al., 2015). However, part of the recent study enriched the PBMCs (n=7) with patient plasma and found that the plasma transformed PBMCs into overproducers of TNF- α ; an effect that was attenuated by the C5a-antagonist, IFX-1. These findings suggest C5a may promote TNF- α production by monocytes.

Analysis of skin and blood transcriptomes, and blood proteome data from HS patients discovered several complement factors that were dysregulated (Hoffman et al., 2018). In the skin transcriptome, there was upregulation of C1q, C2, and factor B, and downregulation of factor H, factor D, and C7. In the blood proteome, only C5a was upregulated, and C4b, C3, C3b, and iC3b were downregulated in HS (Hoffman et al., 2018). Gene Set Variation Analysis (GSVA) also found differential activation of the hallmark complement pathway in HS skin transcriptome, blood transcriptome, and blood proteome.

Proposed Implications of Complement Dysregulation in Hidradenitis Suppurativa

It remains unknown whether the complement dysregulation seen in HS reflects a primary change that drives pathogenesis or presents as an epiphenomena of later stage disease. Since the classical, alternative, and lectin pathways converge to produce C5 convertase and C5a was elevated in the serum, all three pathways may contribute to HS pathogenesis. The primary function of complement includes activation of the immune system via anaphylatoxins, opsonization, and bacterial lysis; all potential early and/or amplifying events in HS (Figure 2). Elevated C5a in HS suggests complement overactivation in these patients. Additionally, specific complement components may be reduced due to consumption. For example, decreased C3 may result from increased complement activity with C3b production for bacterial opsonization and C3/C5 convertase formation. Although the skin transcriptome had decreased C7 (Hoffman et al.,

2018), and the serum had increased soluble C5b-9 (Kanni et al., 2018), the relationship between these findings remains unclear.

Complement and TNF are interlinked inflammatory molecules in innate immune responses. Kanni et al suggested C5a may prime the production of TNF by human monocytes in HS (Kanni et al., 2018). Numerous examples of complement-dependent TNF release have been reported (Page et al., 2018), with C5a as a critical stimulator of TNF release (Liu et al., 2015). Additionally, TNF α may induce complement activation through the upregulation of C3 (Page et al., 2018), alternative pathway components, decreased production of surface regulatory proteins (CD141), and impaired Protein C activation (Sartain et al., 2016). Translational studies in rheumatoid arthritis and psoriasis demonstrated an an overall reduction of C3 and C4 with treatment using anti-TNF agents (adalimumab, infliximab, and etanercept) (Chimenti et al., 2012, Di Muzio et al., 2011). The proposed bi-directional TNF-complement axis in the pathogenesis of HS is indicated in Figure 2.

The role of microbiota in HS pathogenesis

The dysregulated complement signature of HS in conjunction with the critical role of complement in host anti-microbial response warrants reconsideration of the potential role microbial species may play in HS pathogenesis (Belkaid and Segre, 2014, Naik et al., 2019). One explanation for the complement irregularities identified in HS involves the concept of dysbiosis, the alteration of normal commensal microbial composition in an organ (Park and Lee, 2017). The majority of bacteria present in HS are commensal microbes. With the use of modern molecular techniques, an abundance of *Porphyromonas* and *Prevotella* gram-negative anaerobic rods were identified in HS lesions (Guet-Revillet et al., 2014, Ring et al., 2019, Ring et al., 2017). Two

additional taxa of anaerobes, *Fusobacterium* and *Parviomonas*, correlated with clinical severity of HS (Guet-Revillet et al., 2014).

Although HS does not fulfill Koch's postulates of infectious disease, the dysbiosis of commensal bacteria may drive disease pathogenesis (Naik et al., 2019). Contextual pathogenicity depends on a series of host and microbial factors that may manifest as disease (Chen et al., 2018). Atopic dermatitis, inflammatory bowel disease, and sepsis are examples of this concept, (Biedermann and Rogler, 2015, Kobayashi et al., 2015, Maekawa et al., 2014, Singh et al., 2016, Yang et al., 2018). A combination of factors may contribute to dysbiosis, including immune dysregulation, hormonal change, and microbiota imbalance.

Proposed relationship between Genetics and Complement

Mutations of γ -secretase, the trans-membrane protease complex that activates Notch, have been identified in some HS patients (Frew et al., 2017), and γ -secretase loss of function mutations may impair Notch signaling. However, the pathologic significance of these mutations and the close proximity of many identified mutations to the extracellular surface of γ -secretase remains unknown (Frew et al., 2017).

