CHAPTER 8

General discussion
Immunoglobulin A (IgA) represents the most prominent antibody class at mucosal surfaces and the second most prevalent antibody class in human serum. Mucosal IgA is produced by plasma cells in the lamina propria as dimeric molecules with an adjoining peptide (called the J-chain) and is referred to as dimeric IgA (dIgA) (Figure 1)\(^1\). It binds to the polymeric Ig-receptor (pIgR), which is expressed on the basolateral membrane of mucosal epithelial cells, and is subsequently transported through these cells and released into the lumen as secretory IgA (SIgA). Apical cleavage of the pIgR ensures that a part of this receptor, referred to as secretory component (SC), remains attached to SIgA. This provides stabilization and prevents rapid breakdown in the hostile environment of the gut lumen.\(^1\)\(^-\)\(^3\)

SIgA mainly serves as an antiseptic coating of the mucosal wall by neutralizing bacterial toxins and preventing adherence and invasion of microorganisms.\(^4\)\(^,\)\(^5\) Thus, it provides a passive first line of defense against invading bacteria. IgA is traditionally considered as a non-inflammatory antibody,\(^6\)\(^-\)\(^8\) which holds true for SIgA since it is a poor opsonin and unable to fix the classical complement pathway efficiently.\(^9\)\(^,\)\(^10\) Binding of SIgA to the IgA Fc receptor Fc\(_\alpha\)RI is partly hampered as a result of steric hindrance by SC, which prevents induction of inflammatory responses.\(^11\)\(^,\)\(^12\) This is an advantage in secretions that contain a multitude of commensal bacteria and/or environmental antigens, where initiation of an inflammatory reaction would likely affect the integrity of mucosal surfaces.\(^10\)

However, complexes of monomeric or dimeric IgA that do not contain SC can cross-link Fc\(_\alpha\)RI, which vigorously induces inflammatory responses, like phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), respiratory burst, degranulation, antigen-

**Figure 1. Schematic representation of different forms of IgA.** Monomeric IgA is mainly found in the serum. Dimeric IgA is produced by plasma cells in the lamina propria. It then binds to the polymeric Ig receptor, is transported through the epithelial cells and released in the lumen as secretory IgA. A part of pIgR (referred to as secretory component) remains attached. Fc\(_\alpha\)RI; IgA Fc receptor, pIgR; polymeric Ig receptor.
presentation or release of cytokines and inflammatory lipid mediators.\textsuperscript{7,13} Fc\(\alpha\)RI is expressed on cells of the myeloid lineage. It is constitutively expressed on neutrophils, monocytes, eosinophils, and can be induced on some macrophage subpopulations (including Kupffer cells) and interstitial dendritic cells.\textsuperscript{4} The majority of Fc\(\alpha\)RI-positive cells in blood and tissues are neutrophils.\textsuperscript{14} These are the most abundant circulating leukocytes in humans, and the first cells to arrive at a site of infection. This is supported by the severe infections that occur in individuals in which the number of neutrophils is reduced or when neutrophils are unable to fulfil their antimicrobial functions, due to genetic deficiencies.\textsuperscript{15,16} Neutrophils are recruited in response to pathogen- and/or host-derived components, such as bacterial peptides, LTB4 and IL-8, after which they rapidly infiltrate into infected tissues where they are able to kill invading bacteria.\textsuperscript{17} In chapter 2, we identified a previously unrecognized role of IgA and Fc\(\alpha\)RI in mediating neutrophil migration. We described that cross-linking of neutrophil Fc\(\alpha\)RI by IgA-coated beads led to release of LTB4 and neutrophil recruitment, resulting in amplification of neutrophil migration (Figure 2).\textsuperscript{18}

I hypothesize that this may be very efficient for clearing impending mucosal infections. IgG-coated beads did neither induce LTB4 release nor neutrophil recruitment. The IgG Fc receptors Fc\(\gamma\)RIIa and Fc\(\gamma\)RIIIb are constitutively expressed on human neutrophils.

