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The Role of Fc Gamma Receptors in Experimental Arthritis

BY

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Abstract
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Induction of collagen-induced arthritis (CIA), an animal model for human rheumatoid arthritis, is dependent on anti-collagen type II (CII) antibodies. The effector mechanism by which autoantibodies contribute to inflammatory reactions in autoimmune diseases is not well understood. In this thesis I have studied the effector pathways used by IgG anti-CII antibodies to initiate arthritis, namely the IgG Fc receptors (FcγRs) and the complement system. We have found that FcγRIII is crucial for development of CIA, as CII-immunized mice lacking this receptor do not develop arthritis and IgG1 and IgG2b anti-CII antibodies require FcγRIII to trigger arthritis when transferred to naïve mice. The antibody-mediated arthritis was further enhanced in mice deficient in the inhibitory FcγRIIB, indicating that FcγRIIB regulates the activation of FcγRIII. Furthermore, we demonstrate that FcγRIII exist as three distinct haplotypes in mice, FcγRIII:H, FcγRIII:V and FcγRIII:T. Mice expressing the FcγRIII:H haplotype are more susceptible to CIA than mice expressing the FcγRIII:V haplotype, indicating that certain FcγRIII haplotype predisposes for CIA. We also show that the most likely FcγRIII-expressing effector cell in CIA is the macrophage, since FcγRIII-expressing macrophages exclusively can induce arthritis in FcγRIII-deficient mice challenged for CIA.

The complement system was also investigated in development of CIA. We found that this effector pathway is also necessary for onset of arthritis, as CIA was inhibited by treatment with anti-complement factor 5 (C5) antibodies. C5-deficient mice could neither develop CIA unless provided with C5-containing sera.

Taken together, the work presented in this thesis indicates that FcγRs and the complement system are crucial for the induction of experimental arthritis. These findings are important for understanding the mechanisms behind rheumatoid arthritis and blocking of these effector pathways may in the future be used as treatment of rheumatoid arthritis.

Keywords: Rheumatoid arthritis, Antibodies, Fc receptors, Complement, Transgenic/Knockout mice

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“The concept that antibodies, which should protect against disease, are also responsible for disease, sound at first absurd.”

Clemens von Pirquet, 1906

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals

I Diaz de Ståhl, T., Andrén, M., Martinsson, P., Verbeek, SJ. and Kleinau, S.
Expression of FcγRIII is required for development of collagen-induced arthritis.
Eur. J. Immunol. 2002. 32:2915-2922

II Andrén, M., Xiang, Z., Nilsson, G. and Kleinau, S.
FcγRIII-expressing macrophages are essential for the development of collagen-induced arthritis.
Manuscript

III Nandakumar, K.S*., Andrén, M*., Martinsson, P., Bajtner, E., Hellström, S., Holmdahl, R. and Kleinau, S.
Induction of arthritis by single monoclonal IgG anti-collagen type II antibodies and enhancement of arthritis in mice lacking inhibitory FcγRIIB.
Eur. J. Immunol. 2003. 33:2269-2277

IV Andrén, M., Johanneson, B., Alarcón-Riquelme, M., and Kleinau, S.
IgG Fc receptor polymorphisms and C5 influence susceptibility to collagen-induced arthritis.
Submitted for publication

* K. S. Nandakumar and M. Andrén contributed equally to this work.

Contents

Abbreviations

Ab(s)	Antibody (antibodies)
ADCC	Antibody-dependent cell-mediated cytotoxicity
B cell	B lymphocyte
BCII	Bovine collagen type II
CII	Collagen type II
CCP	Cyclic citrullinated peptide
CFA	Complete Freund's adjuvant
CIA	Collagen-induced arthritis
ELISA	Enzyme-linked immunosorbent assay
FcR	Fc receptor
FcγR	Fc gamma receptor
FcRγ	Common γ-chain associated with FcγRI, FcγRIII and FcεRI
FcRn	Neonatal Fc receptor
IC(s)	Immune complex(es)
IL	Interleukin
i.p.	Intraperitoneally
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibition motif
i.v.	Intravenously
K/B×N	Crossing of a C57BL/6 mouse transgenic for a T cell receptor recognizing a bovine ribonuclease peptide with an autoimmune-prone nonobese diabetic mouse.
LPS	Lipopolysaccharide
mAb(s)	Monoclonal antibody (antibodies)
MAC	Membrane attack complex
MACS	Magnetic activated cell-sorting
MASP	Mannose-binding lectin-associated serine proteases
MBL	Mannan binding lectin
MHC	Major histocompatibility complex
RA	Rheumatoid arthritis
SH2	Src homology 2 domain
SHIP	SH2-containing inositol polyphosphate 5-phosphatase
SLE	Systemic lupus erythematosus
T cell	T lymphocyte
Th	T helper cell
TNFα	Tumor necrosis factor-α