CD46 is a transmembrane complement regulatory protein that can be cleaved by γ -secretase (Yamamoto et al., 2013) and *Porphyromonas gingivalis* (Mahtout et al., 2009). This cofactor also mediates complement regulation through inhibition of C3b and C4b. As CD46 binds to the C3b attached to host cells, serine protease factor I cleaves C3b into its inactivated form, iC3b, preventing further amplification of the complement cascade. Polymorphisms of CD46 have been associated with atypical hemolytic uremic syndrome (aHUS), SLE, glomerulonephritis, systemic sclerosis, and other diseases of complement dysregulation (Liszewski and Atkinson, 2015). Furthermore, CD46 interaction with C3b induces intracellular T-cell polarization into Th1 cells,

while simultaneously upregulating IL-10 to increase the negative feedback regulation for Th1 response (King et al., 2016).

These findings suggest a potential link between γ -secretase mutations in HS, commensal bacteria, and the serum complement dysregulation observed in HS. Genetic mutations in γ -secretase may impair CD46-mediated negative feedback, leading to unrestrained T-cell and complement activation that propagates inflammation in the pathogenesis of HS.

Strategies for Anti-Complement Therapies

Recent appreciation for the role of complement in disease pathogenesis has led to the development of novel targeted therapies (Figure 1).

Eculizumab (Soliris, Alexion Pharmaceuticals) is a monoclonal antibody that binds to C5 and inhibits its cleavage by C5-convertase into C5a and C5b. This FDA-approved anti-C5 agent is indicated for paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS) (Morgan and Harris, 2015). In PNH, affected red blood cells (RBCs) lack two glycolipid-anchored complement inhibitory proteins (CD55 and CD59). This deficiency predisposes them to persistent C3 opsonization and MAC-mediated intravascular hemolysis. Two large multicenter trials confirmed the efficacy of eculizumab for PNH with improved clinical response (reduced transfusion needs, hemoglobin stabilization, resolution of hemolysis-related symptoms) (Mastellos et al., 2018). In aHUS, genetic complement regulatory abnormalities and anti-complement antibodies result in continuous spontaneous hydrolysis of C3 and activation of the complement cascade. Unrestrained complement leads to microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. Eculizumab inhibits complement-mediated thrombotic microangiopathy and improves renal outcomes for aHUS patients (Legendre et al., 2013, Noris et al., 2014).

Ravulizumab (ravulizumab-cwvz, Ultomiris, Alexion Pharmaceuticals), a long acting form of the first-generation C5-inhibitor, eculizumab, received FDA-approval for PNH in 2018 (McKeage, 2019). Ravulizumab was noninferior to eculizumab for the treatment of PNH in patients that were complement-inhibitor naïve (Lee et al., 2019) and patients previously treated with eculizumab (Kulasekararaj et al., 2019). The 8-week maintenance frequency of ravulizumab provides a more favorable dosing schedule than the 2-week maintenance frequency of eculizumab. Given the prolonged half-life of ravulizumab, patients may also experience fewer episodes of breakthrough hemolysis (Connell, 2019).

IFX-1 (InflaRx, GmbH) is a monoclonal anti-C5a antibody (Maarouf et al., 2018). In a small proof of concept study, HS patients with advanced disease at a single European site received a weekly infusion of IFX-1. This therapy resulted in 75% of patients meeting the primary clinical endpoint (HiSCR) at 12-weeks, and 83% at the end of the 12-week follow-up period. This biological agent is now in clinical trials for HS in the United States and Canada (NCT 03487276).

BIVV009 (Sutimulimab, originally known as TNT003, Bioverativ) is an antibody against C1s. This approach targets the classical complement pathway, theoretically leaving the alternative and lectin pathways intact. BIV009 has been granted orphan drug status by the FDA in 2017 and is now in clinical trials for bullous pemphigoid (Kushner and Payne, 2018).

PMX-53 (Peptech), a potent C5a-receptor antagonist that results in a dose-dependent inhibition of C5a-mediated activation, had limited success in phase II clinical trials for osteoarthritis, rheumatoid arthritis and psoriasis (Morgan and Harris, 2015). The short half-life and rapid breakdown of this compound may explain the reported inefficacy of this treatment (Holers and Banda, 2018). PMX205, a lipophilic analogue of PMX-53 with improved *in vivo* stability and

efficacy has been suggested as a better drug candidate, particularly for neurological conditions due to increased blood brain barrier permeability (Kumar et al., 2018).

