Chapter 7

General discussion

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The era of the Immunoglobulin A Fc receptor FcαRI; its function and potential as target in disease
Esil Aleyd, Marieke H. Heineke, Marjolein van Egmond
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Immunoglobulin A (IgA) is the most prevalent antibody class at mucosal areas, where it plays an important role in the defense against pathogens. In mucosal areas, IgA is produced in a dimeric form with a J chain (dIgA) by local plasma cells in the lamina propria (Figure 1).

When dIgA binds to the polymeric Ig-receptor (pIgR), which is expressed on the basolateral membrane of mucosal epithelial cells, it is transported through mucosal epithelial cells to the apical site where pIgR is cleaved and the complex is released into the lumen as secretory IgA (SIgA). A part of pIgR, the secretory component (SC), remains attached to SIgA after cleavage. This provides SIgA with stability and protects it from being degraded by enzymes in the harsh environment of the gut lumen. SIgA does not activate the classical complement pathway. The opsonic capacity of SIgA furthermore is poor compared to dIgA and serum monomeric IgA (mIgA), mostly because of the (partial) blockage of the IgA Fc receptor FcαRI binding site on IgA by SC. Its inability to trigger efficient phagocytosis by neutrophils or Kupffer cells supports the anti-inflammatory role of SIgA. The inability of SIgA to trigger immune cell activation is an advantage in an environment where commensal bacteria or antigens are present and initiation of inflammatory responses are undesirable, as this would affect mucosal integrity and barrier function of mucosal surfaces.

However, it was previously shown that both mIgA and dIgA complexes can crosslink the IgA Fc receptor FcαRI and are equally capable of activating neutrophils. FcαRI is expressed on cells of the myeloid lineage, such as neutrophils, monocytes, and eosinophils. The majority of FcαRI-positive cells in blood and tissues are neutrophils, which are the most abundant circulating leukocytes in blood. In homeostatic conditions, no FcαRI expressing cells are observed in the lamina propria. Neutrophils are however quickly
recruited in response to pathogen- and/or host-derived components, such as bacterial peptides, leukotriene B4 (LTB4) and interleukin 8 (IL-8), after which they rapidly infiltrate into infected tissues where they are able to kill invading bacteria. In mucosal areas, antigens can continuously reach the lamina propria, because of the abundance of microbial flora and food components in the intestinal tract. Plasma cells present in the lamina propria produce dIgA that can opsonize these antigens. The formed dIgA-pathogen complexes can crosslink FcαRI and activate neutrophils, and subsequently induce different pro-inflammatory responses, such as the release of cytokines, reactive oxygen species (ROS), inflammatory mediators, phagocytosis and the formation of neutrophil extracellular traps (NETs). Importantly, crosslinking of FcαRI by IgA induces the release of the potent chemoattractant LTB4, and in this way more neutrophils are attracted to the site of infection. This process is specific for IgA, as crosslinking of FcγR by IgG did not induce LTB4 release and consequently could not mediate neutrophil migration. I hypothesize that this process initiates a self-contained positive loop, resulting in the accumulation and activation of neutrophils at the site of infection, until clearance of invading pathogens has been achieved and the mucosal infection has been cleared. The induced inflammatory responses represent a protective mechanism against invading pathogens. After executing their antimicrobial functions and the infection has been cleared, neutrophils will go into apoptosis. This will signal the end of inflammation. Clearance of apoptotic cells by macrophages, referred to as efferocytosis, occurs, which is an anti-inflammatory signal (Figure 2).

The significance of dIgA in protective mucosal immunity is emphasized by the fact that bacterial evolution resulted in the development of IgA-binding proteins. Several IgA proteins that bind to sites in IgA and overlap with the binding site of FcαRI have been described, such as IgA-binding M-like proteins Arp4, Sir22, β-antigen and members of the staphylococcal superantigen-like proteins (SSL) family. These proteins competitively inhibit binding of IgA to FcαRI, hereby circumventing IgA-mediated elimination mechanisms, and obstructing clearance of bacteria. This likely represents an important evasion strategy for pathogens to escape IgA-mediated elimination by FcαRI-expressing phagocytes.