\begin{figure}[h]
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\caption{Schematic model of activation of neutrophils in the intestinal tract following infection. When pathogens breach the mucosal epithelial border, dimeric IgA-pathogen complexes are formed in the lamina propria. Subsequent cross-linking of neutrophil Fc\(\alpha\)RI by these IgA complexes induces a leukotriene (LTB4) mediated neutrophil migration loop, resulting in clearance of invading pathogens.}
\end{figure}
is a glycosyl-phosphatidyl-inositol-anchored receptor, and its signaling pathway is still not completely characterized. FcγRIIa bears an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic tail that is essential for signaling. The cytoplasmic tail of FcαRI does not bear any known signalling motifs and is dependent on association with the FcRγ-chain, which expresses a similar, but not identical ITAM. For instance, FcγRIIa ITAM was able to mediate cytokine release or antigen presentation, which was not triggered via FcγRIIa ITAM. This suggests that the two ITAMs initiate diverse functions, which could be an explanation for the difference we observed in neutrophil migration after triggering via IgA or IgG.

Stimulation of neutrophils with granulocyte-colony stimulating factor (G-CSF) or interferon (IFN)-γ induces expression of FcγRI, which is a high affinity IgG receptor. This receptor, like FcαRI, also associates with FcRγ-chain. It was demonstrated that effector functions such as ADCC by either FcγRI or FcαRI were dependent on the ITAM signaling motifs within the FcRγ-chain. Nonetheless, immature neutrophils that were mobilized from the bone marrow upon G-CSF treatment efficiently triggered migration of neutrophils to tumor colonies and subsequent tumor cell lysis via FcαRI, but not via FcγRI. This suggests that cross-linking of FcαRI induces a different signaling pathway via FcRγ-chain than FcγRI. FcαRI bears a positively charged residue within its transmembrane domain that associates with a negatively charged amino acid of the FcRγ-chain, resulting in a strong electrostatic interaction within the transmembrane regions. FcγRI lacks such a positively charged amino acid in its transmembrane region, which likely induces a weaker association with the FcRγ-chain. This suggests that the different signaling and effector functions via FcγRI on neutrophils may be induced by dissimilarities in interaction between FcαRI or FcγRI with the common FcRγ-chain. This may lead to competition between both FcRs for available FcRγ-chain in favor of FcαRI, hereby diminishing FcγRI function.

Alternatively, FcαRI signaling might be fundamentally different. For instance, it was demonstrated that monovalent targeting of FcαRI with serum IgA inhibits signaling and induces apoptosis in monocytes, which is also dependent on FcRγ-chain ITAM. This inhibitory capacity is referred to as ITAMI, which is different from the classical immunoreceptor tyrosine-based inhibitory motif (ITIM) signaling of inhibitory Fc receptors. An ITIM bearing Fc receptor requires co-engagement to an activated ITAM-containing receptor. Thus interestingly, both IgA-induced activating and inhibitory signals depend on the FcRγ-chain ITAM. However, monomeric serum IgA induces recruitment of tyrosine phosphatases via ITAMI, whereas cross-linking of FcαRI mediates recruitment of tyrosine kinases, respectively. As such, it has been proposed that cross-linking of FcαRI during infection with IgA-opsonized pathogens results in pro-inflammatory responses, whereas naturally occurring serum IgA that is not complexed with an antigen induces inhibitory signals through FcαRI to dampen excessive immune responses.

**IgA-induced skin blistering diseases**

Remarkably, in Chapter 3 we demonstrated that aberrant IgA-antigen complexes from patients with the chronic skin blistering disorder linear IgA bullous disease (LABD) induce excessive inflammatory responses. LABD is characterized by subepidermal blisters
with dense cell infiltrates, dominated by neutrophil presence. In these patients IgA is directed against collagen XVII, which is a transmembraneous hemidesmosomal protein involved in maintaining cell-matrix adhesion of the skin. IgA-autoantibodies are lined along the epithelial basement membrane of the skin and as such form a continuous complex with collagen XVII. Activated and recruited neutrophils will not be able to clear these complexes, which induces a perpetuating inflammatory loop causing severe tissue damage in the form of blisters (Figure 3). This is mainly due to the persistent release of harmful inflammatory cytokines, reactive oxygen species and proteases by the infiltrated neutrophils.