CCX168 (Avacopan, ChemoCentryx) is an orally administered, small molecule selective C5aR inhibitor; it has shown safety and efficacy in a phase II, randomized, placebo-controlled trial (CLEAR) in patients with ANCA-Associated Vasculitis. The primary efficacy measure of greater than 50% reduction in Birmingham Vasculitis Activity Score by week 12 with no worsening of any organ system was achieved in 86.4% ($p=.002$ for noninferiority) of patients treated with avacopan plus 20 mg prednisone and 81.0% ($p=.01$ for noninferiority) of patients solely treated with avacopan, compared to 70.0% in the control group treated with 60 mg prednisone. These findings support avacopan as an effective substitute in replacing high-dose glucocorticoids in treating vasculitis (Jayne et al., 2017, Tan and Zhao, 2018). A phase II study was recently initiated for avacopan in HS (NCT03852472).

The major risk factor associated with inhibition of the terminal complement pathway is *Neisseria meningitidis* infection, over a thousand-fold more frequent in patients with terminal complement deficiencies (Barnum, 2017, Lewis and Ram, 2014). Vaccination is highly recommended prior to commencing treatment targeting the C5 pathway. However, the risk of *Neisseria meningitidis* infection in patients with genetic complement deficiencies is greater than the risk observed in patients with PNH treated with eculizumab, although this difference may be attributed to greater immunization rates and prophylactic antibiotics in the latter. IFX-1 inactivates circulating C5a and may have a better safety profile than agents inhibiting the C5 convertase. Due to the association of early classical pathway component deficiencies (C1q and C4) with lupus-like symptoms, potential treatments targeting these components should be further evaluated.

Conclusion

The complement pathway serves an essential role in immune regulation that may be implicated in the pathogenesis of several inflammatory skin diseases including HS, vasculitis, SLE, and bullous pemphigoid. Several ongoing clinical trials of molecules targeting C5 and C1 will evaluate their potential role in the treatment of HS in the near future. Promising results were obtained from a single center, proof of concept trial using a monoclonal anti-C5a antibody for HS, but larger clinical trials are urgently required to demonstrate the role of complement in the pathogenesis of HS.

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Role of the Complement Pathway in Inflammatory Skin diseases: A focus on hidradenitis suppurativa (HS)

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Table S1. Complement in cutaneous diseases

Disease	Proposed role of complement in pathogenesis
Hereditary and acquired angioedema	C1-inhibitor (C1-INH) deficiency (genetic mutation or acquired autoantibodies) cause overactivated complement and excess bradykinin levels. Bradykinin leads to increased vascular permeability and angioedema (Csuka et al., 2017, Zeerleder and Levi, 2016)
Systemic lupus erythematosus	Autoantibodies directed against DNA and histones may induce uncontrolled complement activation, leading to secondary complement consumption and organ damage; impaired clearance of immune complexes and apoptotic debris may trigger increased autoimmune response (Leffler et al., 2014, Zharkova et al., 2017)
Vasculitis	Perivascular C3d deposits and MAC; C3a and C5a increase endothelial cytokine release and vascular leakage; C5a translocates ANCA to cell surface and stimulates neutrophils leading to endothelial injury and coagulation pathway activation (Chen et al., 2017)
Pemphigus	C1q, C3, C4, and properdin found in intercellular substance; autoantibody activation of complement causes loss of intraepidermal keratinocyte cohesion and blister formation (Panelius and Meri, 2015)
Bullous pemphigoid	Autoantibodies fix complement to basement membrane, increasing inflammation and blister formation (Hammers and Stanley, 2016); C5a-C5aR1 axis is critical for disease pathogenesis in autoimmune blistering murine-model; anti-C5 treatment ameliorates disease (Mihai et al., 2018)
Partial lipodystrophy	Associated with abnormal complement activator, C3 nephritic factor, stimulating C3 convertase and C3b deposition; alternative pathway and MAC damage adipose cells (Panelius and Meri, 2015)

Primary and functional complement deficiency-related skin infections	Pyogenic skin infection associated with lack of C2 and factor I deficiency; factor I inactivates C3b/C4b and decreased factor I leads to secondary C3/factor B deficiency (Panelius and Meri, 2015); deficiency of properdin or terminal complement components (C5b-C9) increases risk of invasive <i>Neisserial</i> infections (Bathum et al., 2006, Schneider et al., 2007)
Hidradenitis suppurativa	Elevated C5a and soluble C5b-9 in the serum of HS patients (Kanni et al., 2018); in the skin transcriptome, C1q, C2, and factor B genes were upregulated, while factor H, factor D, and C7 were downregulated. In the serum proteome, C5a was upregulated, and C4b, C3, C3b, and iC3b were downregulated (Hoffman et al., 2018)