**How potent is IgA in triggering neutrophil activation?**

In chapter 2, we investigated how IgA affects phagocytosis and neutrophil activation after crosslinking of FcαRI by IgA-opsonized bacteria. Activated neutrophils use different killing mechanisms to eliminate microorganisms and phagocytosis is one of these mechanisms. During phagocytosis, the outer membrane of the neutrophil surrounds the pathogens to engulf them and the pathogens are taken up into membrane vesicles, referred to as the phagosomes. Inside the neutrophil, phagosomes fuse with lysosomes, which are intracellular vesicles containing lytic enzymes and other microbicidal proteins, including myeloperoxidase (MPO) and lactoferrin. This fusion results in formation of the phagolysosome, in which pathogens are digested. We demonstrated that IgA potentiates phagocytosis of bacteria by neutrophils when compared to phagocytosis of bacteria in the absence of IgA.
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Figure 2: Schematic model of neutrophil activation at mucosal areas. Left panel: When microbes succeed to infiltrate the lamina propria, they are opsonized by dimeric IgA (dIgA). The formed dIgA-pathogen complexes crosslink the IgA Fc receptor FcαRI and activate neutrophils. Subsequently, this induces phagocytosis, neutrophil extracellular traps (NETs) formation, degranulation and the secretion of the chemoattractant leukotriene B4 (LTB4). More neutrophils are attracted to the site of infection and a migration loop is initiated. This will result in clearance of the infection. Right panel: Newly recruited neutrophils will not longer be activated. Neutrophils will go into apoptosis and this will signal the end of inflammation. Macrophage-mediated clearance of apoptotic cells occur (efferocytosis).

In addition to phagocytosis, neutrophils secrete enzymes derived from intracellular stored granules into the environment, which is an extracellular killing mechanism that neutrophils can employ to eliminate pathogens. Neutrophils can also form NETs. These so-called NETs are web-like structures extruded by neutrophils that can trap pathogens. They contain DNA, histones and several granular and cytoplasmic antimicrobial proteins, like elastase and MPO. Brinkmann et al. first recognized NET formation as a host innate immune defense mechanism against pathogens. The content of the nucleus mixes with granular as well as cytosolic proteins, after which the outer membrane ruptures and NETs are released by activated neutrophils into the extracellular space, resulting in neutrophil death, referred to as NETosis.

Whether IgA affects the formation of NETs by neutrophils was also investigated in chapter 2. We demonstrated that enhanced uptake of IgA-opsonized particles (bacteria and beads) resulted in increased release of NETs, when compared with uptake of non-opsonized particles (Figure 3). Importantly, the observed enhanced IgA-induced phagocytosis and NET formation was mediated by FcαRI, as blocking of FcαRI decreased the formation of...
NETs. There is much debate whether NETs have antimicrobial effects on microorganisms and whether they can kill bacteria directly or just capture or immobilize them. Impairment of microbes survival by NETs has been shown by different groups. However, others showed entrapment of bacteria (in the absence of antibodies) by NETs without killing. The latter also holds true for our experiments. We demonstrated IgA-induced NET formation and catching of live bacteria, but bacterial viability was not diminished and we did not detect bacterial killing. NETs contain different proteolytic enzymes that are harmful to bacteria. Consequently, some bacterial strains have developed mechanisms to avoid capture by NETs, as they express DNases which are able to break down NETs. Therefore, killing of bacteria by NETs might be dependent on the strain that is used. NETs might be able to catch bacteria and inhibit their growth without ultimately resulting in bacteria killing.

In either case, formation of NETs will help to contain infections at mucosal sites. It has been shown that bacterial dissemination from the site of infection could be prevented by the formation of NETs in case of sepsis. I propose that if bacteria succeed to invade the protective barriers of the mucosal wall, they will be opsonized by dIgA (Figure 3). Crosslinking of FcαRI by dIgA-opsonized bacteria will induce potent NET formation, which can immobilize and possibly kill invading microorganisms, preventing systemic spreading. These results support a new and protective mechanism of IgA in boosting the immune response, resulting in clearance of infiltrating pathogens and in maintaining homeostasis at mucosal sites.