Introduction

Every day our body is exposed to a wide variety of different pathogens, such as bacteria and viruses. To be able to survive this, we need a defense system, the immune system, which comprises a set of specific cells and mediators. The immune system can be divided into two branches; the innate and the adaptive immunity. The innate immunity provides the first line of host defense with macrophages, granulocytes and dendritic cells as central players. This part of the immune system is a rather unspecific system with rapid onset, but without memory. The adaptive immunity, in which B and T lymphocytes play a crucial role, is a second line of defense when the innate immunity is not able to clear the infection. This branch has a delayed onset, but it is highly specific and has memory. The memory enables us to mount a more efficient secondary response if we are exposed to the pathogen a second time.

Considering the damage to the body that can be caused by the immune system it needs to be tightly regulated to be able to distinguish self tissue from non-self. T and B lymphocytes that recognize self-antigens are subjected to selection processes and are usually eliminated by apoptosis. However, if this regulation is broken, the body can start to mount an immune response against its own tissue with self-reactive antibodies as important pathogenic factors. The immune response against self-components is called autoimmunity and diseases involving self-reactive lymphocytes are referred to as autoimmune diseases. In this thesis, I have worked with the underlying mechanisms of autoimmunity by studying the involvement of IgG binding Fc receptors in the development of autoimmune arthritis.

Background

Antibodies

Antibodies (Abs), also known as immunoglobulins, have the capacity to bind to foreign substances and in this way, neutralize or eliminate the antigen. Complexes between antigen and Abs are referred to as immune complexes (ICs). Abs are produced as a membrane-bound form on the cell surface of B cells comprising the B cell receptor, and as a secreted form, in body fluid and in tissues. The Ab is composed of two identical heavy chains and two identical light chains (Fig. 1). Each chain consists of a constant part and a variable part. The variable part is the antigen binding region and differs between Ab clones. The Ab class is determined by the Fc part. Five Ab classes are produced; IgA, IgD, IgE, IgG and IgM. The IgG class can be further divided into different subclasses; IgG1, IgG2a, IgG2b and IgG3 in mice and IgG1, IgG2, IgG3 and IgG4 in humans. The Ab classes have distinct structure, biological activities and distributions in the body. For example, IgG, the main Ab class in sera, is important in protection from bacteria and viruses whereas IgE is more important in protection from parasites.

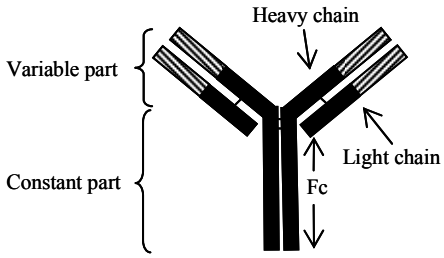

Figure 1. Antibody structure

Abs exert most of their immune functions by linking pathogen with appropriate elimination mechanisms. This includes recognition of the pathogen by the two antigen binding regions and interaction of the Fc region with Fc receptors or components of the complement system on an effector cell.

Fc receptors

Fc receptors (FcRs) recognize the Fc part of the Ab and many of the effector functions of Abs are mediated through interactions with FcRs. FcRs have been described for all classes of Abs; FcαR binds IgA, FcδR binds IgD, FcεR binds IgE, FcγR binds IgG and FcμR binds IgM (reviewed in (1)). Recently, a receptor called Fcα/μR that binds both IgM and IgA was identified (2). FcRs exist primarily as membrane-bound forms but also as soluble molecules in blood. In this thesis I will focus on FcγRs.

Fc receptors for IgG

Three classes of FcγRs have been identified in mice; FcγRI (CD64), FcγRIIB (CD32) and FcγRIII (CD16) (Fig. 2 and Table I). The FcγRs are structurally related and the ligand binding α-chain consists of two (FcγRIIB and FcγRIII) or three (FcγRI) immunoglobulin domains (1). In mice the gene for FcγRI is located on chromosome 3, while those for FcγRIIB and FcγRIII are found close to each other on chromosome 1 (3-7).

