FcaRI and neutrophils: Caught in the act

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Antimicrobial functions of IgA and FcαRI

Mucosal immunity in intestinal areas protects against pathogens from the external milieu, while allowing the uptake of beneficial nutrients and gases. As described in this thesis, immunoglobulin A (IgA) plays an important role in mucosal areas, by forming an anti-microbial layer that protects the epithelial layer against invading pathogens. Moreover, IgA is a potent activator of cells of the immune system, once pathogens have been able to breach the epithelial barrier. This supports a dual role of IgA in mucosal areas such as the gastrointestinal (GI) tract.

The type of IgA present in the lumen of the GI tract is called secretory IgA (SIgA), which is derived from dimeric IgA (dIgA)[1]. The latter is produced by plasma cells that reside in the lamina propria, which underlies the epithelial layer. When dIgA is transported through the epithelial cells to the luminal mucosa, transepithelial transport is carried out by the so-called polymeric Ig receptor (pIgR). At the luminal side pIgR is cleaved and dIgA is released as SIgA. A part of the pIgR remains attached to the dIgA molecule, which is termed the secretory component (SC), together forming SIgA[1, 2].

Antibodies (Ab) consists of Fab-regions that bind to antigens and an Fc-region, which interacts with its prototypic Fc receptor(s), which in case of IgA is Fc alpha receptor I, (FcαRI or CD89)[3]. Binding of an Ab-antigen complex, or immune complex (IC) to an Fc receptor results in activation of Fc receptor-bearing immune cells. The addition of the SC, however, hinders the interaction between the Fc-region of SIgA with FcαRI. Therefore, SIgA is a poor opsonin, and limited immune responses are induced by SIgA. This is beneficial in mucosal areas where also plentiful innocuous antigens (like food antigens or commensal bacteria) are present, as this prevents unwanted damage to the integrity of mucosal tissue. The antimicrobial function of SIgA is therefore mostly passive, consisting of binding antigens in an immunological inert manner and eliminating potential pathogens via the feces. The passive antimicrobial function of SIgA has long been appreciated, and has contributed to the perception of IgA as non-inflammatory Ab. This view is strengthened by the fact that IgA is a poor activator of complement[3].

The complement system is a collection of plasma proteins that, when activated, initiates a signaling cascade that can induce direct lysis of microorganisms, opsonization of pathogens and recruitment of immune cells[4]. Three distinct pathways are observed that induce activation of the complement system. The first, referred to as the 'classical pathway', relies on binding of the complement component C1q to antigen-antibody complexes. The 'mannan-binding (MB)-lectin pathway', is similar to the classical pathway, but depends on the plasma protein mannan-binding lectin (MBL) instead of C1q for activation. The alternative pathway is activated when the component C3 binds to the surface of a pathogen. All three pathways eventually result in the cleavage of C3 into components C3a and C3b. This reaction cascades further into development of the effector mechanisms of the complement system. C3b opsonizes bacteria and plays a role in cleavage of C5 into C5a and C5b, which is essential in the process that leads to direct lysis of pathogens[4].

Neutrophils are the first cells of the innate immune system that are recruited from the circulation in infection and inflammation. The process of cell recruitment, or chemotaxis, can be induced by the complement components C3a and C5a[5]. The Ab classes IgM and IgG are able to bind to C1q [5-7]. IgA, in contrast, is a poor binder of C1q, which has

supported the view of IgA as an immunologically inert Ab. However, this traditional view of IgA was recently more and more challenged, when the role of IgA was investigated in more detail. Both dIgA, which is present in the lamina propria (LP), and serum IgA are not hindered by the presence of SC, and are both able to efficiently opsonize pathogens and bind to FcαRI on immune cells, which induces inflammatory responses[8-10]. In fact, previous research within our group showed that IgA is the most potent Ab to activate neutrophils, and the only Ab that can induce neutrophil migration directly, independent of complement activation[10]. This was due to the release of leukotriene B4 (LTB4) - which is a potent neutrophilic chemotactic agent[10] - after FcαRI triggering, which in turn recruits more neutrophils. In this way a self-contained positive feedback loop for neutrophil influx towards the site of infection is created, until the infectious threat has been eliminated. In this thesis I further investigated the effects that IgA exerts on cells of the immune system and on neutrophils in particular.