**Figure 3: Schematic representation of neutrophil extracellular traps (NETs) formation upon encountering IgA-opsonized bacteria.** Crosslinking of FcαRI by dIgA-antigen complexes induces phagocytosis, followed by the production of intracellular reactive oxygen species (ROS). Granular enzymes mix with nucleus deoxyribonucleic acid (DNA), followed by disruption of the nuclear envelope and cell membrane. Enzymes, such as myeloperoxidase (MPO) and elastase are bound to histones that are subsequently released into the extracellular milieu, representing NETs. Pathogens present in the environment are trapped into this NETs preventing further dissemination.

**Are IgA-induced neutrophil extracellular traps friends or foe?**

NETosis is a protective mechanism that neutrophils use against several infections. However, there is a growing body of evidence in which detrimental consequences for host tissues are described when NETs, containing degrading enzymes, are not removed from tissues or from the circulation. Macrophages are capable of clearing NETs and this occurs in an immunologically silent manner, as this process does not induce proinflammatory
cytokine release. This mechanism would prevent an excess of NET formation, implying that macrophages play an essential role in preventing unnecessary tissue damage. Inefficient elimination of NETs from tissues or from the circulation leads to the presence of DNA in extracellular space, inducing autoimmune responses against these antigens (chromatin and enzymes) and the production of anti-DNA antibodies. In this way, the presence of anti-DNA antibodies in the blood contributes to autoimmune diseases, such as systemic lupus erythematosus (SLE), systemic vasculitis (SVV) or autoimmune lupus nephritis. Furthermore, it has been shown that high levels of NETs can lead to tissue damage in vascular thrombosis or chronic lung inflammation in cystic fibrosis. Moreover, the lack of nucleases, the key regulators of NETs degradation, or presence of non-functional nucleases are associated with the autoimmune disease lupus nephritis. As such, a balance between NET formation and NET degradation defines the protective versus detrimental effects to the host. As evidence showed that NETs can be involved in autoimmune diseases by exacerbation of disease, NETs can act as double-edged sword of the immunity.

It has been shown that NET formation might play a pathogenic role in the chronic autoimmune disease rheumatoid arthritis (RA), which is characterized by inflammation of the joints and the presence of autoantibodies. These include rheumatoid factors (RF), which can be of the IgM, IgG or IgA isotype, directed against the Fc domain of immunoglobulin G (IgG). A second type of RA-related autoantibodies are anti-citrullinated protein antibodies (ACPA). Interestingly, different studies reported a correlation between the presence of IgA RF and ACPA and worse disease prognosis.

As I already demonstrated that NET formation is increased after crosslinking of FcαRI by IgA (chapter 2), I hypothesized that IgA-induced NET formation can potentially be damaging in diseases where IgA autoantibodies are found. In chapter 3, we therefore investigated whether IgA autoantibody complexes in plasma and synovial fluid (SF) of RA patients activate neutrophils resulting in (damaging) NET formation.

We demonstrated that IgA immune complexes, in plasma and SF of RA patients, activate neutrophils as shown by degranulation, secretion of chemotactic factors and the release of NETs. It is feasible that the presence of IgA immune complexes in SF directly contribute to activation and recruitment of neutrophils into the joints of RA patients through interaction with FcαRI. Newly recruited neutrophils will then also encounter these complexes and get activated, which will lead to secretion of more chemoattractants, resulting in amplification of neutrophil migration to the joints of RA patients. Of note, it has been shown that neutrophils are present in high numbers in the SF of RA patients. Normally, neutrophils have a short life span in the circulation (6-18 hours), but within SF, the presence of survival signals in the inflammatory milieu can extend the lifetime of neutrophils. Neutrophil degranulation, release of ROS and NETs may also result in increased destruction of joint tissue, which may explain why RA patients with high levels of IgA RF immune complexes have worse prognosis. As such, IgA autoantibodies can be detrimental to development of erosive disease and tissue destruction in the joints through FcαRI/IgA interactions and NET formation.

