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The Role of the Complement System in Chronic Intestinal Inflammation

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ABSTRACT

Complement is an important component of the innate immunity that can initiate or sustain inflammation but can modulate the developing adaptive immune response against invading foreign pathogens. Chronic inflammation is a characteristic of many long-term clinical disorders. An imbalance in the activation and regulation of the intestinal complement system hampers effective intestinal barrier function and hence contributes to severe intestinal inflammation as observed in inflammatory bowel diseases patients. Animal models have been instrumental in the evaluation of over-activation or impaired activation of the intestinal complement system in determining the extent of chronic intestinal inflammation. This review examines the role and possible therapeutic manipulation of complement system in chronic intestinal inflammation.

Keywords: Chronic inflammation; Complement; Intestinal; Intestinal Complement system.

1. Introduction

Complement activation aids in the recognition of pathogens, has immunoregulatory functions and initiates resolution. It assumes a pathogenic role in a range of progressive diseases such as cancer or autoimmune disease. Unresolved inflammation occurs when microbes are not eliminated, injuries remain unrepaired, apoptotic cells or immune complexes are not sufficiently removed.

The complement system has been indicated to be a double-edged sword since it can promote inflammatory responses that result in tissue damage [1]. Activation of complement during an inflammatory reaction contributes to inflammation driven tissue injury inflammation by generating anaphylatoxins (C3a and C5a) and skewing macrophage activity towards a pro-inflammatory M1 type. Furthermore, alterations in the expression of complement regulatory proteins, which

lead to the excessive complement activation, can also contribute to tissue injury because they no longer protect host cells from self-attack [2].

The complement is considered to serve as the first line of defence [3]. Inflammation is a second line defence mechanism of the immune system; it is response to a physical injury or an infection or to stimulation of damaging, for example, damaged cells, stimuli to pathogens or chemical toxins and radioactivity. The purpose of the process is to eliminate harmful stimuli and start the adaptive immune response, and initiate healing [4-5]. The *inflammatory* response can be either *acute* or *chronic*. Typically, when acute inflammatory responses occur, cellular and molecular immune components lessen injury or infection and cooperate to restore homeostasis by resolving the acute inflammation. But, when the acute inflammatory mechanisms fail to remove tissue damage, the acute inflammation may turn chronic. Chronic inflammation is present in a variety of diseases, for example, cardiovascular diseases, atherosclerosis, type 2 diabetes, rheumatoid arthritis, and cancers [6]. In general, chronic inflammation is a response to a wide range of unresolved or unresolvable factors such as chronic infections, exposure to physical or chemical factors, food carcinogens, continued injuries or shock, moreover, it occurs by digestive fluids, bile acids, urine reflux, and deficiencies of the immune system that normally aid in a timely recognition and removal of stimuli. Recently studies show that complement can be activated and use intracellular biological functions, for instance, in T cells, creating the new term called complosome [7]. The intracellular complosome discusses biological activities together with cell metabolism, autophagy, and worsens damage in pathogen infection, autoimmunity, and cancer development etc. [7-9]. However, up to now, there is no data addresses complosome in the UC development.

2. Complement activation in intestinal inflammation

This review is concerned with inflammatory bowel disease (IBD), which has become an important health problem worldwide, and this disease is expeditiously occurring in both developed and developing countries [10], of which Crohn's disease (CD) and ulcerative colitis (UC) are the most common. Until 2015, an estimated 1.3% of US adults, about 3 million, had been diagnosed with IBD [11]. While intestinal inflammation in UC is limited to the colon, CD encompasses both the small and large intestinal tracts. These diseases IBD, CD, and UC, are heterogeneous states of chronic intestinal inflammation and the reason for their cause is exactly unknown [12-13].

UC pathogenesis is still complicated and unwell understood, and there are factors thought to be related to this disease for example, environmental factors, genetic factors, and the *gastrointestinal* microbiome [14]. UC is known as a chronic relapsing–remitting inflammatory disease where the immune system plays an essential role of regulation [15-16]. Both innate and adaptive immune components are linked with a continued inflammatory microenvironment in the colon [12-13].

IBD are linked to an immunological imbalance of the intestinal mucosa and produce chronic inflammatory conditions. An inappropriate inflammatory response to gut luminal microbial flora is observed [17]; both, CD and UC, are caused via cytokine-driven and non-infectious inflammation of the gut. In CD, there is overproduction of IFN-γ/IL-17 and IL-12/IL-23, whereas UC is related to additional IL-13 [18]. IBD seems to be the cause of a dysfunctional interaction between gut bacteria and the mucosal immune system [19].