FcαRI in protective immune responses

When neutrophils are recruited and activated, three different killing mechanisms can be executed to eliminate microorganisms. One important mechanism is phagocytosis, in which bacteria are taken up in an invaginated membrane vesicle, called a phagosome[4]. In the cytoplasm of the cell, phagosomes fuse with lysosomes, which contain proteolytic enzymes. The thus formed phagolysosomes subsequently digest the bacteria. The uptake of microorganisms is greatly increased when bacteria are opsonized with IgA and interact via FcαRI on the cell membrane, supporting the role of IgA in bacterial clearance (**Figure 1,** and Van der Steen et al.[10]).

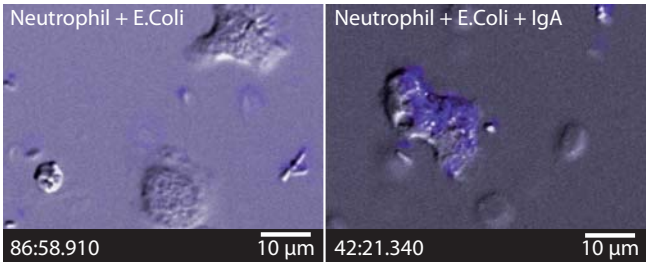

Figure 1. Phagocytosis of E.Coli bacteria (labelled blue fluorescent) by neutrophils in the absence of IgA (left panel) compared to uptake of bacteria opsonized with IgA (right panel). More phagocytosis is observed when IgA is present. Time is displayed as min:sec.msec

In addition to phagocytosis, neutrophils can release the contents of different granules, which reside in their cytoplasm, into the exterior milieu via degranulation. The release of these proteolytic agents into the environment acts as an extracellular killing mechanism that the neutrophils can employ to eliminate pathogens, but can also cause harm to the surrounding environment.

In **chapter 2** we described that IgA enhances the formation of neutrophil extracellular traps (NETs), which is the third killing mechanism that neutrophils can employ. Neutrophils can, upon activation, expel decondensated nuclear chromatin, which is decorated with lytic proteins that include MPO, neutrophil elastase (NE) and histones, as web-like structures into the milieu. NETs trap extracellular bacteria and possibly directly kill them. Two types of NET formation have been described.

First, the release of nuclear DNA results in neutrophil cell death, which is termed NETosis[11]. This type of NET formation is dependent on the generation of reactive oxygen species

(ROS)[12]. We demonstrated that uptake of IgA-opsonized bacteria resulted in enhanced ROS production (**Chapter 2**), which likely explains the augmented NET release after stimulation via FcαRI. Moreover, in our experiments release of NETs was accompanied by neutrophil death. This is in contrast to the second form of NETs release that does not involve dying of neutrophils. In the latter case, NETS are rapidly released (in minutes) in a ROS independent way. This type of NETs contains less proteolytic enzymes, and as such may be a mechanism that mainly immobilizes bacteria, preventing pathogens spreading throughout the body. The prevention of dissemination of the bacteria also suggests that the two types of NET formation supplement each other. At later time point, more toxic, NETosis-inducing NETs are generated, that are better suited to eliminate the bacteria that are ensnared by the rapidly formed NETs[13]. While these processes both co-exist because of the differential activation methods that result in the 'rapid' versus 'slow' NET formation, it is more interesting to look at actual bacterial killing by NETs. The first article that described NETs, by Brinkmann et al., reported bacterial eradication by the expressed NETs[11]. However, we did not detect bacterial killing in our experiments, and also in literature antimicrobial effects on microorganisms are debated[14]. NETs do contain histones and proteolytic enzymes, such as elastase that pose a threat to the bacteria. Consequently, some bacterial strains have developed mechanisms to avoid capture by NETs, as they express DNAses, that break down NETs[15]. Therefore, bacterial killing by NETs may be possible in strains that did not evolve these mechanisms. However, irrespective of the killing ability of NETS, their capacity to constrain bacteria is also important. For instance, it was shown that in sepsis NETs prevented bacterial dissemination from the site of infection[16]. This facilitates clearance of immobilized pathogens by other (living) neutrophils or macrophages. Therefore, I propose that IgA-induced NET formation may play an important protective role in mucosal immunity.

The role of FcαRI in induction of adaptive immune responses

In addition to induction of direct bactericidal effector functions, the effect of FcαRI cross-linking on neutrophils by IgA also affects other cells of the immune system. The positive neutrophil chemotaxis loop that neutrophils initiate by release of LTB4 after triggering of FcαRI was previously described[10]. In **chapter 3** we discuss the chemotactic effect of this lipid mediator on monocyte migration (**Figure 2**). LTB4 induces monocyte chemotaxis only at higher concentrations.