Although blocking NET formation might be risky as susceptibility to certain infections...
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may increase, blocking NET formation can be a therapy in autoimmune diseases. In this case, the harmful activities of NETs outweigh their protective benefits. DNase or anti-NET-associated proteins/enzymes therapies are already considered to get rid of excessive NET formation in patients with different autoimmune diseases. For example, it has been shown that a significant amount of extracellular DNA in the sputum of cystic fibrosis patients originates from NET formation. Hereby, neutrophil elastase (NE) promotes chromatin decondensation in sputum by cleaving DNA-bound histones and enhancing the ability of exogenous DNase to reduce sputum viscosity. NE inhibitors have been used in clinical trials and showed reduction of pulmonary NE activity, but are only moderately beneficial to patients. Furthermore, it was demonstrated that sera from a subset of patients with active SLE had a reduced ability to degrade in vitro generated NETs. This was mostly restored when these patients were in remission, implying that a therapy that has an effect on degradation of DNA could be beneficial for patients with active disease. A clinical trial with inhaled DNase therapy showed improvement of lung function and reduction of infectious exacerbations in patients with cystic fibrosis.

We showed that IgA-induced NET formation was prevented when FcαRI was blocked using an anti-FcαRI mAb. Thus, blocking FcαRI in RA patients may represent a more specific therapy. Anti-FcαRI therapy can prevent excessive and harmful NET formation and tissue damage induced by IgA autoantibodies in IgA-mediated autoimmune diseases, presumably with less side effects compared to DNase therapies.

When IgA-induced neutrophil activation continues to cause havoc

An in vivo role for neutrophils in initiating and maintaining inflammatory processes in the joint has been identified by using the autoantibody-induced K/BxN serum-transfer arthritis model, in which it was shown that early clinical signs of inflammation in the joints correlated with the presence of neutrophils. Additionally, it was demonstrated that the signal transduction molecules Syk, PLCγ2 and Src-family kinases play a critical role in the development of the autoantibody-induced K/BxN serum-transfer arthritis model, whereby activated neutrophils and macrophages release proinflammatory chemokines, cytokines and lipid mediators that attract further neutrophils and monocytes from the circulation resulting in the generation of an inflammatory environment.

The contribution of FcαRI-mediated neutrophil activation in RA is difficult to investigate, as in vivo studies are hampered since mice lack the expression of a FcαRI homologue. Two human FcαRI transgenic mouse models have become available. In the first model, the CD11b promoter was used resulting in human FcαRI expression on monocytes and macrophages. In the second model, a cosmid clone bearing the human FcαRI gene and regulatory elements was used. The latter resulted in human FcαRI expression on cells of the myeloid lineage, mostly neutrophils, which corresponds more closely with the human situation. These mice were crossed with mice that were human IgA (hIgA) knock-in to generate human FcαRI transgenic mice that produce human IgA (FcαRI/hIgA mice). This made it possible to investigate the in vivo role neutrophils and FcαRI/IgA interactions in autoimmune diseases.

As such, in chapter 4, I further investigated the hypothesis that IgA autoantibodies are
involved in causing joint damage in RA using an experimental model of RA. Arthritis can be induced by systemic administration of an IgG cocktail of monoclonal antibodies that target various regions of collagen type II (COL2, present in articular cartilage), referred to as collagen antibody induced arthritis (CAIA) \(^{66}\). An advantage of this model is that autoantibodies can initiate disease independently of B and T cells during the effector phase of arthritis.

We adapted CAIA for our FcαRI/hIgA mouse model. As human FcαRI transgenic neutrophils are not able to bind mouse IgA, we immunized hIgA knock-in mice with bovine collagen II to obtain hybridoma cell lines producing hIgA antibodies against bovine collagen II (anti-bCOL2 hIgA). These antibodies were injected in FcαRI/hIgA mice and their presence in blood was monitored for a few days. Unfortunately, we observed short half-life of hIgA antibodies in mice, compared to the half-life of IgG autoantibodies generally used in the CAIA model (commercial available cocktail of anti-COL2 IgG antibodies). The short half-life of hIgA antibodies in mice precluded the use of CAIA as a model, a this would require daily injections of antibodies for multiple days to induce arthritis. Antibody engineering strategies to improve the in vivo half-life of IgA have been described \(^{67,68}\). Implementation of these strategies may result in prolonged in vivo half-life of our anti-bCOL2 antibodies, but this is currently not available.