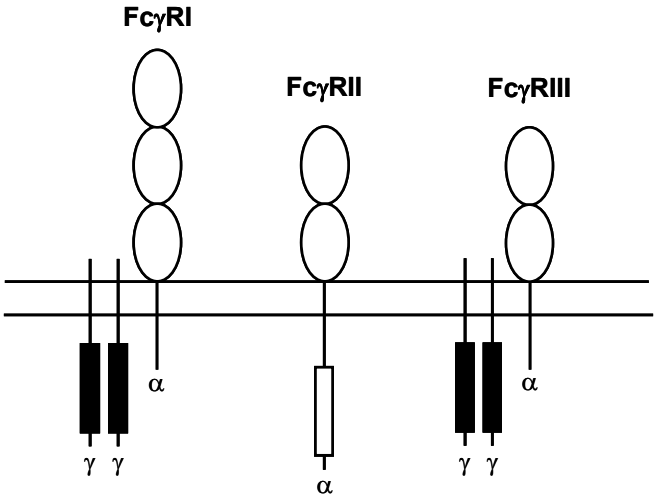

Figure 2. Structure of the murine Fc receptors for IgG

FcγRI is a high affinity receptor that binds monomeric IgG and ICs and is expressed on macrophages, monocytes and dendritic cells (1, 8). The low affinity receptors, FcγRIIB and FcγRIII bind IgG in the form of ICs. FcγRIIB is expressed on a broad range of hematopoietic cells and FcγRIII is expressed on macrophages, mast cells, neutrophils, NK cells and on γδ T cells (1, 9). In addition to the variation in affinity among the receptors, each FcγR binds the IgG subclasses with distinct specificities (Table I).

Table I. Distribution and specificities of murine and human FcγRs (1, 10).

Receptor	IgG subclass specificity	Expression pattern
Mouse		
FcγRI	IgG2a > IgG2b > IgG1	Macrophages, monocytes and dendritic cells
FcγRIIB	IgG2b > IgG1 >> IgG2a	B cells, macrophages, monocytes, mast cells, neutrophils, dendritic cells, Langerhans cells, follicular dendritic cells and early thymocytes
FcγRIII	IgG1 > IgG2a > IgG2b	Macrophages, mast cells, NK cells, neutrophils and γδ T cells.
Human		
FcγRI	IgG3 > IgG1 > IgG4 >>> IgG2	Macrophages, monocytes, neutrophils (i), eosinophils (i), dendritic cells and mast cells (i)
FcγRIIA	IgG3 > IgG1 >>> IgG2, IgG4	Macrophages, monocytes, neutrophils, eosinophils, basophils, platelets, dendritic cells (subsets), Langerhans cells and T cells (subsets)
FcγRIIB	IgG3 > IgG1 > IgG4 >> IgG2	B cells, mast cells, basophils, macrophages, eosinophils, neutrophils, dendritic cells and Langerhans cells
FcγRIIIA	IgG1, IgG3 >>> IgG2, IgG4	Macrophages, monocytes (subsets), NK cells, eosinophils, dendritic cells, Langerhans cells and T cells (subsets)
FcγRIIIB	IgG1, IgG3 >>> IgG2, IgG4	Neutrophils

(i) = inducible

FcγRI and FcγRIII are activating receptors and associated with their ligand-binding α-chain is a signal transducing subunit, the γ-chain (FcRγ), the gene for which is located on chromosome 1 (11, 12). FcRγ contains an immunoreceptor tyrosine-based activation motif (ITAM). The FcRγ is important for efficient assembly and cell-surface expression of FcγRI and FcγRIII but also of FcεRI (13). Cross-linking of two activating receptors results in phosphorylation of ITAM by members of the src kinase family. This leads to recruitment of SH2-containing signaling molecules that bind the phosphorylated ITAM and subsequently activate different kinases, depending on the cell type activated by the FcγR. These events lead to degranulation, phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC), transcription of cytokine genes or release of inflammatory mediators (14, 15).

In addition to FcγRIIIA, one additional FcγRIII exists in humans, called FcγRIIIB. FcγRIIIB contains no signaling component; instead it exhibits a glycosyl-phosphatidyl-inositol linkage in the cytoplasmic membrane and is exclusively expressed on neutrophils (16). Moreover, FcγRIIIA exists as two polymorphic forms in humans; FcγRIIIA-158V and FcγRIIIA-158F (17). The different polymorphic forms have been suggested to alter ligand affinity and receptor-mediated effector functions (18, 19).

FcγRIIB is a single chain inhibitory receptor containing an immunoreceptor tyrosine-based inhibition motif (ITIM) in the ligand-binding α-chain. Murine FcγRIIB exists in four isoforms (B1, B1´, B2 and B3) generated by cell type specific alternative splicing of the transmembrane and cytoplasmic exons (20). B1, B1´ and B2 exist as membrane bound receptors whereas B3 is produced as a soluble molecule by macrophages. FcγRIIB1 is preferentially expressed on B cells whereas FcγRIIB2 is predominantly expressed on monocytes and macrophages (1). FcγRIIB also exists as two polymorphic forms in mice; Ly-17.1 and Ly-17.2 (21). Polymorphism in the FcγRIIB gene has also been shown in humans, affecting receptor-mediated functions (22). Cross-linking of an ITIM-bearing receptor with an ITAM-containing receptor leads to phosphorylation of ITIM, resulting in recruitment of the inhibitory signaling molecule, SHIP, and subsequent abrogation of ITAM-triggered activation and proliferation (14, 23). The inhibitory and the activating FcγRs are thus usually expressed together on the same cell and appear to act in concert, composing a regulatory pair, and are therefore considered critical in regulating the inflammatory response. In addition, homoaggregation of FcγRIIB can induce apoptosis in B cells independently of the ITIM (24).