Moreover, monocyte migration is slower, compared to LTB4-induced neutrophil migration. In this way, neutrophils will constitute the first wave of recruited cells during mucosal infection. When more neutrophils arrive and are activated via FcαRI, concentrations of LTB4 will increase, which will attract monocytes as second cell type that is recruited towards the site of infection. Upon arrival recruited monocytes differentiate into macrophages.

During immune responses, activated monocyte-derived macrophages can be differentiated into distinct phenotypes, as a result of differential cytokine stimulation[17]. Analogous to the induction of either a T helper cell 1 (T_H1) or a T helper cell 2 (T_H2) response in naïve T cells, the extremes of these phenotypes are referred to as M1 and M2[18]. The M1 phenotype, or 'classically activated' macrophage, is characterized by IL-12^{high}, IL-23^{high}, IL-10^{low} expression and participates in driving T_H1 responses that generally promotes the

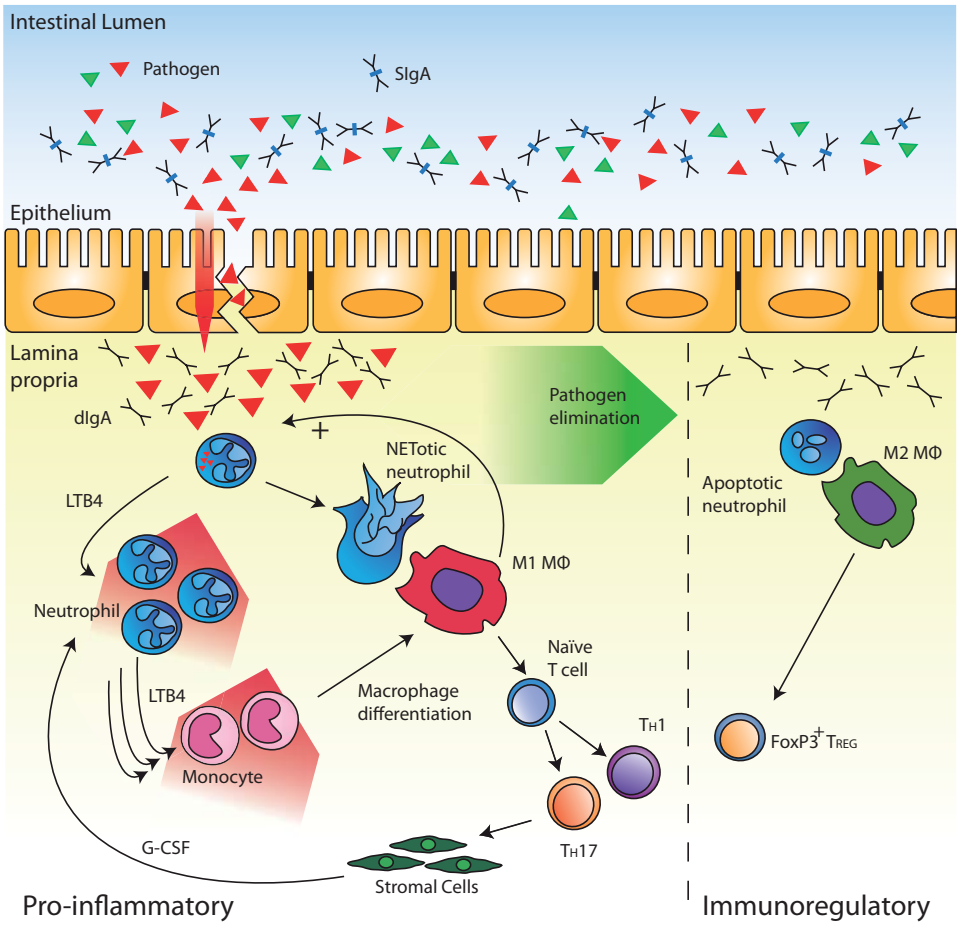

Figure 2. Model of neutrophil and monocyte recruitment following IgA mediated immune activation (Left panel). During intestinal infection, pathogens that are present in the intestinal lumen, infiltrate the lamina propria, and are opsonised by dimeric IgA, leading to binding to FcαRI on neutrophils. LTB4 release by neutrophils attracts more neutrophils and eventually also monocytes from the circulation. IgA-mediated neutrophil activation furthermore results in NET formation and NETosis. The inflammatory environment induces monocyte differentiation towards M1 macrophages (M1 MΦ), and induction of TH1 and TH17 cells Via G-CSF expressing stromal cells, activated by TH17 cells, granulopoiesis is promoted. (Right panel) After pathogens are cleared from the tissue, neutrophils undergo apoptosis. The uptake of apoptotic neutrophils induces an immunoregulatory M2 phenotype in macrophages, and a FoxP3+ regulatory T cells (FoxP3+ TREG) response. This will result in resolution of immune responses, tissue repair and restoration of homeostasis.