We therefore switched to another commonly used animal model for RA, referred to as collagen induced arthritis (CIA). Immunization with heterologous COL2 induces CIA in mice \(^{69}\). Immunized mice will themselves start to produce autoantibodies against COL2, which generates a high autoantibody titer in blood. Immunized mice produced anti-bCOL2 hIgA antibodies that were cross-reactive with mouse collagen II. Immunofluorescence staining of knee cryosections of immunized mice showed binding of hIgA antibodies to the cartilage, where COL2 is expressed in the joints. A significant increase in blood and bone marrow GR-1 positive neutrophils was observed at 3, 5, 7 and 10 weeks after immunization. A small number of neutrophils migrated to the knees of immunized FcαRI/hIgA mice, but this was not sufficient to induce clinical signs of arthritis, such as inflamed paws. The lack of neutrophil migration and accumulation in the joints may explain the lack of joint inflammation and paw swelling in these mice. However, 10 weeks after immunization, we did detect a significant decreased functionality/strength of the joints of mice by measuring the ability of mice to hold on to a grid. Control mice were able to hold on to the grid throughout the entire assay period, while in contrast, the majority of the immunized mice did not succeed in holding on to the grid, suggesting the initiation of loss of joint function, a feature also used as clinical parameter to assess severity of disease in RA patients. This suggests that in this pilot arthritis model, the inflammatory process of the joints just started, and did not lead to overt clinical symptoms.

As we did not observe joint inflammation and swollen paws, in future experiments, mice should be followed for a longer period than 10 weeks to see whether they will develop more signs of disease on a later time point. It is conceivable that the use of IgA autoantibodies induces a different onset and duration of arthritis in mice compared to classical CIA mediated by IgG autoantibodies. This needs to be further investigated to fully establish the in vivo role of neutrophil FcαRI/IgA interactions in the pathogenesis of RA.
Neutrophil activation through IgA autoantibodies and via FcαRI might also play a role in other IgA-autoantibody mediated diseases, such as Linear IgA Bullous Disease (LABD). This is a rare autoimmune skin blistering disease, which is associated with aberrant deposits of IgA autoantibodies directed against collagen XVII, a surface protein of keratinocytes in the epidermis. LABD is furthermore characterized by inflammatory infiltrates, predominantly neutrophils. Considering the high neutrophil influx in the skin of LABD patients and the presence of IgA autoantibodies, I hypothesize that excessive neutrophil activation leads to tissue damage in LABD. Our group previously showed that incubation of normal skin cryosections with neutrophils and serum from LABD patients (containing anti-collagen XVII IgA autoantibodies) induced separation of the dermis and epidermis \textit{ex vivo}, which reflects blister formation. Tissue damage was induced by activated neutrophils that secreted elastase and ROS. Importantly, IgA-induced tissue damage was prevented by blocking FcαRI using an anti-FcαRI antibody, indicating that these events are dependent on the interaction of IgA autoantibodies with neutrophil FcαRI.

In chapter 5, we developed a novel mouse model to investigate the \textit{in vivo} role of neutrophil human FcαRI/IgA interactions in inducing tissue damage in LABD. As human FcαRI is able to bind to human IgA, but poorly to murine IgA, we immunized hIgA knock-in mice with proteins of mouse collagen XVII to generate a hybridoma cell line that produces hIgA antibodies against mouse collagen XVII (mCOL17). Injection of anti-mCOL17 hIgA in the ears of FcαRI/hIgA mice resulted in massive recruitment and accumulation of neutrophils in the ears. Neutrophil migration and accumulation could be prevented using a FcαRI blocking antibody. Moreover, in case of an already existing inflammation, blocking FcαRI decreased accumulation of neutrophils and consequently the induction of chronic inflammation and tissue damage.