Of note, IL-13, Th2 cytokine, may protect cells against complement mediated lysis [20], and the generation of complement anaphylatoxins has been related to production of IFN-γ and IL-17 in other disease states [21] that may require simultaneous triggering of TLR activities [22]. C5a stimulation, via IL-10 secretion may exert an inhibitory role on the IL-17/IL-23 axis [23].

There are differences in the type and extent of intestinal complement activation between UC and CD patients. The activation of the classical pathway of the complement system was shown in patients with UC at the apical epithelium, whereas in CD patients increased activation of the alternative pathway of the complement system in intestinal epithelium was observed [24], and may point to initiators of the alternative pathway being more important in Crohn's disease than in UC. The levels of jejunal complement were normal in the first-degree relatives of patient population with CD [25]. However, another study reported that the levels of complement activation of the alternative pathway were reduced in patient with CD and their first-degree relatives [26]. It has been suggested that early and late phase complement activation occurs at the luminal face of the epithelium in Crohn's disease.

The expression of intestinal IgM and B cells was increased in remission of CD patients in concert with early classical complement pathway components. The presence of intestinal C1q and C3 crosslinking of the IgM-B-cell receptor by C1q binding, in addition to B-cell activation by C3 binding to CR1 and CR2, may result in an enhanced induction of the humoral immune response in CD patients [27].

Expression of CD55, also termed decay-accelerating factor, was shown to be highly up-regulated on the apical membrane of intestinal epithelial cells from IBD patients. In patients with CD, the intestinal epithelial cells show a loss of expression of basolateral CD59, whereas in UC there is apical or absent expression of CD59. Moreover, the components of the complement system of C1q, C3, C4, factor B and CD46 show high expression level in intestinal epithelial cells. Strong apical C3b and C4c depositions have been detected exclusively on intestinal epithelial cells in patients of CD compared to healthy individuals and patients with UC (Figure 1). C3aR1 is expressed in the lower crypts on intestinal stem cells, whereas C5aR1 is expressed on the cell surface of intestinal M cells [28].

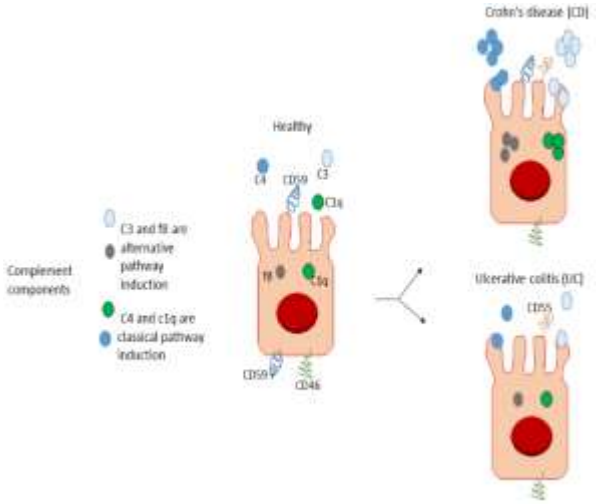

Fig.1. Diagrammatic comparison between Crohn's disease (CD) and ulcerative colitis (UC) in the changes in epithelial intestine of complement components.

Sufferers with IBD have a much higher risk of colorectal cancer development than the general population [29]. The most serious problem of IBD is Colitis-associated colorectal cancer (CAC). Long-standing ulcerative colitis prompts to development CAC the main source of death in patients with UC. The CAC development is associated to overproduction of pro-inflammatory cytokines by immune and non-immune cells, for example TNF-a, IL-6, and IL-17A [30-32]. UC is linked with increased production of cytokines with anti-inflammatory effects, for example IL-10, and proinflammatory effects, for example interleukin (IL)-1b, IL-13, IL-6, IL-8, TNF-α and IL-

17A [18, 33]. Serum IL-17A has recently been suggested as a possible predictive marker at onset of UC [34]. The role of the complement system was investigated in a mouse model of chronic colitis driven colorectal carcinogenesis triggered by dextran sodium sulfate (DSS) and the carcinogen azoxymethane. In this model, C3 deficient mice displayed reduced colonic pro-inflammatory cytokine IL-1β levels, lower cell proliferation in colonic epithelial cells as well as a significantly lower tumor load compared to wildtype mice [35].