Humans express one additional form of FcγRII known as FcγRIIA. FcγRIIA is an activating receptor with an ITAM in the ligand-binding α-chain (25). It is a low affinity receptor that is expressed on most inflammatory cells.

In addition to FcγRs, the neonatal FcR (FcRn) binds IgG. This receptor is a heterodimer composed of an α-chain, related to the α-chain of the major histocompatibility complex (MHC) class I molecule, in association with β_2-microglobulin (26, 27). FcRn has been identified as the receptor that transports maternal IgG across neonatal intestine and the fetal yolk sac (28-31). This receptor has also been shown to be expressed in the placenta, the major site for maternal transfer of IgG in humans (32). In addition, FcRn is responsible for maintenance of serum IgG levels by inhibiting degradation of IgG in lysosomes (33, 34). A recent study shows that FcRn is also expressed on human monocytes, macrophages and dendritic cells and has been suggested to be involved in maintenance of IgG levels in peripheral blood (35).

Recently several receptors related to FcγR have been identified in humans on the basis of their Ig-like domain homology with the classical FcR (36, 37). These receptors are preferentially expressed by B cells but their functions remain to be resolved. However, they do express ITAM which indicates a role in cell signaling.

FcγR-deficient mice

The current understanding of the functions of FcγRs has been greatly enhanced by the generation of mice deficient in the different FcγRs. The first FcγR-deficient mice produced were deficient in FcRγ, and therefore lacking FcγRI, FcγRIII and FcεRI (38). Macrophages from these mice are unable to phagocytose IgG-opsonized particles. These mice also exhibit a defective NK cell-mediated ADCC and mast cell-mediated allergic response (38). FcRγ has also been shown to play a major role in IC-driven inflammatory reactions, since FcRγ-deficient mice exhibit an impaired Arthus reaction (39). In the Arthus reaction, antigen is deposited in a desired tissue, followed by systemic administration of the related Ab. This results in an IC-mediated inflammatory reaction within 4-8 hours. The importance of FcRγ in IC-mediated inflammation has also been shown in an animal model for Goodpasture's syndrome, where FcRγ-deficient mice are protected from fatal glomerulonephritis (40). Protection from glomerulonephritis is also seen in a model for systemic lupus erythematosus (SLE) (41). Furthermore, cytotoxic Abs trigger inflammation through FcRγ, since FcRγ-deficient mice are protected from experimental immune hemolytic anemia (42) and experimental immune thrombocytopenia (42). FcRγ is also important for induction of experimental autoimmune encephalomyelitis, a model of multiple sclerosis (43). However, in recent years it has become evident that the FcRγ-deficient mice do express functional FcγRI at about one fifth of the normal level and that FcγRI-mediated endocytosis is functional in these mice (44).

To be able to distinguish between the functions of the two activating receptors, knock-out mice lacking exclusively FcγRI or FcγRIII have been generated (44-46). Mice lacking the ligand-binding α-chain of FcγRIII exhibit impaired NK cell-mediated ADCC and phagocytosis of IgG1-coated particles by macrophages (45). In addition, these mice lack IgG-mediated mast cell degranulation and they show an impaired Arthus reaction. Furthermore, FcγRIII-deficient mice are protected from experimental immune hemolytic anemia in an isotype-dependent manner, since protection is only seen when the disease is induced with IgG1 anti-red blood cells Abs but not with IgG2a Abs (47). Mice deficient exclusively in the high-affinity receptor, FcγRI, show impaired hypersensitivity responses and are highly susceptible to bacterial infections (46). In addition, FcγRI is important for antibody-dependent killing by macrophages and the mice also show an elevated Ab response after immunization with an antigen (44).

In contrast to mice deficient in the activating receptors, targeted disruption of the FcγRIIB gene in mice results in elevated IgG levels in response to thymus-dependent and thymus-independent antigens (48) and also to IgG IC (49). Deficiency in FcγRIIB also contributes to an enhanced IgG-mediated

passive cutaneous anaphylaxis reaction (48). The severity of experimental autoimmune encephalomyelitis in these mice is also increased (43). FcγRIIB-deficient mice, in contrast to wild type mice, have also been shown to develop Goodpasture's syndrome (50). In addition, it has been indicated that FcγRIIB is crucial for maintaining self-tolerance. Thus, depending on the genetic background, FcγRIIB-deficient mice can develop a spontaneous autoimmune disease (51).