These results demonstrate that the presence of IgA-antigen complexes in the skin directly contribute to activation and recruitment of neutrophils into the skin through interaction with FcαRI. Newly recruited neutrophils will then also encounter these IgA-antigen complexes and get activated, which will potentially lead to secretion of the chemoattractant LTB4 resulting in amplification of neutrophil migration \textit{in vivo}. For inflammatory arthritis and allergic skin inflammation, it has been shown that the neutrophil LTB4 receptor BLT1 and neutrophil-derived LTB4 are required to develop disease. Thus based on \textit{in vitro}, \textit{ex vivo} and \textit{in vivo} data we demonstrated that in the onset and progression of LABD, IgA-mediated activation of neutrophils via FcαRI results in neutrophil accumulation and tissue damage (Figure 4). This process might also play a role in the pathogenesis of the skin blistering disease dermatitis herpetiformis (DH). This is the skin manifestation of celiac disease, which is triggered by gluten exposure. DH is characterized by accumulation of neutrophils and the presence of aberrant IgA autoantibodies against tissue transglutaminase (tTG). Anti-tTG antibodies are formed in the gut and can cross-react with epidermal-TG in the skin. Possibly, the deposition of these immune complexes in the skin contribute to activation of neutrophils in DH.
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Figure 4: Schematic model of detrimental effects of IgA autoantibodies in inducing neutrophil activation and tissue damage in Linear IgA Bullous Disease (LABD). IgA autoantibodies directed against collagen XVII bind to the basement membrane of the skin. These IgA-antigen complexes crosslink the FcαRI on neutrophils resulting in cell activation and release of the potent neutrophil chemoattractant leukotriene B4 (LTB4). This way, a neutrophil migration loop is initiated, resulting in tissue damage and blister formation in LABD patients.

The current treatment of neutrophil-mediated chronic inflammatory skin diseases is general suppression of immune responses with corticosteroids and immunosuppressive drugs or dapsone \(^{77-79}\). In most cases, long-term treatment is necessary, making the side-effects of these therapies considerable and a major disadvantage as they are often poorly tolerated \(^{80}\). The side-effects associated with the use of dapsone include hemolytic anemia, methemoglobinemia and agranulocytosis \(^{81,82}\). A more specific treatment for FcαRI/IgA interactions is desirable for IgA-mediated diseases. In this case, blocking the interaction between IgA and neutrophil FcαRI represents a novel therapeutic strategy for these patients. For skin blistering diseases, anti-FcαRI therapy could be administered locally in the skin which would have the benefit to avoid side effects caused by systemic delivery of anti-FcαRI.

Antibodies are not absorbed through the skin because of their big size. For this reason, peptide mimetics, which are small enough to pass the epidermis and which can block neutrophil FcαRI/IgA interactions may be good candidates. A such, we investigated the potential of anti-IgA and FcαRI peptides in blocking IgA-induced neutrophil migration. One linear IgA peptide and one FcαRI peptide effectively reduced neutrophil migration. Furthermore, IgA-induced neutrophil migration was completely blocked in the presence of several peptides in ex vivo skin experiments (M. Heineke et al, in revision). As FcαRI blocking decreased inflammation, peptide mimetics of FcαRI in an ointment represent a novel therapeutic and specific treatment for IgA-induced skin blistering diseases. However, local administration of anti-FcαRI would be more difficult for RA patients, which makes systemic administration necessary.
Collectively, this emphasizes that FcαRI represents a promising new therapeutic target to reduce inflammation and tissue damage in IgA-mediated diseases. A more detailed understanding of activation of FcαRI by IgA gives insight for the development of new therapies.