IgA-induced neutrophil migration may also play a role in the pathogenesis of the skin blistering disease dermatitis herpetiformis. This disease is characterized by granular deposits of IgA autoantibodies directed against epidermal transglutaminase 3 (TG3). Similar to LABD large neutrophil infiltrates are observed in the dermal papillary tips of the skin. Interestingly, dermatitis herpetiformis is associated with celiac disease, since anti-TG3 IgA autoantibodies show crossreactive epitopes with tissue transglutaminase 2 (TG2). Celiac disease is a chronic, multifactorial inflammatory disease of the small intestine, and can develop in genetically susceptible individuals upon ingestion of wheat gluten and related cereal proteins. The inflammatory reaction appears to be driven by activation of Th1 cells that recognize both gluten peptides and gluten peptides deamidated by TG2. Celiac disease is characterized by the presence of anti-TG2 IgA autoantibodies, but is not considered a disease mediated by IgA-induced neutrophil migration. However, anti-TG2 IgA deposits were demonstrated at the epithelial basement membrane of the small intestine, including in patients with early onset celiac disease. The intensity of the IgA deposits increased as enteropathy progressed on a gluten-containing diet, and decreased on a gluten-free diet. Moreover, genome-wide
expression profiling demonstrated enhanced neutrophil infiltration in active celiac disease patients that was still present in patients in complete remission.\textsuperscript{45} It was furthermore demonstrated that a significant neutrophilic infiltrate can be seen in duodenal biopsies from patients with celiac disease, and that the severity correlates with the overall disease activity.\textsuperscript{46} Therefore, it is intriguing to speculate that IgA-induced neutrophil recruitment plays a role in celiac disease, comparable to dermatitis herpetiformis. Importantly, we showed that \textit{ex vivo} IgA-induced neutrophil migration and tissue damage were inhibited by blocking Fc\textalpha\ RI, indicating that these events are dependent on the interaction of IgA autoantibodies with Fc\textalpha\ RI. Thus, interrupting the neutrophil migration loop by blocking Fc\textalpha\ RI might reduce tissue damage in diseases with aberrant IgA-immune complexes. In chapter 4, we developed an LABD mouse model to further investigate the role of IgA induced tissue damage \textit{in vivo}. For this model transgenic mice with LysEGFP (Lysozyme-Enhanced Green Fluorescent Protein) expressing neutrophils were crossbred with human Fc\textalpha\ RI transgenic mice (Fc\textalpha\ RI-LysEGFP).\textsuperscript{25,47} This gave us the opportunity to study migration of neutrophils with intravital imaging microscopy. We generated human IgA antibodies anti-mouse collagen XVII (COL17) -which is the target antigen in LABD-, and injected this in the ears of mice. Because mouse ears are almost translucent, intravital microscopy of blood vessels is possible. We observed rolling and extravastion of Fc\textalpha\ RI-expressing neutrophils in response to the presence of IgA anti-COL17, which resulted in massive accumulation of neutrophils in blood vessels and tissue, in contrast to control ears (injected with irrelevant IgA). Pilot data demonstrated that also \textit{in vivo} IgA-induced neutrophil-migration could be blocked with anti-Fc\textalpha\ RI monoclonal antibodies (mAbs). This could be an improvement over current therapies for chronic blistering diseases. These therapies mainly consist of administration of general immunosuppressive medications with concomitant side effects.\textsuperscript{48,49} However, systemic delivery of anti-Fc\textalpha\ RI mAbs may be accompanied with unwanted side effects as well, and a topical treatment of a skin disease is preferable. However, considering the size of the mAbs, which is 150 kDa, absorption through the skin is expected to be negligible. Therefore, peptide mimetics that block IgA-Fc\textalpha\ RI interactions and are small enough to pass the epidermis may be good candidates. Fortunately, we could develop IgA- and Fc\textalpha\ RI-based peptides since the structure of the interaction of IgA and Fc\textalpha\ RI is known.\textsuperscript{50-52} In chapter 5, we explored the potential of these peptides to block IgA-induced neutrophil migration with the ultimate goal to use these peptides as a therapeutic tool for IgA-induced skin blistering diseases. Peptide mimetics currently gain a lot of interest for drug development, and have already been used for vaccination strategies.\textsuperscript{53-55} They were furthermore demonstrated to successfully inhibit functions of the human epidermal growth factor receptor 2 (HER2/neu), platelet-derived growth factor (PDGF),tumor necrosis factor alpha (TNF\textalpha) or caspase-1/interleukin-1\textbeta-converting enzyme (ICE) \textit{in vitro} and \textit{in vivo}.\textsuperscript{56-59} In chapter 5, we demonstrated blocking of IgA-induced neutrophil migration in \textit{ex vivo} skin grafts with IgA- or Fc\textalpha\ RI based peptides. Screening of card-linked Fc\textalpha\ RI-peptides showed that peptides containing five amino acids involved in interaction with IgA (LKFWNETDP) demonstrated stronger binding compared to card-linked peptides based on a sequence with three of the residues involved in
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binding (YRIGHYRFR). Nonetheless, linear and cyclic soluble FcαRI-peptides based on the latter sequence demonstrated excellent blocking of IgA-FcαRI interactions in ex-vivo skin migration experiments. It is possible that soluble peptides based on the amino-acid sequence LKFWNETDP, may result in even better blocking of IgA-induced neutrophil migration, which will be analysed in future experiments.

