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The Role of the Complement in Chronic Intestinal Inflammation

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ABSTRACT

Complement plays a crucial role in innate immunity that can initiate or sustain inflammation but can also influence the developing adaptive immune response against invading foreign pathogens. Chronic inflammation is characteristic of many long-term clinical disorders. One of the reasons for the impairment of the effective intestinal barrier function is the imbalance in the regulation and activation of the gut complement, and this in turn causes serious inflammation, as seen in patients with inflammatory bowel diseases. Animal models have been instrumental in the evaluation of over-activation or impaired activation of the gut complement in determining the extent of chronic intestinal inflammation. This review examines the role and prospective cure manipulation of the complement in chronic gut 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, and apoptotic cells or immune complexes are not sufficiently removed.

The complement system has been indicated to promote inflammatory responses that lead to tissue damage [1]. The complement activation through an inflammatory reaction leads to tissue injury by inflammation via generating allergen toxins (C3a and C5a) and skewing macrophage activity towards a pro-inflammatory M1 type. Furthermore, changes in protein regulation of complement and their expression lead to extreme complement activation, which can lead to tissue injury

since 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 immunity; it is response to a physical damage 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 disorders, atherosclerosis, diabetes type II, rheumatoid, and tumors [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. Recent studies have shown that complement can be activated and used in several biological functions within cells, for instance, in T-cells, which has led to a new term called the complosome [7]. The complosome within cells involves biological activities together with the metabolism of the cell, autophagy, and aggravated damage in pathogen infection, autoimmunity, cancer development, etc. **[7**, 8, 9]. Nevertheless, until now, no data addressing the complosome in the development of ulcerative colitis.

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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]**.** The most prevalent forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Until 2015, an assessed 1.3% of US adults, about 3 million, had been analysed with IBD [11]. While intestinal inflammation in UC is finite 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 disorder, where the immune system plays a crucial regulatory role [15, 16]. Components of both innate and adaptive immunity responses are associated with the persistent inflammatory microenvironment observed in the colon [12, 13].

IBD is linked to an immunological imbalance of the intestinal mucosa and produces chronic inflammatory conditions. An inappropriate inflammatory reaction to intestinal luminal microbial normal flora is observed [17]; both CD and UC result from cytokine-driven and non-infectious gut inflammation. In CD, there is an overproduction of IL-17 and IFN-γ, IL-12 and IL-23, whereas UC is related to additional IL-13 [18]. IBD seems to be caused by an abnormal interaction between gut bacteria and mucosal immunity [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 produce 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 extent of complement gut activation and the type between UC and CD patients. Activation of the classical complement pathway has been shown in patients with UC at the apical epithelium, whereas in CD increased activation of the alternative complement pathway in the gut epithelium cells has been observed in patients with CD [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 relatives of the patient with CD [25]. However, another study reported that the levels of complement activation of the alternative pathway were reduced in patients with CD and their relatives [26]. It has been suggested that initial and late-phases in complement activation in Crohn's disease occur at the luminal surface of the epithelium.

The expression of intestinal IgM and B-cells was increased in remission of CD patients which aligned with the early components of the classical pathway of complement. The presence of intestinal C1q and C3 crosslinking of the IgM and B-cell receptor by binding of C1q, in addition to B-cell activation by binding of C3 to CR1 and CR2, may result in 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 membrane of gut 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 complement components of C1q protein, C3, C4, factor B and CD46 proteins show high expression levels in intestinal epithelium cells. Strong C3b and C4c depositions have been identified exclusively on cells of the intestinal epithelial in patients with CD paralleled to healthy individuals and patients with UC (Figure 1). C3aR1 is expressed in the lower crypts of intestinal stem cells, whereas C5aR1 is expressed on the surface of the cell of intestinal M cells [28].

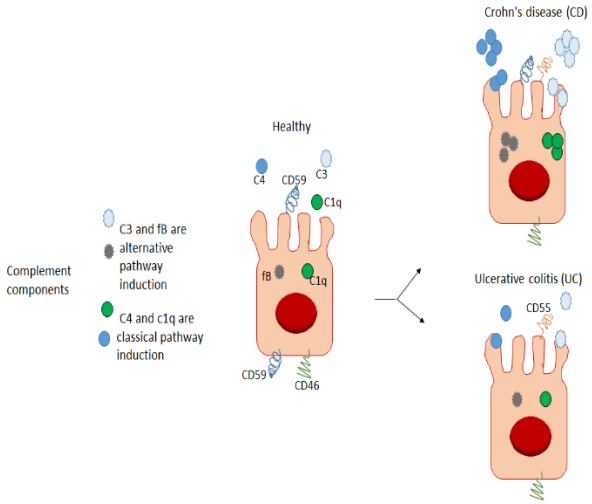

Fig.1. Diagrammatic comparison between CD and UC in the changes in epithelial intestine of complement components.