**IgA and IgG-induced neutrophil activation, two sides of the same coin?**

To better understand the process of FcαRI activation, we investigated the activation of neutrophil FcαRI (IgA) and compared this with Fcγ (IgG) receptor activation in chapter 6. IgA and IgG induced phagocytosis, NETosis and ROS production to a similar extent. However, stimulation of neutrophils with IgA resulted in increased production of TNF-α, MIP-1α, MIP-1β, MCP-1 and IL-8, which are potent cytokines and chemokines that attract and activate other immune cells. Additionally, several arachidonic acid metabolites were measured using metabolomics, since inflammatory lipids can induce cell signaling and we previously measured LTB4 induction after IgA triggering \(^8\). Only IgA potently induced series 2 prostaglandins and leukotrienes. These results suggest that only IgA stimulation of neutrophils leads to the release of proinflammatory lipids, whereas IgG does not. Neutrophils express a higher number of Fcγ receptors compared to FcαRI \(^84,85\), and therefore the number of receptors cannot explain why only IgA induces several inflammatory functions, like enhanced release of cytokines, chemokines, inflammatory lipids and a stronger calcium release compared to triggering of FcγRIIA receptors by IgG. Interestingly, phosphorylation of tyrosines was rapid and short after FcγRII crosslinking, while FcαRI crosslinking led to a stronger and more sustained phosphorylation pattern, which implies a differences in signaling patterns after crosslinking of FcαRI (CD89) or FcγRIIA (CD32A). These findings suggest that although FcαRI and FcγRIIA signal via FcR gamma chain (γ chain) and similar routes, differences exist. FcαRI signals via ITAMs-γ chain complexes, while FcγRIIA signals via a similar, but non-identical intracellular ITAM located in its cytoplasmic tail.

We observed no difference in phagocytosis of IgA- and IgG-coated beads, while only IgA induced cytokine and chemokine release. Studies with B-cell transfectants previously showed that FcγRIIA was not capable of inducing cytokine release upon stimulation with IgG, while swapping its ITAM with the ITAM of FcRγ chain resulted in potent IL-2 production \(^86,87\), suggesting that the latter can only be mediated through FcRγ chain ITAM.

However, IgA stimulation of monocytes also induced cytokine release. According to the literature, only a subset of monocytes was found to express a high number of FcγRIII \(^88\), while all monocytes express FcγRII, FcγRI and FcαRI \(^84,89,90\). Thus next to FcγRIIA, resting monocytes express the activating high-affinity receptor FcγRI, which signals via the FcRγ chain. Although IgG-complexes were taken up as effectively as IgA-complexes, we found a difference in IL-6 and TNF-α cytokine production after stimulation of monocytes. The capacity of IgA to induce a greater cytokine response than IgG seems to be present in both neutrophils and monocytes, hinting towards an intrinsic mechanism of FcαRI to induce more potent cellular responses.

Thus, we have shown that triggering of FcαRI by IgA of both neutrophils and monocytes
results in different cellular responses than FcγRI triggering by IgG. The exact molecular mechanisms underlying the differences between the two receptors are yet to be discovered. It is however conceivable that IgA can play an important role systemically than has been thought of, because of its potent ability to trigger proinflammatory functions.

**FcaRI in anti-tumor immunotherapy**

When we reflect on the potential of neutrophils to induce severe tissue damage after crosslinking of FcaRI by IgA antibodies, it is attractive to consider targeting FcaRI for cancer treatment, aiming to destruct tumor cells by activation by IgA antibodies. In vitro experiments using therapeutic IgA or targeting FcaRI provided promising results. The ability of IgA to induce antibody-mediated tumor cell killing by neutrophils via FcaRI has been demonstrated for multiple tumor-associated antigens in vitro, such as HER2/neu (on breast carcinoma), EpCAM (colon carcinoma), EGFR (epithelial carcinoma and renal cell carcinoma), HLA class II (B cell lymphoma), CD30 (T- and B-cell lymphoma) and carcinoembryonic antigen, which was less pronounced using IgG antibodies. It has been shown that targeting FcaRI, but not FcγR, resulted in neutrophil migration. Destruction of either mamma carcinoma or colon carcinoma colonies in three-dimensional culture systems was observed due to LTB4 release and concomitant neutrophil accumulation after crosslinking of FcaRI. It was furthermore demonstrated that targeting FcaRI on neutrophils induces autophagic tumor cell death and to a lesser extend tumor necrosis. As such, it might be particularly interesting to recruit neutrophils as effector cells into tumors that are more resistant for apoptosis induction or their use for blood cancers.

