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The intestinal complement system in inflammatory bowel disease: Shaping intestinal barrier function

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ABSTRACT

The complement system is part of innate sensor and effector systems such as the Toll-like receptors (TLRs). It recognizes and quickly systemically and/or locally respond to microbial-associated molecular patterns (MAMPs) with a tailored defense reaction. MAMP recognition by intestinal epithelial cells (IECs) and appropriate immune responses are of major importance for the maintenance of intestinal barrier function. Enterocytes highly express various complement components that are suggested to be pivotal for proper IEC function. Appropriate activation of the intestinal complement system seems to play an important role in the resolution of chronic intestinal inflammation, while over-activation and/or dysregulation may worsen intestinal inflammation. Mice deficient for single complement components suffer from enhanced intestinal inflammation mimicking the phenotype of patients with chronic inflammatory bowel disease (IBD) such as Crohn's disease (CD) or ulcerative colitis (UC). However, the mechanisms leading to complement expression in IECs seem to differ markedly between UC and CD patients. Hence, how IECs, intestinal bacteria and epithelial cell expressed complement components interact in the course of IBD still remains to be mostly elucidated to define potential unique patterns contributing to the distinct subtypes of intestinal inflammation observed in CD and UC.

1. The complement system

The complement system was named according to its ability to "complement" the antibacterial activity of antibodies and constitutes a highly conserved part of the innate immune system. Due to its main functions in the detection, opsonization and elimination of bacteria as well as of apoptotic or malignant cells, the complement system is crucial for the efficient clearance of invading bacteria and for tissue homeostasis [1,2].

The complement system can be activated *via* three different pathways: the classical, the lectin and the alternative pathway. While binding of the initial complement component C1q to the Fc portion of target cell bound antibodies activates the classical pathway of complement system, the lectin pathway is primarily immunoglobulin-independent. The lectin pathway recruits germ line-encoded pattern-recognition receptors (PRRs) such as mannose-binding lectin (MBL), that recognize microbial-associated molecular patterns (MAMPs) like cell surface exposed mannose residues. The alternative pathway of complement activation is evoked by spontaneous hydrolytic cleavage of C3 to C3a and C3b in the fluid phase, leading to complement activation and/or amplification on, amongst others, bacterial surfaces. After initiation of complement activation, C3 and C5 convertases are assembled on target cells' surfaces and induce cleavage of either C3 or C5, respectively. Notably, composition but not function of C3 and C5 convertases of the classical or lectin pathway differ from the alternative pathway. Together, activation of the complement system leads to the formation of anaphylatoxins (C4a, C3a, C5a), to lysis of target cells by the membrane attack complex (MAC; C5b-9) or to the opsonization of targets with opsonins (such as C3b) [3–5]. Of note, it has recently become clear that non-canonical activation of complement through single proteases is an additional means of complement activation that contributes to several diseases states such as intestinal ischemia or arthritis [6–8].

Because complement, once activated, is basically non-discriminatory, healthy and malignant cells typically express different complement regulatory proteins, which protect host cells against

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deleterious effects of the complement system. Among the best characterized are CD46, CD55 and CD59. While CD46 (membrane cofactor protein, MCP) acts as a co-factor of the serine protease "factor I" to degrade C3b or C4b, CD55 (decay-accelerating factor, DAF) prevents the formation of new and accelerates the decay of preformed C3 and C5 convertases. At the final step of the proteolytic complement cascade, CD59 blocks the assembly of the MAC by displacement of C9 [4]. Overexpression of these molecules has been described as one of the mechanisms by which tumor cells evade the attack of the immune system [5,9,10].

2. The intestinal barrier

Maintenance of physical integrity at the intestinal barrier is a pivotal element for the survival of higher organisms. The intestinal barrier builds up a primary field of continuing battles between the immune system of an organism and the surrounding microbiota [11]. The immunological barrier is formed by cellular (*i.e.* epithelial- and mesoderm-derived professional immune cells) and non-cellular components (*e.g.* antimicrobial peptides, cytokines and antibodies). The orchestration of the interplay between different factors like mucosal immune cells and epithelial cells within the intestinal barrier organ is crucial for its proper function [12].