3. Complement and anti-microbial peptides in intestinal inflammation, including deficiencies

During inflammation, the cellular composition of the intestinal mucosa changes significantly. Complement and anti-microbial peptides (AMPs) are involved in inflammation and share lytic activities, phagocytic, and chemo-attractant activities [36]. The family of AMPs includes β-defensins (HBD-1,2,3,4), RegIIIα, and LL 37/human cationic AMP 18 [37-39]. AMPs are secreted from Paneth cells which is important in maintaining spatial segregation of the intestinal microbiota from the epithelium [40]. While levels of HBD-1 were unchanged between the healthy and inflamed gut, they have been shown to decline in UC [41-43]. Moreover, the levels of HBD-2, 3 and 4 showed an increase in UC but not CD [38]. LL-37 expression was unchanged during gut inflammation [44-45]. The expression of murine RegIIIγ also known as human hepatocarcinoma-intestine pancreas/pancreatitis-associated protein was increased during bacterial exposure and mucosal inflammation, and the expression of human RegIIIα was increased in patients with IBD [46-47]. There are differences between the healthy and chronic inflamed intestinal epithelium impacting on expression of AMPs. AMPs are increased in abundance in metaplastic Paneth cells in the colon [48-49]. Colonic metaplastic Paneth cells produce α-defensins, lysozyme, and $sPLA_2$ [48]. Secretory phospholipase A2 ($sPLA_2$) is not detected in the healthy colon but is expressed by metaplastic Paneth cells in addition to some colonocytes throughout inflammation [50]. Alpha-defensins are also induced in the large intestine during inflammation, related with metaplastic Paneth cells [48]. Mutations in microbe detecting molecules in Paneth cells are assumed to directly impact defensin production. But, the particular microbiome has been realised to be an essential factor in influencing defensin production in mice with defects in these detecting molecules [51].

AMPs are pivotal for host defense and have numerous important links with human disease. Chronic intestinal inflammation related to defective antibacterial mechanisms, perhaps because of changed expression and secretion of AMPs. CD of the ileum has been related with a deficiency in Paneth cell α-defensins. CD of the colon is related with a low-gene copy number polymorphism of the human α-defensin locus, this lead to a reduced epithelial induction of α-defensin AMPs in the colonic mucosa. Defensin deficiency weakens the antimicrobial defense properties of the intestinal mucosa, leading to changes in the composition of intestinal flora that ultimately may enhance bacterial attack of the mucosa and predispose to the chronic inflammation of CD [52-53].

Primary complement deficiency has been diagnosed in patients who presented with IBD, as IBD can be an early sign of an underlying immunodeficiency, e.g, MASP2 deficiency, Ficolin 3 deficiency and CD55 [54, 55], although CD55 is not expressed by healthy colonic epithelial cells [55-56]. The identification of MASP2 deficiency highlighted the possibly vital role of intact activation of the complement system in colitis [57].

A deficiency of complement in the intestine leads to significantly lower IL-1β using mouse model of chronic colitis driven colorectal carcinogenesis

triggered by DSS and the carcinogen azoxymethane. IL-1β is a proinflammatory cytokine with a wide range of systemic and local biological functions and has been described in enhancing chronic inflammation and developing malignant growth it [58-59]. The intestinal IL-1β is essential for build-up of IL-17A-secreting innate and adaptive immune cells in the intestine on normal state and during chronic inflammation [60-61]. Complement activation components C5a and C3a represent an important component for Colitis-associated colorectal cancer (CAC) formation by activating and regulated strong production of proinflammatory IL-1β by neutrophils thus eliciting protumor IL-17A response in intestinal myeloid cells and promoting CAC development.

Defensins play a role in colonic infection and inflammation. During inflammatory processes, many AMPs are expressed and can work as disease biomarkers, such as in IBD. Human beta-defensin 2 (HBD-2) is weakly expressed in normal colon but significantly increased in inflamed colonic epithelium of patients with IBD [62]. But, plasma levels of HBD-2 in IBD patients were unchanged [63]. the role of HBD-2 in the pathophysiology of colitis and colitis-associated microflora has been shown as HBD-1, exposure of human colonic epithelial Caco-2 and HT-29 cells to proinflammatory IL-1α and/or enteroinvasive *Escherichia coli (O29:NM)* significantly increase HBD-2 expression. Also, HBD-3 and HBD-4 expression are increased in colonic crypts of UC patients, but not CD patients [38]. Chronic inflammation prompts Gankyrin (GK) and stress response proteins that promote the development of CAC [64, 65]. GK (also known as PSMD10, p28 and Nas6p) is an oncoprotein which regulates inflammatory responses and its inhibition is believed as anti-inflammatory treatment for IBD. In the colon, Gankyrin up-regulates the expression of pro-inflammatory cytokines (TNF-α - IL-17), markers of stem cell, Bmi1 and sex-determining region Y (SRY) - box 9 (Sox9). Deficient of GK mice in the upper small bowel augmented inflammatory activity paralleled with healthy mice when colitis was prompted with dextran sodium sulfate. Biochemical analyses have discovered deficient of GK mice to have caused decreased in the expression of antimicrobial peptides, α-Defensin-5 and -6, in the upper small bowel. This result revealed the decreased expression of GK in the small bowel is related to colonic involvement in human CD [66-67].