inflammatory response[19]. In contrast, M2 or ´alternative activated´ macrophages, express a IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype, and T_H2 responses[20], which are associated with immunoregulation and restoration of tissue homeostasis[21]. An important function of macrophages within the immune response, is to clear cell remnants and debris to prevent excessive collateral damage. Eventually they will down-regulate immune responses, initiate wound healing and restore homeostasis. It is important that immune reactions are not inhibited too early in the process, when pathogens are still present.

Activated neutrophils release pro-inflammatory cytokines that promote pro-inflammatory macrophage activation. For example, IFNγ is released that directly activates macrophages[22]. It was furthermore demonstrated that released MPO (possibly via NETs)

interacts with macrophage mannose receptors (MMRs) on resident macrophages. This in turn induces production of ROS and pro-inflammatory cytokines including TNFα, IL-1, IL-6, GM-CSF and IL-8 by macrophages[23, 24]. In turn, activated macrophages express the cytokines IL-12 and IL-23, which results in the induction of a T_H1 and T helper 17 (T_H17) cell response[25]. It was reported that histones in NETs antagonize the process of efferocytosis, wherein macrophages take up cellular remnants of dead cells[26]. As seen in our experiments **(chapter 2)**, only when no pathogens are present to eliminate, neutrophils die via apoptosis. The uptake of apoptotic neutrophils by macrophages is reported to induce an anti-inflammatory, M2 state in the cells, characterized by IL-10 and TGF-β expression, stimulation of $FoxP3^+$ regulatory T cells, hereby down-regulating the immune response and restore homeostasis[27-29].

Thus, I propose that during an inflammatory response, neutrophils will die via apoptosis only after all pathogens have been eliminated, which will signal the induction of down-modulation of immune responses. In contrast, as long as the infectious threat is present, neutrophils will be activated by dIgA-opsonized pathogens. This will maintain pro-inflammatory immune responses, by recruiting more neutrophils, and monocytes from the circulation, but also by inducing adaptive immune response, by means of T_H1 and T_H17 responses **(Figure 2)**.

In vivo models

Almost all knowledge concerning IgA and FcαRI has been based on *in vitro* experiments, which provide good insights in cellular mechanisms, but discard many factors that affect these mechanisms in the *in vivo* situation. *In vivo* studies have however been hampered due to lack of suitable mouse models. Mice do not express an FcαRI homologue, but have Fcμ/αR and transferrin receptors (TfR), which bind to IgA, but do not have similar functions compared to human FcαRI[30]. To overcome these limitations we created an *in vivo* model to study the protective effects of the human IgA and FcαRI in infection. A knock-in mouse model that produces human IgA[31], was crossed with a transgenic mouse model, which expresses human FcαRI on neutrophils[32]. I next tested the hypothesis that the expression of both hIgA and FcαRI would protect mice from a challenge with *Salmonella* bacteria in **chapter 4**. Surprisingly, we observed the opposite, as a detrimental reaction to the infection occurred in hIgA/FcαRI mice, which in fact died earlier than mice without FcαRI. Several reasons may explain these results. First, as *S.Typhimurium* has developed mechanism to survive and grow within phagocytes, enhanced uptake of IgA-opsonized bacteria by $FcαRI^+$ neutrophils and Kupffer cells may in fact provide a survival benefit. Additionally, *S.Typhimurium* can utilize enhanced ROS production after triggering of neutrophil FcαRI, as this will generate increased tetrathionate, which *S.Typhimurium* uses as electron acceptor, resulting in growth advantage[33]. Either situation may lead to augmented bacterial load, systemic dissemination, and sepsis. Alternatively, excessive inflammatory responses through enhanced neutrophil activation may have led to increased morbidity and mortality of hIgA/FcαRI mice. Either way, since infection with *S.Typhimurium* was not limited to the gastro-intestinal tract, the model proved unsuitable (with hindsight) to investigate the potential protective function of IgA and FcαRI at mucosal sites.