Intestinal epithelial cells (IEC), so called enterocytes, are the main cell entity of the intestinal barrier and build up a physical barrier against luminal bacteria and their components such as lipopolysaccharides (LPS). Moreover, these cells express various protective factors, such as scavenger receptor proteins, which bind bacteria and prevent bacterial translocation into the mucosal wall [13]. Secretory epithelial cells can be mainly divided into Paneth cells and goblet cells that occur at distinct locations of the gastrointestinal (GI) tract. While Paneth cells, which secret antimicrobial peptides and also maintain the stem cell niche in the gut, are located only in the small intestines, mucin producing goblet cells, are expressed at different quantities also in the large intestines [11]. Goblet cells produce and secrete highly glycosylated proteins, so called mucins, with mucin 2 (Muc2) being the most abundant one in the colon and small intestine. Colonic mucus is composed of two structurally and functionally different strata: a firm inner layer and a loose outer layer. In the colon, the mucosa-attached inner layer, that contains many antimicrobial peptides, scavenger molecules and secretory IgA antibodies, has been shown to exclude a majority of bacteria, whereas the loose outer layer serves as a habitat for the intestinal commensal microbiota [14]. The importance of an intact mucus layer for intestinal homeostasis has been demonstrated in various animal models. Spontaneous colitis development of Muc2 deficient mice is associated with bacteria found in direct contact with the epithelium, an observation also made in UC patients [15]. Nevertheless, mucus glycans do not only serve as a barrier towards epithelial cells, they can also be bound by bacterial adhesins and act as a nutrient source for bacteria, which hydrolyse mucins through glycosidases. Therefore, mucus producing goblet cells might have an impact on the microbiome, since a certain mucus composition might favor or hamper the growth of certain bacteria [16].

Regeneration of the intestinal barrier is based on the proliferation of intestinal stem cells (leucine-rich repeat-containing G-protein coupled receptor 5 $(Lgr5)^{+}$ cells) which are located at the crypt basis. The transit amplifying zone is located next to the stem cell niche at the lower hemicrypt. Here, progenitor cells undergo rapid proliferation before differentiation. After differentiation the cells migrate along the crypt-villus axis to the top of the crypts (colon) or to the tips of the villi (small intestines), respectively. After 34 days, differentiated epithelial cells undergo apoptosis and are shed into the gut lumen. Interestingly, various defects at the distinct differentiation steps have been found to cause barrier dysfunction leading to intestinal inflammation and cancer development [17].

The human intestinal tract is colonized by a large number of different microbes, the gut micobiota. It is known that this dense and diverse microbial ecosystem provides an important role in protecting the epithelial barrier function. Microbial imbalance or so called dysbiosis in the gut strongly correlates with intestinal inflammation [18]. Epidemiological studies show that alterations of the bacterial composition, potentially leading to impaired physiological function, have been associated with a range of different human diseases, ranging from metabolic disorders and different cancer entities to chronic inflammatory barrier diseases such as inflammatory bowel disease (IBD) [19]. IBD is classified into the two well-defined subtypes Crohn's disease (CD) and ulcerative colitis (UC). Although IBD has been linked to genetic variants of genes belonging to the innate immune system (such as Nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and 2), the exact etiology of IBD is still unresolved [20]. UC is restricted to the colon and presents severe mucosal inflammation that is accompanied by mucosal and submucosal ulcerations. CD in contrast is characterized by discontinuous, transmural inflammation that may affect all layers of the intestine in the whole gastrointestinal tract [21].

Various independent microbiota studies in IBD showed a reduced bacterial diversity in patients with CD and UC. At species level certain bacteria such as Proteobacteria or Firmicutes have been found to be over- or underrepresented, respectively [22]. The underlying mechanisms resulting in the shift of the intestinal microbiota are still under debate including the high impact of nutrition on the course of IBD [23].

Apart from the fecal microbiome, which is, at least in the healthy gut, separated from the cells of the intestinal mucosa [24], mucosa-adhesive and mucosa-invasive bacteria are a second field of investigation. In contrast to studies on the fecal microbiome, investigations on mucosa-adhesive and mucosa-invasive bacteria are rare. As these mucosa-associated bacteria are in close contact with the intestinal epithelial cells they constitute an important group which is suggested to play a major role for inflammation seen in IBD patients [11]. Aligning with this notion, Swidsinski et al. [25], demonstrated increased numbers of mucosa-associated bacteria in IBD patients *vs*. controls and that the count of these bacteria positively correlated with disease activity in IBD patients. However, whether mucosa-associated bacteria are primarily a result of impaired barrier function and mucus depletion in IBD patients, or also causative for mucosal inflammation is still unknown.

3. The intestinal complement system in health

It is known that intestinal epithelial cells are able to monitor the intestinal surface and detect microbes by PRRs and, if necessary, to trigger an immune response through the innate immune system [26]. Interestingly, while the efficacy and importance of the complement system in the systemic antimicrobial defense in the blood stream has been investigated in detail, the contribution of the complement system to intestinal barrier function still remains mostly elusive.

The complement components circulating in the serum are mainly produced by the liver. However, non-hepatic pathways enabling the presence of complement factors in the intestine, have already been identified. For example, secretion of complement factors into the duodenum *via* the pancreatic compartment has been described [27]. Furthermore, IECs also have been identified as a primary source of production of distinct complement components throughout the gastrointestinal tract [28].

3.1. Intestinal synthesis of complement factors

In 1966, Colten et al. presented data that demonstrated hemolytically active C1 to be mainly synthesized in the small intestine of guinea pigs when compared to other tissues such as the stomach, the colon or the liver [29]. The same group unraveled two years later murine small intestinal epithelial cells as the only C1 expressing cell type in the small intestine [30]. However, in humans Colten et al. identified the colon and the ileum as the sole synthesis sites of hemolytically active C1, with