Currently, microneedles ('tatooing') are seen as a promising tool for the (trans)dermal delivery of peptide drugs. This array of microscopic needles is sufficiently long to penetrate the epidermis but small enough to do not cause skin injury and pain feeling. However, this approach is not feasible for multiple delivery of blocking peptides in skin of patients who already have damaged skin and blisters. For these patients, the optimal administration of blocking peptides would be topical application of an ointment. We demonstrated that an ointment containing a skin permeation enhancer resulted in penetration of the epithelial barrier and delivery of IgA-peptide mimetics in a dose dependent manner. Moreover, the concentration of peptides in the receptor fluid, which is a measure of systemic delivery, was negligible, supporting limited systemic exposure. Successful delivery of peptides through the epidermis has been demonstrated in vivo.

Pemphigus vulgaris is a life-threatening autoimmune skin blistering disease, caused by IgG autoantibodies targeting the keratinocyte surface antigens desmoglein 3 and desmoglein 1, leading to loss of keratinocyte cohesion. A peptide targeting desmoglein was used and it was demonstrated that when applied topically in an ointment, it abrogated autoantibody-mediated skin blistering in a pemphigus mouse model. As such, peptides blocking IgA-induced neutrophil migration topically applied in the form of an ointment may be considered a new approach in receptor-specific therapeutics for IgA-induced blistering diseases.

**IgA-induced neutrophil migration in other diseases**

FcαRI induced tissue damage may also apply to diseases in which aberrant IgA complexes have not yet been recognized as potentially damaging. 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 although Fcμ/αR and transferrin receptors (TfR) which bind to IgA, have been described. However, these receptors do not have similar functions compared to human FcαRI. To overcome this limitation human FcαRI transgenic mice were generated, in which FcαRI expression, regulation, interaction with human IgA, and function mimic the human situation. These mice were crossbred with a knock-in mouse model that produces human IgA (hIgA). This resulted in the generation of two groups of mice. In one group mice only produced hIgA that were used as control. In the second group mice produced hIgA and expressed FcαRI on neutrophils, so only in these mice in these mice complexed hIgA can cross-link FcαRI and subsequently activate and recruit neutrophils.