4. Animal models of intestinal inflammation

In addition to investigating the changes in levels of complement protein in serum, the animal models were used to identify whether different complement activation pathways exerted a predominately inflammatory or anti-inflammatory effect in disease pathogenesis.

To study intestinal inflammation, Okayasu *et al*., 1990 [68] employed a dextran sodium sulfate (DSS) induced barrier dysfunction model and presented that oral administration of 5% DSS in drinking water to BALB/c mice was able to induce acute or chronic colitis after multiple cycles of DSS [68]. An advance in appreciation of the role of the complement system in the intestinal inflammatory response during chronic inflammation came through the use of gene deficient animals in models of DSS induced mucosal toxicity. Using mice deficient in C1q, MBL and C3 in a model of chronic DSS-induced colitis, an essential role for these opsonins and/or the classical pathway C3 convertase was demonstrated to provide a protection against mucosal injury and infection. However, deficiency mice of the alternative pathway (fB) had significantly less impact on injury profiles [69].

Macrophages are considered the primary source of complement components C1q in inflamed colon tissue [70] deficiency of C1q worsens intestinal damage and

inflammatory infiltration in DSS-induced acute colitis [71]. In the model of chronic DSS-induced colitis, mice deficient in C1q and MBL died at the beginning of the experiment, whereas mice deficient in C5aR1 or C3 showed stronger intestinal inflammation and reduced survival rates compared to wild-type mice [69, 72]. Mice deficient in C3 showed in a mouse model of chronic colitis-driven colorectal carcinogenesis triggered by DSS and the carcinogen azoxymethane reduced colonic pro-inflammatory cytokine IL-1β levels, and less cell proliferation in colonic epithelial cells as well as lower tumor load significantly compared to wild-type mice [35].

The role of the alternative pathway of complement was studied further in the pathogenesis and the shaping of an inflammatory response in chronic DSS-induced colitis. Mice deficient in Factor B, one of the serine proteases essential for alternative pathway activity, displayed lower chronic colitis in contrast to wild-type mice and showed the essential role of the alternative pathway in enhancing inflammation in chronic colitis [69]. In a separate study, a fusion construct of complement receptor 2 and Factor H, CR2-fH, given during DSS treatment periods, inhibited alternative pathway activity [69]. Alternative pathway inhibition significantly changed the inflammatory response in the chronic state and led to a reduction in B cells, macrophages, and mature dendritic cells within the lamina propria. The treatment led to reduced tissue inflammation and injury, reduced the fibrosis levels, and changed the local immune response compared to basal levels and DSS treated wild-type mice. In chronic inflammation, unnecessary wound healing can cause the development of fibrosis, and lead to surgery in human IBD patients [73]. M1 macrophages have a pro-inflammatory phenotype that can lead to tissue damage, whereas M2 macrophages are usually anti-inflammatory and play an essential role in wound healing. A timely resolution of the injury reduces a fibrotic reaction.

5. Implications from human array and association studies

Microarrays have been used to identify genes that might be involved in perpetuating inflammatory disease progression in IBD. Overexpression of IL-8 mRNA in both CD and UC and TNF-α mRNA in CD was observed in samples of 31 patients were used to analysed by cDNA microarrays for microarray studies [74].

To identify gene expression outlines particular for UC and CD, in endoscopically affected and normal intestinal colonic mucosa from IBD patients. The study evaluated a panel of 84 genes related to the IBD-inflammatory pathway on 21 UC and 22 CD paired inflamed and not inflamed mucosa and on age-matched normal mucosa from 21 non-IBD controls. CCL11 and MMP10 mRNA genes in the UC, and C4BPB (Complement component 4 binding protein beta) and IL1RN (interleukin 1 receptor antagonist) mRNA genes in the CD showed an up-regulation trend in both non-inflamed and inflamed mucosa when compared to controls. These genes appear to be particular markers of UC and CD inflammation levels. This study suggested that the transcript levels of CCL11, MMP10, C4BPB, and IL1RN (but not C3 or CR2) are candidate biomarkers that could help in clinical practice for the differential diagnosis between UC and CD and could guide new research on future therapeutic targets [75].