Sufferers with IBD have a much increased danger of colorectal cancer development than the regular 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 with pro-inflammatory cytokines by immune and non-immune cells, for example, TNF-a, IL-6, and IL-17A [30, 31, 32]. UC is linked with higher cytokines production 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].

IL-17A serum has been recommended as a probable predictive receptor at the beginning of UC [34]. Using models of the mouse, the role of the complement was studied in colon colitis colorectal carcinogenesis activated by dextran sodium sulfate (DSS) and colon azoxymethane. This model, mice deficient in C3 showed decreased levels of pro-inflammatory IL-1β cytokine in the colon and reduced cell reproduction in colon epithelial cells, in addition to a significantly reduced load of tumors paralleled to mice of wild-type [35].

3. Link of complement and anti-microbial peptides in gut inflammation, including deficiencies

The cell structure of the gut mucosa through inflammation changes dramatically. Complement proteins and anti-microbial peptides (AMPs) are involved in inflammation and share lytic activities, phagocytic, and chemo-attractant activities [36]. The family of AMPs includes types of β-defensins (hBD)-1,-2,-3, and -4, hRegIII-alpha, and human cathelicidin, LL-37 and AMP 18 [37, 38, 39]. AMPs are secreted from Paneth cells, which is essential in keeping spatial separation of the gut microbes of epithelium [40]. While levels of HBD-1 were unchanged between the inflamed gut compared to healthy, they have been revealed to decrease in UC [41, 42, 43]. Moreover, the levels of HBD-2, 3 and 4 showed an increase in UC but not CD [38]. The expression of LL-37 was unchanged during gut inflammation [44, 45]. The expression of murine RegIIIγ through exposure to bacterial and mucositis was increased, and the expression of hRegIII-alpha in IBD patients was also higher [46, 47]. There are differences among the normal and chronically inflamed gut epithelial cells that influence the expression of AMPs. AMPs are increased in profusion in colonic Paneth cells [48] [49]. and colonic Paneth cells secrete lysozyme, alpha-defensins, and phospholipase A2 (PLA2) [48]. The secretory of PLA2 is undiscovered in the normal colon; however, it is expressed via Paneth cell, in addition to certain colonocytes during inflammation [50]. Alpha-defensins are prompted during inflammation in the gut, linked with Paneth cells [48]. Microbial mutation-detecting molecules in Paneth cells are assumed to influence sources of defensin. Nevertheless, the particular microbiota has been recognized to be an essential factor in prompting the production of mice defensin, leading to flaws in molecules detected [51].

AMPs are essential for host defense and have numerous important links with diseases that cause humans. Chronic gut inflammation is related to the mechanisms of defective antibacterial, perhaps because of the changed expression and secretion of AMPs. CD of the ileum has been related to the Paneth cell alpha-defensins deficiency. CD is related to a low copy-number gene polymorphism of the site α-defensin in humans; this leads to a decreased epithelial cell stimulation of α-defensin AMPs in the mucous membrane of the colon. The lack of defensin results in impaired antimicrobial defense possessions of the gut mucosa, causing changes in the expression of gut normal flora that enhance the attacking of bacteria in the mucosa and increase the risk of infection with the chronic inflammation of CD [52, 53].

Primary complement deficiency has been diagnosed in patients who presented with IBD, where IBD could be an early sign of primary immune-deficiency, e.g MASP2 deficiency, Ficolin 3 deficiency, and CD55 [54, 55], even though CD55 is unexpressed in a healthy colon by epithelium cells [55, 56]. The identification of MASP2 deficiency has an essential role in complete normal activation of the complement system in colitis [57].

A deficiency of complement in the intestine leads to a significantly lower level of IL-1β, as demonstrated using a model of chronic colon and rectum mouse

carcinogenesis caused by DSS and the chemical azoxymethane. IL-1β is one of the proinflammatory cytokines with a wide variety of systemic and local biological roles and has been described as enhancing chronic inflammation and developing malignant growth [58, 59]. The gut IL-1β is essential for a build-up of IL-17A-producing innate and adaptive immune cells in the gut on normal and through chronic inflammation [60, 61].). Complement activation components C5a and C3a represent an important component for Colitis-associated colorectal cancer (CAC) formation by activating and regulating strong secretion of proinflammatory IL-1β by neutrophils which thus triggering a protumor IL-17A response in gut myeloid cells and promotes CAC development.