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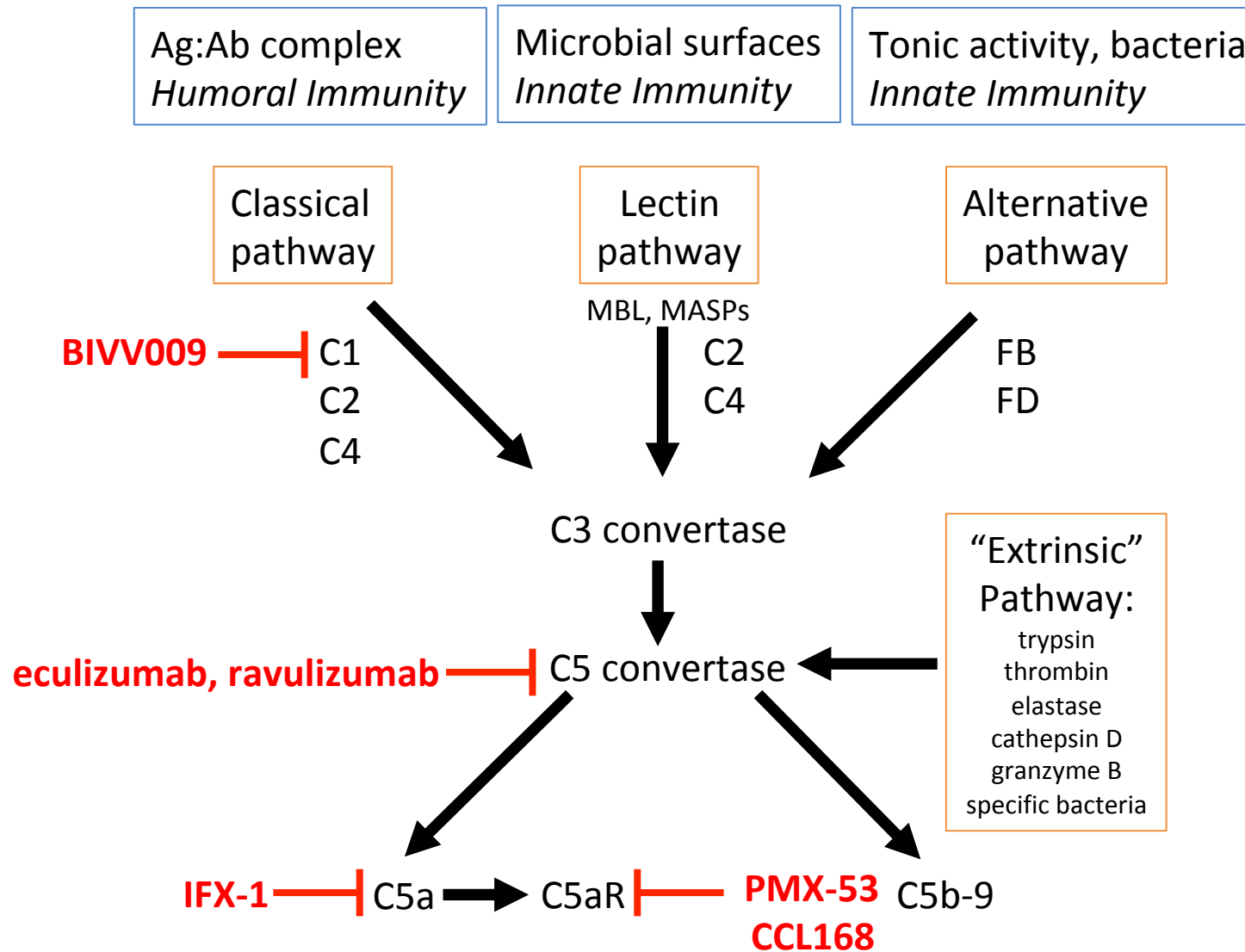


Figure 1. Complement pathways and inhibitors. Classical, lectin, alternative pathways converge to produce C3 convertase (C4b2a and C3bBb), which then produces C5 convertase (C4b2a3b and C3bBb3b). Various enzymes and bacteria can directly cleave C5 via the “extrinsic” pathway. Inhibitors of complement effectors that are currently in development are shown in red.