Since we previously demonstrated that neutrophils in the colon of ulcerative colitis (UC) patients had taken up IgA complexes (chapter 2), we hypothesized that cross-linking of
FcαRI by aberrant IgA-antigen complexes might be a key process in causing severe tissue damage in UC, which was further investigated in chapter 6. Ulcerative colitis is a subtype of chronic inflammatory bowel disease with a largely unknown etiology that presents in susceptible hosts. It is characterized by superficial, continuous inflammation that is limited to the colon. One of the hallmark features of UC are large neutrophil infiltrates. In active UC, histological evidence of high-density neutrophil accumulation in the intestinal lumen correlates with epithelial injury and clinical disease activity. Because the mucosal barrier is impaired in these patients, leading to translocation of luminal contents, it is likely that excessive IgA-antigen complexes are formed.

In chapter 6, we used our novel mouse model to analyze whether the expression FcαRI induces a more severe colitis. We induced experimental colitis by administering dextran sulphate sodium (DSS) in the drinking water of the mice. We demonstrated that hIgA/FcαRI mice experienced a more severe colitis compared to their FcαRI-negative littermates. Although administering DSS is an approved method of inducing colitis, the mechanism of action remains unknown. It was recently demonstrated however that vesicles of DSS complexed to medium-chain-fatty-acids (MCFS) were taken up by mucosal epithelial cells. It was suggested that the arrival of these DSS/MCFS vesicles in the cytoplasm may activate intestinal inflammatory signaling pathways, and consequently reduces intestinal barrier functions. This may result leakage of luminal antigens into the lamina propria with concomitant accumulation of IgA-antigen complexes and IgA-induced neutrophil migration. This is supported by our data demonstrating extensive tissue damage and large immune cell infiltrates that mostly comprised of neutrophils, in cryosections of the colon of hIgA/FcαRI mice. This indicates that FcαRI induced neutrophil recruitment and subsequent tissue damage may contribute to the pathogenesis of UC (Figure 4).

In chapter 7, we investigated the induction of adaptive immune responses in our experimental colitis model. We observed an increased number of CD4-positive T cells (T helper cells) in hIgA/FcαRI mice. Even though we were not able yet to identify different T helper subsets, mRNA analyses demonstrated enhanced expression of chemokine (C-C motif) ligand (CCL) 2, interleukin (IL)-1β and IL6 in IgA/FcαRI mice that are involved in the induction of Th17 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 Th17 responses. Recently, it was demonstrated that the percentage of circulating Th17 cells was significantly increased in patients with active UC, compared with patients with CD. Moreover, increased transcripts for Th17-related cytokines were found in UC compared to CD. Interestingly, a reciprocal crosstalk between neutrophils and Th17 cells has been described. It was demonstrated that IL-17 upregulates the production of granulocyte-colony stimulating factor (G-CSF), which stimulates the production of neutrophils. Additionally, Th17 cells secrete the neutrophil chemokine (C-X-C motif) ligand (CXCL) 8 or IL-8. In turn, neutrophils attract Th17 cells via release of CCL2 (monocyte chemoattractant protein; MCP-1) and CCL20 (macrophage inflammatory protein; MIP-3α) that bind to the receptors CCR2 and CCR6 on Th17 cells. This interaction between neutrophils and Th17 cells may explain the increased number of Th17 cells in patients with UC.
Thus, in contrast to the general believe of neutrophils as end-stage cells without the capacity for biosynthesis, it is now clear that neutrophils can release a plethora of cytokines and chemokines that activate or attract other cells of the immune system. When neutrophils are recruited towards a site of infection, interactions with various resident or recruited immune cells occur. For instance activated neutrophils have been shown to synthesize and secrete high levels of B-cell-activating factor (BAFF), and ‘a proliferation-inducing ligand’ (APRIL).73,74 Both cytokines play a fundamental role in differentiation, proliferation and immunoglobulin production of B cells.74 It has been demonstrated that splenic neutrophils induced immunoglobulin class switching, somatic hypermutation and antibody production by activating B cells of the marginal zone. Furthermore, patients with neutropenia had fewer and hypomutated marginal zone B cells.75 In addition, neutrophils were the only source of APRIL in the lower part of mucosal associated lymphoid tissue.
(MALT) indicating that neutrophils may create plasma cell niches in the lamina propria to sustain local antibody production by B cells. As such, neutrophils may play an important role to prime adaptive immune responses.