Over 230 IBD-associated loci comprising about 300 potentially associated genes were identified by research using genome-wide association studies (GWAS). These include the pattern recognition receptors Nucleotide-binding oligomerisation domain 2 (NOD2) and Caspase recruitment domain-containing protein 9 (CARD9). NOD2 was initially reported as a susceptibility gene for Crohn's disease. CARD9

signaling is contributory in the innate immune response against assured intracellular bacteria [76, 77]. It has been reported that CARD9 is necessary for NOD2-mediated activation of Mitogen-activated protein kinas (MAPK) signalling components, for example, p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun-NH2-terminal kinase (JNK). After treatment with muramyl dipeptide, CARD9-deficient macrophages display a selective defects in p38 and JNK signaling [78]. CARD9 signaling is critical for intestinal immune homeostasis affecting innate lymphoid cell and T-helper 17 cell responses, has a role in the composition of gut microbiota, intestinal epithelial cell restitution and barrier maintenance, production of microbial metabolites, and overall sensitivity to colitis [79-80]. It is interesting to note that CARD9 and complement receptor 3 (CR3) cooperate in the phagocytosis of fungi [81].

6. Conclusion in the microbial context

IBD is one of the many chronic inflammatory disease states that the complement system has been assigned an important pathogenic role in. Complement shows an important role in the pathogenesis of IBD through the direct effect of complement activation products on tissue injury and by effects of membrane-bound complement receptors or glycoproteins on inflammation and the following shaping of immunity. Apart from its role in the innate immune system, Complement is involved in adaptive immunity. Complement stimulates the differentiation of regulatory T cells [82-83], which are relevant in the maintenance of mucosal tolerance [84-85].

IBD is thought to be eventually driven by separated T cells reactive in part to enteric antigens. The T cells include for example, Th1 cells which produce IFNγ; Th17 cells which produce IL-17; or Th2 cells which produce IL-4, IL-5, or IL-13. Microbially produced glycans (MPGs) play critical roles in regulating the immune responses to the microbiota in the intestine: Microbiota of the gut use MPGs to inhibit innate immune responses via reducing the production of pro-inflammatory cytokines and increasing the production of anti-inflammatory cytokines through innate immune cells. Also adaptive immune responses can be modulated by MPGs; for example MPGs can block B cell and antibody responses, particularly by covering surface antigens. MPGs can direct differentiation of polyclonal T cells into regulatory T cells (T_{regs}) that suppress effector T cells in addition to intestinal inflammation. *Bacteroides fragilis* PSA prompts the differentiation of IL-10 producing FoxP3+ T_{regs} in mice in a TLR2-dependent manner, which can defend compared to TNBS-induced colitis and experimental colitis prompted by *Helicobacter hepaticus* [86-88]. MPGs on external membrane vesicles (OMVs), that are released by numerous Gram-negative bacteria, are similarly able to regulate T cell responses.

Reduction of complement C3 resulted in an increased abundance of *E. coli* in the intestine of DSS-treated compared to wild-type mice. C3 controls the number of *E. coli* in the intestine only under colitic conditions as C3 can be found in the intestinal content [89-90], the effect of C3 to regulate *E. coli* abundance is presumably mediated by C3 released into the intestinal lumen. Adherent-invasive *E. coli* (AIEC) is a group of intestinal pathogenic *E. coli* (InPEC) that is linked with IBD including CD and UC [91-92]. AIEC expresses surface adhesins including type I fimbriae that are essential for its capacity to interact with host cells and for biofilm formation [93]. Numerous IBD-associated loci are known to regulate the recognition and killing of bacteria in addition to the function of immune cells [94].

For instance, *IL23R* and *TNFSF15* that are critical for the activation of innate lymphoid cells 3 (ILC3) and Th17 cells, these cell are essential for the maintenance of the intestinal barrier and tissue repair, and linked

with IBD susceptibility [95-96]. Complement C4B gene numbers are linked to pediatric IBD; C4B, through formation of ester bonds to carbohydrate targets, is likely involved in the aberrant inflammatory response of the host to the microbiota [97].

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