INITIATING FACTORS

Genetic factors

Hair follicle contents:

- commensal microbiota, dysbiosis
- keratin
- PAMPs/DAMPs

Exogenous factors:

- smoking
- obesity
- mechanical

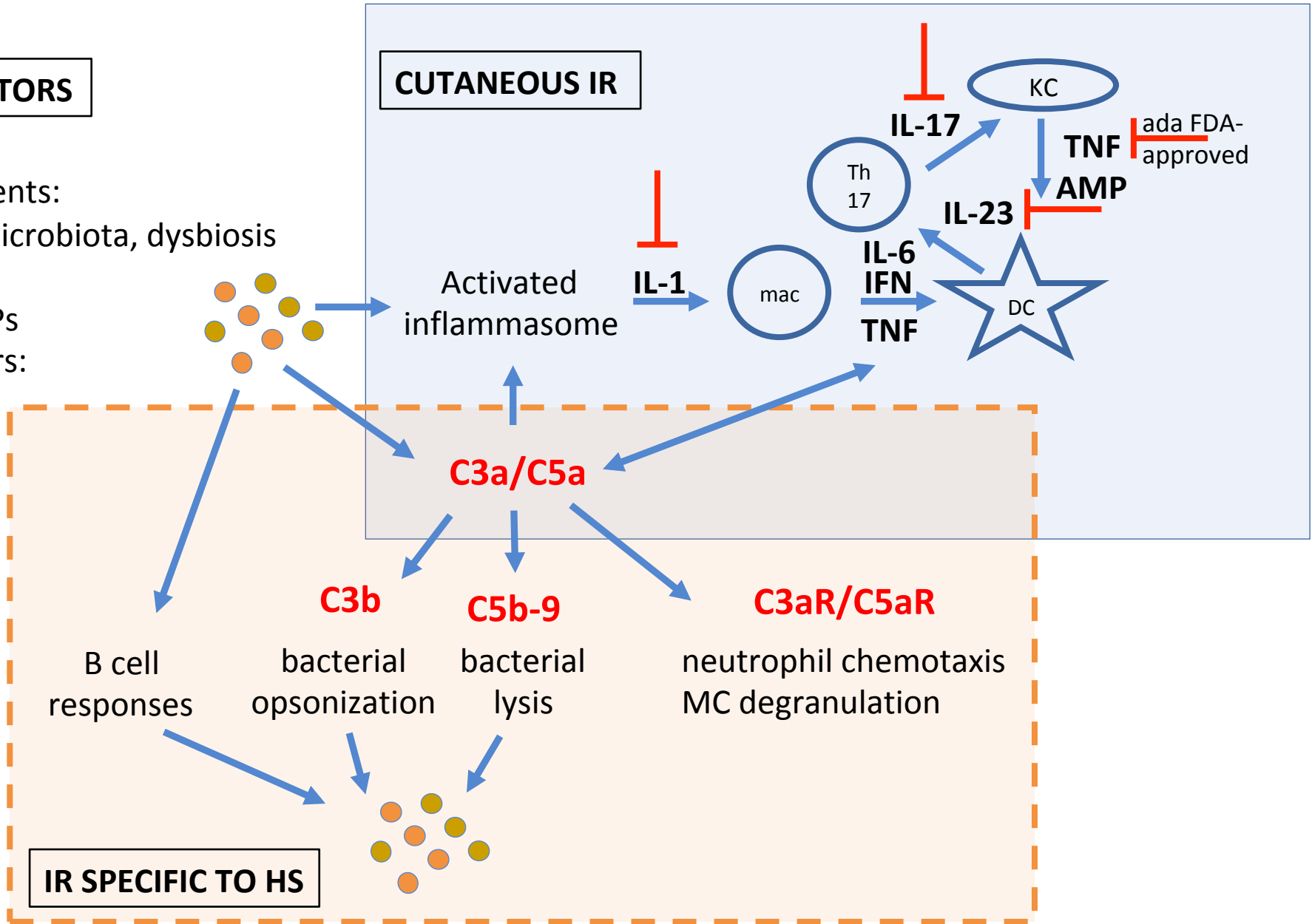


Figure 2. Proposed simplified role of the complement pathway in hidradenitis suppurativa (HS) pathogenesis.

Initiating factors are strong inducers of innate immune responses (IR) in patients with a predisposition for HS.

Spontaneous immune responses (blue box) lead to keratinocyte (KC) activation with abundant cytokine and antimicrobial peptide (AMP) production. Complement has known pro-inflammatory and anti-bacterial effects and relationships (dotted orange box) although they have not yet been proven in HS. Targeted anti-cytokine biologics are shown in red.

Abbreviations: ada (adalimumab); mac (macrophages), DC (dendritic cells)