Previous research supported that IgA-FcαRI interaction is important for protection against pathogens in humans and *in vivo* models. For instance, IgA deficiency (IgAD)

is the most common primary immunodeficiency in Caucasians[34]. Although not life threatening, IgAD is associated with respiratory and GI tract infections, autoimmunity and allergies[35-37]. The incidence of these disorders might be a consequence of a diminished epithelial barrier function, caused by the absence of SIgA. Additionally, due to the abundance of food components and microbial flora in the lumen, antigens continuously reach the lamina propria through diffusion or transcytosis. Dimeric IgA can interact with these antigens, leading to the formation of immune complexes, which are either internalized by phagocytes or transported back into the lumen via pIgR[38]. In this way, dIgA may provide an effective means of eliminating (excessive) immune complexes. The absence of this immune complex elimination role of dIgA, might result in ill-suited responses against commensal flora or nutrient components. It was suggested that this mechanism may also play a role in other mucosal disorders, including gluten-sensitive enteropathy and inflammatory bowel disease[6, 36], supporting an important protective role for IgA. Furthermore, transgenic mice expressing human FcαRI were protected from a lethal intranasal challenge with *S. pneumoniae* when recombinant human IgA was administered intraperitoneally[39]. Similarly, human FcαRI expressing mice, but not FcαRI negative littermates were protected by recombinant IgA against pulmonary infection with *Mycobacterium Tuberculosis* [40].

The role of FcαRI in disease

Even though IgA-induced neutrophil migration may help to eliminate invading pathogens, it can also lead to tissue damage in case of an aberrant IgA immune response. For instance, recent research within our group showed that IgA and FcαRI interaction on neutrophils has a detrimental effect on skin tissue of patients with linear IgA bullous disease (LABD)[41]. This is an auto-immune disease that is characterized by IgA autoantibodies against collagen XVII, which is present in the basement membrane separating the dermis and the epidermis[42, 43]. Neutrophils in the skin are activated by IgA, leading to degranulation and recruitment of more neutrophils. Consequently, a perpetuating neutrophil migration loop is initiated that ultimately results in tissue damage and separation of the dermis en epidermis, leading to severe blistering of the skin. Blocking anti-FcαRI monoclonal antibodies that interfere with the binding between IgA and FcαRI were able to reduce neutrophils activation and migration as well as tissue damage *ex vivo*[41].

Because ulcerative colitis (UC) too is characterized by a prominent neutrophil accumulation in the colon (where IgA is the most prominent antibody), we investigated whether IgA-induced neutrophil recruitment may contribute to the pathology of colitis in our novel human IgA/FcαRI mouse model. As such, we examined FcαRI- mediated damage of intestinal tissue in a dextran sodium sulfate (DSS) colitis mouse model in **chapter 5**[44, 45]. We observed that hIgA/FcαRI mice had more morbidity and mortality, compared to mice that did not bear FcαRI. Afflicted mice were also characterized by an increased neutrophil influx in damaged tissues. Thus, FcαRI-induced neutrophil migration likely plays an important role in inducing inflammation in colitis.

Tissue damage may be due to degranulation of neutrophils, which releases proteolytic enzymes in the extracellular milieu. However, as described in **chapter 2**, IgA-mediated activation of neutrophils can also result in NET formation. A role for NETs in UC has been suggested[46]. After activation, neutrophils can release large amounts of pentraxin 3 (PTX3). This protein activates the classical complement pathway and can induce

pathogen recognition by macrophages and DCs as well as apoptotic cell clearance[47]. PTX3 co-localized with NETs in patients with UC, which may contribute to maintaining the immune response in these patients[46, 48]. It was demonstrated that chromatin and histones, as detected in NETs, can cause damage to healthy tissue[49, 50]. It is therefore not surprising that NET formation can be observed in other diseases, including systemic lupus erythematosus (SLE), thrombosis and sepsis. It was demonstrated that DNAse of patients suffering from SLE cannot efficiently degrade NETs[51]. Additionally, increased DNAse inhibitors have been described[52]. As such, the inability to degrade NETs presumably induces deposition of NET-bound autoantibodies and prolonged activation of the complement system, exacerbating the disease[52]. NET formation has also been linked to thrombosis, as histones on NETs were reported to aggregate platelets, whereas the chromatin fibers can act as scaffold, thus creating a thrombus[53]. It was demonstrated that in severe sepsis, NETs are formed in the vasculature[54]. However, in contrast to SLE and thrombosis, NETs exert also a protective role, by trapping bacteria in liver sinusoids and pulmonary capillaries, hereby preventing dissemination and septic shock. This protective effect, however, comes at the expense of tissue damage caused in the host, indicating a dual role for NETs during sepsis.