The complement system

Abs can also exert their effector functions through interaction with the complement system. The complement system comprises a set of more than 30 serum and cell surface proteins, which eliminate microorganisms and other antigens from tissues and blood. The complement system achieves this alone or in cooperation with Abs and/or cells that express complement receptors. The complement response is a cascade that can be activated either, by the *classical pathway*, the *alternative pathway* or by the *lectin pathway* (reviewed in (52)) (Fig. 3).

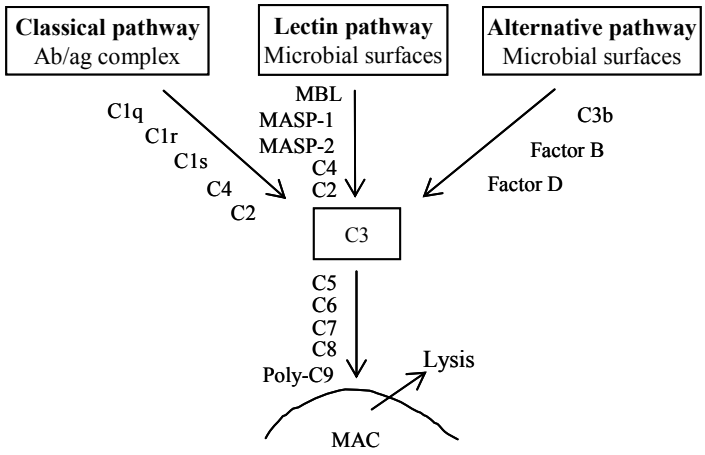

Figure 3. Overview of the complement system

The classical pathway recognizes antigens through bound IgM or IgG Abs, while the alternative pathway and the lectin pathway are triggered by direct recognition of certain microbial surface structures, in the absence of Abs. The first part of the classical pathway consists of the proteins C1 – C4, catalytic enzymes that can activate C3 to C3b. C3b can bind to the existing C3 convertase and alter its specificity to C5 instead, by creating a C5 convertase. This C5 convertase cleaves C5 to C5a and C5b. Finally the late steps in complement activation are initiated and C6 through C9 bind sequentially to

C5b, forming the membrane attack complex (MAC), which causes lysis of the antigen.

Studies of complement-deficient mice have been performed to elucidate the role of these components in the immune response. It has been shown that C5a receptor (C5aR)-deficient mice demonstrate a highly reduced Arthus reaction in skin, lungs and peritoneum (53). C5a is an anaphylatoxin that causes increased vascular permeability and promotes migration and activation of leukocytes (54). An impaired Arthus reaction is also seen when the interaction between C5a and C5aR is blocked by a C5aR antagonist (55). In contrast, the Arthus reaction is not inhibited in mice lacking C3 or C4 (56). This indicates that C5aR plays a major role in IC-mediated inflammation.

Autoimmune diseases

Most of the time the immune system works as it is supposed to, but sometimes things go wrong and the body starts to mount an immune response against its own tissues. The immune response against self-components is called autoimmunity. Normally the mechanism of self-tolerance protects an individual from potentially self-reactive lymphocytes, but if this protection is broken, cell-mediated and humoral immune responses can be generated against self-antigens (57). When self-reactive Abs bind to self-antigens they can cause serious damage to cells or organs. The autoimmune diseases (reviewed in (58)) are poorly understood disorders, but both genetics and environmental factors are believed to contribute. Clinically, autoimmune diseases are divided into systemic or organ-specific diseases. Insulin-dependent diabetes mellitus and myasthenia gravis are examples of organ-specific diseases, whereas rheumatoid arthritis and SLE are systemic diseases. In these latter diseases, Abs, in the form of ICs, have been shown to be important in triggering inflammation. The complement system and FcγRs are two pathways that are believed to contribute to IC-driven inflammation.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a common autoimmune disease that affects up to 1 % of the population worldwide and has an unknown etiology (reviewed in (59, 60)). It is a disease with a clear gender bias; women are affected 2.5 times more often than men. The disease can occur at any age, but it is most common among those aged 40 – 70 years and the incidence increases with age. Early clinical indications of RA are swelling, pain and stiffness in finger and toe joints, but other onset symptoms including weakness, malaise, fatigue and fever can also be observed (59). RA is a systemic disease, meaning that parts of the body other than the joints, such as the skin and the heart, can

become affected. The diagnosis of RA is based on seven criteria (Table II) (61), of which four need to be fulfilled.