Defensins are important in colonic infection and inflammation. During inflammatory processes, various AMPs are expressed and could work as disease biomarkers, particularly in IBD. Beta-defensin 2 (hBD 2) in humans shows low expression in a healthy colonic, nevertheless, is very much elevated in the colitis of IBD patients [62 But, plasma hBD 2 levels in patients with IBD do not show significant changes [63]. The role of hBD 2 in the pathophysiology of colitis and colitis that is related to normal micro-flora has been shown as hBD 1, with studies indicating that exposure of human colon epithelial cells (Cancer coli-2 and HT-29) to IL-1α pro-inflammatory and enteroinvasive *Escherichia coli* serotype O29: nm markedly increased hBD 2 expression. Also, expression levels of hBD 3 and hBD 4 are elevated in the colonic catacombs of UC patients while not observed in CD patients [38]. Chronic inflammation prompts Gankyrin (GK) and stress response proteins that promote the development of CAC [64, 65]. GK, an oncoprotein that organizes and inhibits inflammatory responses, is believed to be a potential anti-inflammatory approach for IBD. In the colon, Gankyrin up-regulates the cytokines expression (TNF-α and IL-17), as well as protein markers of stem cells like BMI 1 and SOX 9. Mice lacking GK in the duodenum exhibited increased inflammation similar to healthy mice when UC was prompted with DSS. Biochemical analysis has discovered deficient GK mice lead to decreased antimicrobial peptide expression, specifically α-Defensin-5 and -6, in the jejunum. This result revealed the decreased expression of GK in the small intestine is related to colonic disease in human CD [66, 67].

4. Animal models and inflammation of the gut

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 orally giving DSS 5% in drinking water to mice BALB/c could lead to induced either acute or chronic colitis after several DSS cycles [68]. An advance in appreciation of the importance of the complement in the gut inflammatory response throughout chronic inflammation came through the use of gene deficient animals in models of DSS induced mucosal toxicity. In an experiment using mice lacking C1q, MBL, and C3 in a chronic DSS-due colitis model, a critical role of these opsonins and the classical pathway C3 convertase was demonstrated to provide defense from mucosal damage and infection. However, deficiency mice of the alternative pathway exhibited less pronounced injury profiles [69].

Macrophages are considered the primary source of complement components C1q in inflamed colon tissue [70] A lack of C1q exacerbates gut injury and inflammatory infiltration in DSS-due acute colitis [71].

In chronic DSS-induced colitis models, mice deficient in C1q and MBL died at the beginning of the experiment, whereas mice deficient in C5aR1 or C3 demonstrated heightened gut inflammation and reduced survival paralleled to wild type counterparts [69, 72].). Mice deficient in C3, showed in a chronic colitis mouse model driven by DSS and the azoxymethane, exhibited lower levels of the pro-inflammatory IL-1β cytokine, and less cell reproduction in colonic epithelial cells, and a significantly decreased tumor load relative to wild type mice [35].

The role of the alternative pathway was studied further in the pathogenesis and inflammatory response in DSS-prompted chronic colitis. Mice lacking 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 importance of the pathway of alternative in enhancing chronic colitis inflammation [69 In a separate study, a fusion construct of complement receptor 2 and Factor H, CR2-fH, given throughout DSS treatment periods, inhibited the alternative pathway activity [69]. The inhibition of alternative pathway significantly changed the inflammatory response in the chronic and led to a reduction in B-cells, macrophages, and dendritic cells within the mucosae The treatment led to decreased tissue inflammation and damage, lower fibrosis levels, and modification to the local immunity compared to both baseline and DSS-cured wild-type mice. Excessive injury healing can lead to fibrosis in chronic inflammation, potentially necessitating surgical intervention in human IBD patients [73]. M1 macrophages, characterised by a pro-inflammatory phenotype, may contribute to tissue injury, whereas M2 macrophages typically exhibit anti-inflammatory properties and are critical for wound healing. A timely resolution of the injury reduces a fibrotic reaction.

5. Implications from human array and association studies

Microarrays have been utilized to pinpoint genes that might be linked to 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 and was used to analyse by cDNA microarrays for microarray studies [74].