In addition to large neutrophil infiltrates, aberrant adaptive immune responses are also observed in UC. Traditionally, UC was regarded as a T_H2 cell mediated immune disease[55]. However, more recently a role for T_H17 cells was reported in UC[56], as increased concentrations of IL-17 were found in UC, but not in CD[57, 58]. While T_H2 responses involve the cytokines IL-4 and IL-25, which can inhibit the T_H17 pathway[59], several studies have reported the concomitant presence of T_H17 response-associated cytokines IL-17A, IL-22 and IL-26 in UC[60-62]. Interestingly, crosstalk between neutrophils and T_H17 cells has been described[63]. T_H17 cells can recruit neutrophils via IL-8 and induce production of G-CSF by stromal cells. In turn, neutrophils attract T_H17 cells via release of CCL2 (MCP-1) and CCL20 (MIP-3α) that bind to CCR2 and CCR6 receptors on T_H17 cells.

We therefore investigated the induction of adaptive immune responses in our colitis models **(chapter 6).** We observed an increased number of CD4-positive T helper cells in hIgA/FcαRI mice. Even though we were not yet able to identify different T helper subsets,

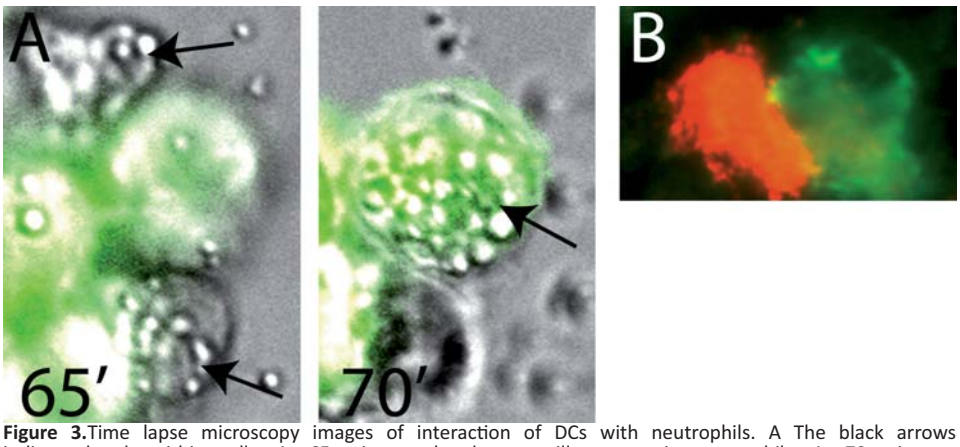

Figure 3. Time lapse microscopy images of interaction of DCs with neutrophils. A The black arrows indicate beads within cells. At 65 minutes, beads are still present in neutrophils. At 70 minutes, beads have been transferred into a dendritic cell (green). B Red fluorescent stained (CD66b) neutrophil in contact with a DC (green fluorescent; DC-SIGN) in inflamed colon of a patient with UC.