Table II. Classification of RA according to the 1987 revised criteria defined by the American Rheumatism Association (61).

Criteria	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour
2. Arthritis of 3 or more joints	At lest 3 joint areas simultaneously have had soft tissue swelling or fluid observed by a physician
3. Arthritis of hand joints	At least 1 area swollen in a wrist, MCP or PIP joint
4. Symmetric arthritis	Simultaneous involvement of the same joint area on both sides of the body
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxta-articular regions, observed by a physician.
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor
7. Radiographic changes	Radiographic changes typical of RA, which must include erosions or unequivocal bony decalcification

MCP = metacarpophalangeal joints, PIP = proximal interphalangeal joints

Histologially RA is characterized by proliferation of the synovial membrane, with infiltration of inflammatory cells from the blood. The lining layer of the synovial membrane is increased from 1 – 2 cells to 6 – 8 cells thick and is composed mostly of activated macrophages (type A synoviocytes) with an underlying layer of fibroblast-like cells (type B synoviocytes). The inflammatory cells that are most abundant in the inflamed synovial membrane are macrophages, T cells and plasma cells. The junction between the inflamed synovial membrane and the cartilage and bone is the major site of damage in RA. This region is called the pannus, a tissue very characteristic of RA. The pannus is rich in macrophages and these cells migrate over the underlying cartilage and bone causing erosion of these tissues.

The disease is also accompanied by an abnormal production of autoantibodies (reviewed in (62)). Most of these Abs are not specific for RA because they also occur in other inflammatory conditions (RA-associated Abs), whereas other Abs appear to be more specific and are present almost exclusively in RA patients (RA-specific Abs). The most well-known autoantibodies in RA are reactive with the Fc part of IgG and are known as rheumatoid factors (reviewed in (63)). These rheumatoid factors are present in approximately 70 % of RA patients and are used as a diagnostic factor. However, they are also found in patients with other autoimmune diseases. Other RA-associated Abs include those that are reactive with type II collagen (64). In

recent years it has become evident that Abs directed against cyclic citrullinated peptides (CCP) are highly specific for RA and these Abs are present in about 80 % of patients (65). In addition, it has been shown that the presence of anti-CCP Abs can predict RA several years before disease onset (66). Other specific Abs in RA patients include those against glucose-6-phosphate isomerase (67).

Furthermore, genetic factors have been shown to play a role in susceptibility to RA and the disease has been linked to genes in the MHC region. The majority of RA patients express HLA-DR4, HLA-DR1 or HLA-DR10 allels (68). These genes are referred to as the shared epitope genes and they share a highly conserved sequence of amino acids in the third hypervariable region of their β1 chains of the MHC molecule (69). Studies have shown that presence of anti-CCP Abs together with shared epitope genes is associated with a very high risk of future development of RA (70).

Animal models of rheumatoid arthritis

Animal models of autoimmune diseases have been developed for a number of human disorders in the hope that the study of these models will lead to the development of effective and safe therapies. In most cases, these experimental diseases are induced by immunization with an antigen suspected to play a role in the analogous human disease. Several animal models are used to study RA. All of these models closely mimic the human disease. The most widely used model for RA is collagen-induced arthritis (CIA), which will be discussed more thoroughly in the next section. Other mouse models for RA include pristine-induced arthritis (71) and proteoglycan-induced arthritis (72) where arthritis is induced using the immunological adjuvant, pristane and cartilage proteoglycan respectively. One recently described model is the spontaneously triggered arthritis seen in K/B×N mice (73). This model is generated by crossing a C57BL/6 mouse transgenic for a T cell receptor recognizing bovine ribonuclease peptide with an autoimmune-prone nonobese diabetic mouse. The offspring develop a spontaneous arthritis starting at 3 to 4 weeks of age. The disease can be passively transferred to healthy mice with serum from arthritic mice (74), indicating the important role of Abs in this model. Glucose-6-phosphate isomerase has been demonstrated to be the autoantigen responsible for the disease (75). Other arthritis models frequently used to study RA include the antigen-induced arthritis model, induced with methylated bovine serum albumin in complete Freund's adjuvant (CFA) (76) and the IC-mediated arthritis model, induced by lysozyme-antilysozyme IC (77). Many of the new treatments, such as cytokine inhibitors, developed for RA in recent years, have merged from studies in these animal models.