In vivo evidence on the potential of targeting FcaRI on neutrophils as effector cells to eliminate tumor cells and their use in cancer therapy is still limited. It was shown that IgA2 EGFR anti-tumor mAbs mediated tumor cytotoxicity in vivo in FcaRI transgenic mice by macrophages, which was significantly decreased in the absence of FcaRI. However, the short half-life of IgA was a problem in this study. The major difference with the half-life of IgG made it difficult to compare efficacy of IgG and IgA in vivo, using similar antibody conditions in the tumor environment. To overcome this hurdle, many attempts are currently being made to circumvent the short half-life of IgA in patients and mice. Recently it has been shown that this limitation can be successfully addressed by antibody engineering strategies to prolong the in vivo half-life of IgA. Furthermore, a ‘cross-isotype’ antibody IgAG was described that combines several functions of IgG and IgA. These engineered antibodies bind both FcaRI and FcγRs and increased complement-dependent cytotoxicity of tumor cells, as IgAG can bind C1q (in contrast to IgA).

Additionally, both neutrophils and macrophages efficiently induced antibody-dependent cellular cytotoxicity and antibody-dependent phagocytosis of tumor cells in the presence of IgAG. Similarly, an IgG1/IgA1 tandem antibody format was described that was based on the IgG anti-HER2 mAb trastuzumab. The IgG1/IgA2 tandem antibody augmented myeloid cell-mediated effector functions via FcaRI, but also retained the optimal pharmacokinetics of the parental IgG. Thus, engineering IgA in order to improve half-life may unleash the potent capacity of targeting FcaRI on macrophages and neutrophils.
Conclusion and future perspectives

IgA has been thought of as a non-inflammatory antibody that helps to maintain homeostasis at mucosal surfaces, without initiating proinflammatory functions (Figure 5, homeostasis). This thesis demonstrates that crosslinking of neutrophil FcαRI by IgA complexes induces a variety of processes, such as phagocytosis, production of ROS, the release of cytokines and NET formation. These functions are beneficial processes in case of an infection, as clearance of invading pathogens will occur and disease will be prevented. However, we demonstrated that in the presence of IgA complexes, neutrophils are continuously activated, which becomes harmful (Figure 5, disease). This accumulation of neutrophils will ultimately result in tissue damage. This is clearly the case in patients with IgA-mediated autoimmune skin blistering diseases, but may also hold true for patients with rheumatoid arthritis. FcαRI/IgA interactions might also play an important role in diseases, such as ulcerative colitis and dermatitis herpetiformis. The use of anti-FcαRI therapy to block FcαRI/IgA interactions can break the continuous activation of neutrophils at inflamed sites, hereby alleviating disease.

To summarize, based on the data presented in this thesis, I conclude that anti-FcαRI therapy is a novel and specific approach for IgA-mediated diseases. The present work leads to a better insight in the role of FcαRI and IgA interactions in infection and inflammation. These novel findings and future work, addressing the role of IgA autoantibodies and FcαRI interactions, will lead to the development of novel strategies to limit inflammation. Thus, this might not only be helpful for patients with IgA-mediated disease, but also for cancer patients. On the other hand, this mechanism can be enhanced to target tumors.
Figure 5: Schematic overview of neutrophil activation by IgA complexes at mucosal surfaces, joints and skin (1) In homeostatic conditions dimeric IgA (dIgA) is produced by local plasma cells in the lamina propria. (2) Invading bacteria are opsonized by dIgA. Crosslinking of the IgA Fc receptor FcαRI receptor by these IgA complexes induces neutrophil activation; phagocytosis, neutrophil extracellular release (NETs), degranulation and the release of the chemoattractant leukotriene 4 (LTB4). This initiates a neutrophil migration loop and clearance of pathogens. (3) IgA complexes, present in the synovial fluid of RA patients activate neutrophils via FcαRI and induce the formation of NETs. (4) In IgA skin diseases, neutrophil activation by IgA autoantibodies induce the release of LTB4 resulting in continuous neutrophil migration and ultimately in blister formation. All these processes of excessive neutrophil activation might play an important role in inducing tissue damage at mucosal surfaces, joints and other tissues.
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