Like UC, is Crohn’s disease (CD) a subtype of chronic inflammatory bowel disease but with distinct characteristics. CD is characterized by transmural, and non-continuous inflammation usually confined to the terminal ileum. Remarkably, CD is characterized by large infiltrates of mononuclear cells, whereas in UC neutrophil infiltrates are found. However, in chapter 2, we demonstrated that patients with active CD had infiltrates of neutrophils and that these neutrophils had taken up complexed IgA, albeit in lower numbers than in colon tissues of UC patients. Moreover, jejunal tissues of CD patients contained enhanced release of neutrophil granule components compared to healthy control. Still, neutrophils are not the dominant cell type in infiltrates of CD patients. The inflammation in CD is mainly confined to the ileum that has lower bacterial counts compared to the colon where inflammation of UC is confined. Therefore, activation of neutrophils could be dependent on the bacterial load of the intestines. It was demonstrated that the numbers of bacteria associated with biopsies from UC patients were approximately double of those associated with samples from CD patients. Moreover, there was an almost complete absence of IgA-producing cells in affected areas of the lamina propria of CD patients. This may result in less accumulation of IgA-pathogen complexes during inflammation in the lamina propria and, hence, less neutrophil recruitment. This is supported by the data of Cambridge et al. demonstrating that 54% of patients with UC contained anti-neutrophil serum antibodies, compared to 10% of CD patients. Thus, IgA-induced neutrophil recruitment plays a more prominent role in the pathogenesis of UC than in CD.

Interestingly, several studies have demonstrated that the presence of IgA-rheumatoid factor in patients with rheumatoid arthritis (RA) is linked to a more severe disease and that it augments neutrophil degranulation. Moreover, it was suggested that granulocyte recruitment to inflamed joints was mediated by LTB4. RA is a complex inflammatory disease of synovial joints, that is characterized by joint destruction and chronic disability. It is conventionally considered to be a disease that is mediated by Th1 cells. Additionally, macrophages, mast cells, and natural killer cells are found in the synovial membrane. Neutrophils have been found to reside mainly in the synovial fluid and do seem to play a role in the pathogenesis of RA. We propose that IgA-FcγRI interactions may induce neutrophil activation and recruitment to the joints of RA patients, leading to degranulation and subsequent tissue damage.

IgAN is a common primary glomerulonephritis and induced by IgA, but neutrophils are not considered to play a role in this disease. Mechanisms involved in the pathogenesis of IgAN are not completely understood. A reduced expression of FcγRI was observed on the surface of circulating monocytes of IgAN patients in spite of normal FcγRI transcription levels. This was due to shedding of the extracellular domain of FcγRI after interaction with abnormally glycosylated polymeric IgA (pIgA), leading to the formation of soluble FcγRI/pIgA complexes (sFcγRI/pIgA). These complexes then bind to the transferring receptor (Tfr, CD71) on mesangial cells which initiates proinflammatory responses and leukocyte infiltration. Next to monocytes and macrophages, neutrophils have been
found in infiltrates of renal biopsies of IgAN patients.\textsuperscript{98-100} However, they do not seem to play a prominent role in the pathogenesis of this disease. This may be the result of the occupation of the IgA-binding sites by soluble FcαRI in the sFcαRI/pIgA complexes. Consequently, binding and cross-linking of FcαRI on neutrophils is not feasible and the detrimental neutrophil-migration loop will not be induced. However, this is only one possible explanation and it would be challenging to further investigate a possible role of IgA-induced neutrophil recruitment in IgAN.

**Conclusion and future perspectives**

FcαRI has a dual role in immunity since it mediates either inflammatory or anti-inflammatory signalling, depending on multivalent or monovalent receptor targeting.\textsuperscript{28,101} While monomeric IgA induces inhibition of heterologous receptors and apoptosis, multivalent aggregation of FcαRI by IgA-antigen complexes triggers several activating functions, including phagocytosis, superoxide production, and inflammatory cytokine secretion such as tumor necrosis factor-\(\alpha\) (TNF\(\alpha\)) and IL-6.\textsuperscript{28,10} Moreover, via the release of LT\(\beta\)4 a neutrophil migration loop is induced, which may lead to efficient clearance of pathogens that have invaded the mucosal tissue.