Collagen-induced arthritis

CIA was first described in 1977 by Trentham and coworkers. They observed an inflammatory arthritis affecting the peripheral joints in rats after a single intradermal injection of type II collagen (CII), a major component of joint cartilage, emulsified in CFA (78). The model was later also induced in mice (79) and in primates (80, 81). CIA can be triggered using native homologous or heterologous CII and is specific to CII, since arthritis can not be induced with type I or type III collagen (78, 79). In rats, CII, in combination with either CFA or incomplete Freund's adjuvant, can trigger arthritis (78), while in mice, CFA, which contains *Mycobacterium tuberculosis,* is needed (79). Immunization of mice with CII/CFA results in a polyarthritis with the first clinical symptoms, redness and swelling of the paws, visible after 3 – 4 weeks.

The histolopathology of joints with CIA resemble the abnormalities observed in RA patients, such as synovial hyperplasia, mononuclear cell infiltration, pannus formation and cartilage and bone destruction (82).

Like RA, CIA is linked to MHC class II genes and mouse strains with haplotype H-2^q or H-2^r are susceptible to disease (82, 83). The involvement of T cells has been implied by the fact that only mice with certain MHC class II molecules develop disease. The observation that administration of anti-CD4 Abs can suppress CIA further indicates the involvement of T cells (84). However, transferring CIA with $CD4^+$ T cells has proven to be difficult (85). Studies have also investigated the contribution of the different $CD4^+$ subpopulations of T cells, T helper type 1 (Th1) cells and T helper type 2 (Th2) cells, for development of CIA (reviewed in (86, 87)). CIA has always being regarded as a Th1-related disease. However, it has been shown that Th1 cells, producing mainly INFγ and IL-2, are important at the beginning of the disease, whereas Th2 cells, producing mainly IL-4, are involved in the later stages (88). Furthermore, onset of CIA is characterized by high titers of anti-CII Abs (82, 89). The anti-CII Abs cross-react with autologous mouse CII, resulting in an autoimmune reaction. In contrast to transfer of CII-reactive T cells, passively transferred IgG anti-CII Abs induce a severe arthritis in naive mice within a few days of transfer (90-92). This indicates the more important role for B cells and Abs over T cells in CIA. The importance of B cells has further been proven by the fact that B cell-deficient mice are protected from the disease (93). Although T cells play a role in the autoimmune response in CIA, B cells and autoantibodies against CII appear to be the primary effector mechanisms in this model.

Moreover, tumor necrosis factor α (TNFα) and interleukin (IL)-1 are proinflammatory cytokines shown to be crucial in the regulation of joint in-

flammation and tissue destruction in CIA (94, 95). TNFα has been shown to accelerate CIA in rats when administrated in the joint (96, 97) and in contrast, the disease is inhibited when mice are treated with anti-TNFα Abs (98, 99). Transgenic mice, expressing human TNFα, can spontaneously develop arthritis (100). Like TNFα, IL-1 can also accelerate CIA when administrated in the joints (101). Moreover, the development of CIA is inhibited when mice are treated with anti-IL-1 Abs (102) or an IL-1 antagonist (103). IL-6 and IL-12 are also important in CIA, since mice lacking either of these cytokines are protected from disease (104, 105). However, IL-12 may have a dual role in CIA, since early administration of IL-12 enhances disease whereas chronic administration may be anti-inflammatory (106).

FcγR in autoimmune arthritis

In the past ten years, several studies have tried to elucidate the impact of FcγRs on IgG-mediated inflammation and an important role for these receptors has been established (Table III).

Table III. Contribution of FcγR to development of different arthritis models.

Arthritis model	FcRγ	FcγRI	FcγRIIB	FcγRIII	References
Collagen-induced arthritis	++		-		(107, 108)
K/B×N serum arthritis	++	0	0/-	+	(109, 110)
Antigen-induced arthritis	+	+	-	0	(46, 111, 112)
IC-mediated arthritis	++	+	-	++	(113, 114)
Proteoglycan-induced arthritis	++		-		(115)

++ = crucial for induction of disease, + = contribute to disease induction, - = important for suppression of the disease, 0 = do not play a part in the disease.

Our group has previously shown that mice deficient in FcRγ are almost completely protected from CIA, even though they produce the same amount of potentially arthritogenic anti-CII Abs as wild type littermates (107). FcRγ has also been shown to be crucial in other models of arthritis. In experimental antigen-induced arthritis, it has been shown that joint swelling and cartilage erosion are significantly reduced in FcRγ-deficient mice (111). FcRγ has also been shown to play an important role in IC-mediated arthritis, where it was shown that inflammation and cartilage destruction were completely absent in the FcRγ-deficient mice (113). In the K/B×N model of arthritis, no clinical signs of arthritis were observed in the FcRγ-deficient mice, indicating the importance of FcRγ also in this model (109). In addition, FcRγ has been demonstrated to be important in proteoglycan-induced arthritis (115) and in antibody-induced arthritis (116).