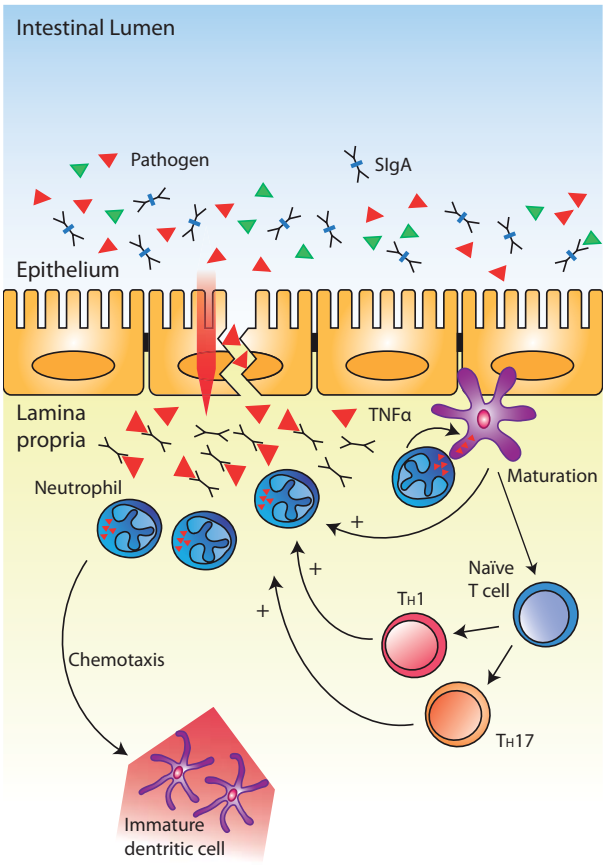

Figure 4. Schematic overview of the interaction of neutrophils and DCs during the immune response in the intestinal tract following impaired barrier function. IgA in the lamina propria activates neutrophils via FcαRI, inducing recruitment of immature DCs. Neutrophil stimulation of immature DCs via antigen transfer and TNFα expression. The DC maturates and expresses pro-inflammatory cytokines that act on neutrophils, but also on naïve T cells, that differentiate into TH1 and TH17 cells, which in turn also promote the immune response.

mRNA analyses demonstrated enhanced expression of CCL2, IL-1β and IL6 in IgA/FcαRI mice, which are involved in the induction of T_H17 cells. Activation of neutrophils via FcαRI furthermore resulted in release of these cytokines *in vitro*, which suggest that triggering neutrophil FcαRI may influence the development of T_H17 responses.

When we performed co-culture experiments of neutrophils and DCs, we observed transfer of IgA-coated beads that had been taken up by neutrophils, into DCs (**Figure 3A**). Direct interactions of neutrophils and DCs via CD18 and CAECAM on neutrophils and DC-SIGN on DCs have previously been reported[64, 65]. Neutrophil activation resulted in DC maturation via cell-cell contact and release of TNFα[66,67]. DCs in turn promote maturation and proliferation of naïve T cells towards the T_H1 and T_H17 cell phenotype, via antigen presentation in lymphoid tissue. Activated neutrophils also release the chemokines CCL2, CCL3, CCL4, CCL5 and CCL20 that attracts immature DCs from the circulation[68-71]. We showed that neutrophils released CCL2, CCL3, CCL4, and TNFα after activation through IgA. As such, I hypothesize that neutrophils may act as sentinels at mucosal sites, by recruiting and alarming DCs for impending infections. Subsequently, transferred antigens can be presented to T cells by dendritic cells (**Figure 4**). This mechanism may also play a role in UC, as we observed close contact between dendritic cells and neutrophils in inflamed colon of patients (**Figure 3B**). However, even though FcαRI stimulation of

neutrophils resulted in TNFα release *in vitro*, we did not observe increased TNFα in hIgA/FcαRI mice with colitis. As such, the precise role of FcαRI-activated neutrophils in initiation of adaptive immune responses needs to be established.

Conclusion and future perspectives

Activation of the immune system, via IgA - FcαRI interaction, results in pro-inflammatory processes, such as recruitment of neutrophils, phagocytosis, degranulation and NET formation. Furthermore, release of cytokines by activated neutrophils leads to migration of monocytes and induction of adaptive immune responses. In mucosal areas, impaired barrier function leads to large neutrophil infiltration and activation, which in turn can induce recruitment and activation of other immune cells, including DCs, T_H1 cells, T_H17 cells and IgA producing plasma cells (**Figure 5**). Consequently, invading pathogens can be efficiently eliminated from sites of infection in mucosal areas in an orchestrated way that aims to minimize collateral tissue damage. However, it is of utmost importance that IgA-mediated immune responses are carefully balanced. When this balance is disturbed