To explore gene expression outlines particularly for UC and CD, researchers used an endoscopic procedure to examine the inside of the body affected and normal colon mucosa from patients with IBD. The study assessed 84 genes associated with the IBD inflammation, analysing 21 UC and 22 CD patients` mucositis and non- mucositis alongside normal mucous membranes appropriate for age from 21 non-IBD controls. CCL11 and MMP10 mRNA genes in the UC, in addition to mRNA genes of C4BPB (C 4 bound protein beta) and IL1RN (interleukin 1 receptor adversary) in the CD showed an up-regulation in inflamed and non-inflamed mucosa when paralleled to controls. These genes appear to be particular markers for levels of inflammation of UC and CD. The finding suggested that the levels of transcription all of CCL11, MMP10, C4BPB, and IL1RN (but not C3 or CR2) are biomarkers for differentiating between UC and CD in clinical practice and may inform future research on therapeutic targets [75].

Research utilizing GWAS has recognized over 230 sites related to IBD including approximately 300 potentially related genes. Among these are include the pattern recognition receptors, nucleotide bound oligomerisation NOD 2, and CARD 9. NOD2 was initially reported as a gene linked to susceptibility in CD. CARD9 signaling has a vital function in the innate immunity to certain intracellular bacteria [76, 77]. It has been shown that CARD9 is essential for NOD2-mediated stimulation of MAPK signaling components,

for example, p38 MAPK and JNK protein, after treatment with muramyl dipeptide, macrophages lacking CARD9 exhibit selective deficiencies in p38 and JNK signaling [78]. CARD9 signaling is critical for intestinal immune homeostasis affecting the responses of innate lymphoid cells and T-helper 17 cells. It plays a role in shaping gut microbiota, intestinal epithelial cell restitution, and barrier maintenance, producing microbial metabolites, and overall sensitivity to colitis [79, 80]. Notably, 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 conditions where the complement system has been an important pathogenic. Complement shows an important contribution to IBD`s development through the direct impact of its activation products on tissue damage, as well as through membrane-bound complement receptors or glycoproteins that influence inflammation and immune responses. Apart from its role in innate immunity, the complement also interacts with adaptive immunity, stimulating the differentiation of regulatory T cells [82, 83], which are crucial for maintaining mucosal tolerance [84, 85].

IBD is thought to be eventually driven by activated T cells that respond to particular enteric antigens. These T cells for example, Th1 cells producing IFNγ, Th17 cells generating interleukin-17, and Th2 cells that secrete IL-4, IL-5, or IL-13. Microbially produced glycans (MPGs) are vital in modulating immune responses to gut microbiota. These glycans can inhibit innate immune responses via reducing pro-inflammatory cytokines and enhancing anti-inflammatory cytokines production from innate immune cells. Also, MPGs can influence adaptive immune responses; for example, they may block B cell activity and antibody production, particularly by covering the antigen surface. MPGs can also encourage the differentiation of polyclonal T cells into regulatory T cells (T_{regs}), which help suppress effector T cells and alleviate gut inflammation. An instance is *Bacteroides fragilis* PSA which prompts the formation of IL-10-producing FoxP3+ T_{regs} in a TLR2-dependent manner and offers defence compared to TNBS-induced colitis and colitis induced by *Helicobacter hepaticus* [86, 87, 88]. Similarly, MPGs that are found on external membrane vesicles (OMVs) released by various Gram-negative bacteria can regulate T-cell responses.

The reduction of complement C3 led to an increase of *E. coli* abundance in the intestine of DSS-treated compared to wild-type mice. The numbers of *E. coli* were controlled by C3 in the intestine only under colitic conditions. Because C3 can exist in the intestinal content [89, 90], it is hypothesized that the effect of C3 in regulating the abundance of *E. coli* is mediated by C3 released into the intestinal lumen. Group Adherent invasive *E. coli* (AIEC) is a group of intestinal pathogenic E. coli (InPEC) that is linked with IBD containing CD and UC [91, 92]. The expression of AIEC surface adhesins containing fimbriae of type I is essential for its capacity to cooperate with host cells and for the formation of biofilm [93]. Numerous IBD-associated are known to regulate the recognition and killing of bacteria in addition to immune cell function [94].

For instance, both IL23R and TNFSF15 are very important for the activation of both innate lymphoid cells 3 (ILC3) and Th17 cells; these cells are essential for the maintenance of the intestinal barrier and repair of the tissues and linked with susceptibility to IBD [95, 96]. Complement C4B gene numbers are linked to pediatric IBD; C4B, through the formation of ester bonds to carbohydrate targets, is likely involved in the aberrant inflammatory response of the host to the microbiota [97].

7. Conflict of interest statement

The author declare that there is no conflict of interest.

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