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, induction of immune responses through IgA-FcαRI interactions may help to fight (mucosal) infections. For instance, it was demonstrated that bacteria opsonized with dIgA are efficiently phagocytosed by neutrophils.\textsuperscript{18,102} Furthermore, when human FcαRI transgenic mice were infected with *Bordetella pertussis* that had been opsonized with human IgA, enhanced bacterial clearance in lungs of these mice was observed compared with non-transgenic littersmates.\textsuperscript{103} Moreover, it was recently shown that passive transfer of human IgA monoclonal antibodies against *Mycobacterium tuberculosis* protected human FcαRI transgenic mice, but not FcαRI-negative control mice, against *M. tuberculosis* infection.\textsuperscript{104} Mucosal administration of an HIV-1 vaccine resulted in resistance to the virus and production of virus-specific IgA with HIV-1 transcystosis-blocking properties in *Macacamulatta* monkeys.\textsuperscript{105} It was recently demonstrated that targeting FcαRI directed neutrophils to destroy HIV-infected target cells.\textsuperscript{106} Because *M. mulatta* monkeys express FcαRI, an active role for FcαRI in eliciting protection is suggested, in addition to HIV neutralizing IgA Ab. Therefore, IgA-FcαRI interactions may function as an active second line of defense at mucosal surfaces by recruiting neutrophils. As such, vaccination strategies inducing a mucosal IgA response may be a good approach to protect against mucosal pathogens. We will further investigate the protective role of FcαRI in infections in our novel hIgA/FcαRI Tg mouse model.

On the other hand, patients with conditions that are characterized by IgA-antigen complex driven excessive immune responses (such as LABD and UC), would greatly benefit from selective inhibition of the IgA-FcαRI interaction, which would counteract the inflammatory processes. I have already shown that tissue damage in LABD is reduced with specific anti-FcαRI monoclonal antibodies (chapter 3). This mechanism of action might also prove effective to dampen inflammatory responses in patients suffering from
UC, or other diseases not considered as strictly mediated by neutrophils, like RA. This would be an improvement over current therapies that mainly consist of administration of general immunosuppressive medications.\textsuperscript{107} The specific blocking of FcαRI and IgA could overcome systemic side effects. However, the potential of FcαRI on neutrophils to induce severe tissue damage after cross-linking by specific IgA-antibodies, 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 CEA.\textsuperscript{108-113} Furthermore, as only FcαRI induces neutrophil recruitment, 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 colonies.\textsuperscript{18,114} Crosstalk between neutrophils and endothelial cells was observed as well, as the latter cells released CXCL8 (IL-8), which is a prototypic neutrophil chemokine.\textsuperscript{114} Moreover, neutrophils mainly facilitate autophagy to induce tumor cell death rather than the more commonly recognized apoptotic cell death mechanisms induced by NK cells or cytotoxic T cells. This is particularly interesting for treating malignancies with apoptosis-resistant tumor cells.\textsuperscript{115} Thus, FcαRI represents a promising candidate molecule for targeting tumors with IgA monoclonal antibody therapy.

In conclusion, IgA mediated neutrophil recruitment is crucial in mucosal areas. However, detrimental effects arise in diseases where aberrant IgA-antigen complexes are found and a continuous loop of neutrophil migration is induced. Understanding this balance will allow future specific therapeutic interventions in the immune response when either induction or inhibition of immunity is required.
REFERENCES


27. Otten, M.A., et al. FcR gamma-chain dependent signaling in immature...


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82. Green, F.H. & Fox, H. The distribution of mucosal antibodies in the bowel of patients
106. Duval, M., Posner, M.R. & Cavacini, L.A. A bispecific antibody composed of a nonneutralizing antibody to the gp41 immunodominant region and an anti-CD89 antibody directs broad human