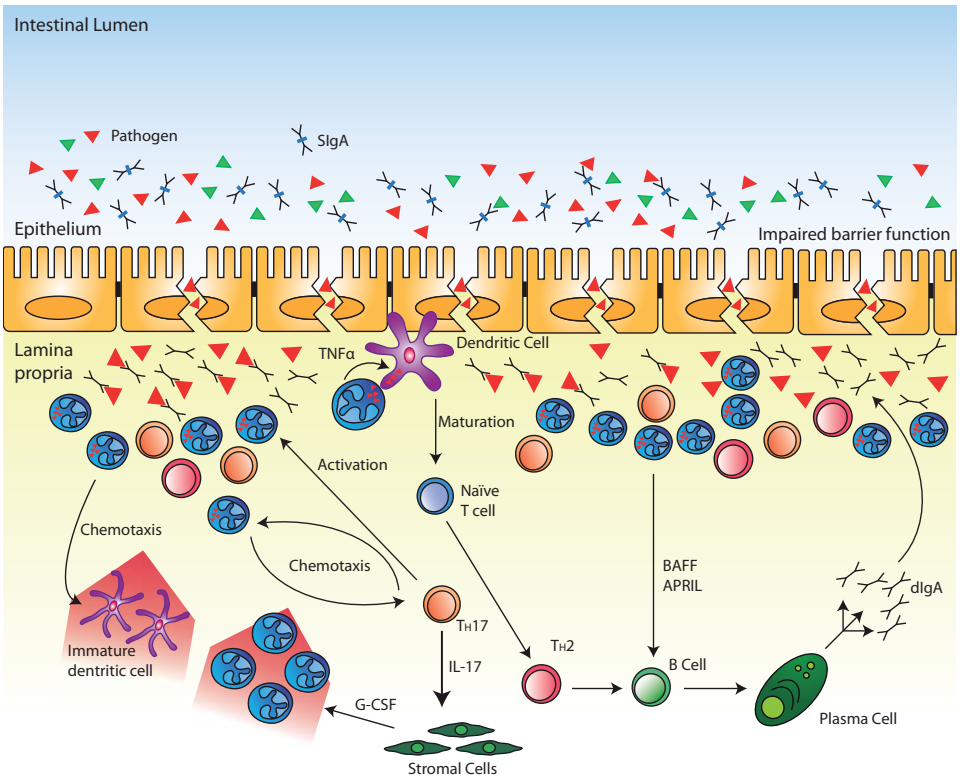

Figure 5. Schematic overview of the immune response in the intestinal tract following impaired barrier function. In the case of ulcerative colitis (UC), IgA immune complexes are over abundantly present in the lamina propria. This causes continuous activation of neutrophils via FcαRI, that leads to activation of DCs and maturation of T cells. The immune response in UC is characterized by presence of TH17 and TH2 cells. The latter cell type promotes B cell differentiation towards IgA producing plasma cells. Neutrophils also contribute to this by release of B cell stimulating cytokines BAFF and APRIL. The IgA in turn will form new IgA immune complexes that react with neutrophils, resulting in a persistent immune response. TH17 cells induce stromal cell stimulation, via IL-17, causing G-CSF release. This subsequently attracts new neutrophils from the circulation towards the site of infection and activates them. Also TH17 cells are attracted by chemokines released by neutrophils.

towards either too little or too much activity, detrimental conditions arise.
The understanding of the IgA – FcαRI interactions and their subsequent effect on neutrophils will facilitate development of new therapeutic strategies for conditions in which IgA and FcαRI play a decisive role. On the one hand, patients with conditions such as LABD and UC that are characterized by IgA-auto-Ab driven excessive immune responses would greatly benefit from selective inhibition of the interaction between IgA and FcαRI that would counteract the inflammatory processes. Research within our group has already shown that tissue damage in LABD is reduced with specific anti-FcαRI monoclonal Abs[41]. This mechanism of action might also prove effective to dampen inflammatory responses in patients suffering from UC. This would be an improvement over current therapies that mainly consist of administration of general immunosuppressive medications. The specific blocking of FcαRI and IgA could overcome systemic side effects[72] .
On the other hand, the induction of immune responses by specific IgA-antibodies combined with the potential of FcαRI on neutrophils to induce tissue damage, may prove a useful tool in anti-cancer therapy. It has been shown that FcαRI is the most potent Fc receptor to induce antibody-mediated tumor cell killing by neutrophils, which has been described for a multitude of tumor-associated antigens, such as Ep-CAM, HER-2/neu, EGFR, HLA class II, CD20, CD30 and CEA73-78. Furthermore, as only FcαRI induces neutrophils recruitment10, targeting of FcαRI, but not of IgG Fc receptors led to accumulation of neutrophils in 3-dimensional tumor colonies, ultimately resulting in complete destruction of tumor colonies79. Crosstalk between neutrophils and endothelial cells was observed as well, as the latter cells released CXCL8, which is a prototypic neutrophil chemokine. Thus, FcαRI represents a promising candidate molecule for targeting tumors with IgA monoclonal antibody therapy.

In conclusion, IgA mediated immune responses are crucial in mucosal areas. However detrimental effects arise in case of unbalanced immune responses. Understanding this balance will allow future specific therapeutic interventions in the immune response, when either induction or inhibition of immunity is required.

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