To further elucidate the role of activating FcγRs in arthritis, studies have been performed to investigate the roles of FcγRI and FcγRIII, exclusively in arthritis. In IC-mediated arthritis it has been shown that influx and activation of inflammatory cells, as well as cartilage destruction, were decreased in FcγRIII-deficient mice (114). In the K/B×N model, FcγRIII-deficient mice could develop arthritis when they received K/B×N sera, but to a significantly lesser degree than the wild type controls (109, 110). In contrast, FcγRI-deficient mice developed arthritis to the same extent as wild type controls (109). However, FcγRI has been shown to be involved in the development of severe cartilage destruction in antigen-induced arthritis and in IC-mediated arthritis (112, 114).

In addition to the involvement of the activating FcγRs in arthritis, the contribution of the inhibitory FcγRIIB has also been extensively studied. Mice with the arthritis-resistant H-2^b background can develop CIA when they have a deletion of the FcγRIIB gene (108). FcγRIIB-deficient mice also display an augmented CIA with an enhanced anti-CII Ab production (107). Likewise, in IC-mediated arthritis and antigen-induced arthritis, enhanced joint inflammation and severe cartilage destruction were seen in FcγRIIB-deficient mice compared to wild type mice (112, 114). An enhanced disease was also observed in proteoglycan-induced arthritis (115). In the K/B×N model, different observations have been made. One group claims that there is no difference between FcγRIIB-deficient and wild type mice in arthritis development (109) while another group reports that enhanced arthritis is seen in FcγRIIB-deficient mice (110).

Altogether, these data strengthen the view that activating FcγRs is necessary for triggering arthritis and that the inhibitory FcγRIIB down-regulates joint inflammation.

Furthermore, the expression of FcγRIIB and FcγRIII on synovial macrophages and peritoneal macrophages has been analyzed in arthritis-susceptible DBA/1 mice compared to less susceptible C57BL/6 mice. The results show that synovial macrophages and peritoneal macrophages from the arthritis-susceptible strain express significantly higher levels of FcγRIIB and FcγRIII then the less susceptible strain (113). In another study it has also been shown that the balance between inhibitory and activating FcγRs is skewed towards activating FcγRs in arthritis-susceptible DBA/1 mice compared to arthritis-resistant BALB/c and C57BL/6 mice (117). This suggests that a higher expression of FcγRIII may lead to increased activation and hence enhanced inflammation in DBA/1 mice.

The processes by which IC localize to the joints during inflammation are not completely clear but in a recent report it was demonstrated that FcγRIII are essential for this process during development of K/B×N arthritis, since mice deficient for FcγRIII exhibited a complete block in localization of ICs to the joints (118). This further strengthens the view that FcγRs are crucial for arthritis induction.

In humans, FcγRs have also been shown to be associated with autoimmune disease, since a polymorphism of FcγRIIIA has been linked to RA (119, 120) and SLE (19, 121). This polymorphism is a single nucleotide substitution resulting in an amino acid substitution, valine (V) to phenylalanine (F) at position 158 (17). The FcγRIIIA-158F allotype binds less IgG1, IgG3 and IgG4 than the FcγRIIIA-158V allotype (18, 19). In humans, the polymorphisms of the promoter region and the transmembrane region of FcγRIIB have been associated with SLE (122, 123). In mice, it has been shown that mouse strains that spontaneously develop autoimmune disease usually bear the Ly-17.1 allele of FcγRIIB (21). One additional polymorphism has been described in the transcription regulatory region of the FcγRIIB gene in several autoimmune mouse strains, but not in non-autoimmune disease-prone strains (124, 125).

The complement system in autoimmune arthritis

Many studies have focused on the role of the complement system in arthritis. The aim of these studies has been to reveal which activating pathway of the complement system is most important for arthritis development. Recently, it was indicated that the alternative pathway is crucial for K/B×N serum-induced arthritis (109). Mice lacking Factor B were almost resistant to the disease, whereas mice deficient in components of the classical pathway (lacking C1q or C4) or the lectin pathway (lacking MBL-A) developed arthritis similarly to wild type mice. Factor B has also been shown to play a role in development of CIA, since Factor B-deficient mice were completely protected from the disease (126). In this study, they also show that the Factor B-deficient mice exhibited an impaired humoral response to CII, which could be one reason for the resistance to CIA seen in these mice. Downstream components of the complement cascade have also been shown to be important. C3 has been shown to play a part in arthritis development both in CIA and in the K/B×N model (109, 126). In both these studies, C3-deficient mice could develop arthritis but to a lesser extent than wild type mice. In addition, C5 are important for arthritis development, since C5-deficient mice are protected from development of both K/B×N-arthritis (109) and CIA (127). The importance of C5 has further been shown by the inhibition of

CIA in mice treated with anti-C5 monoclonal Abs (128). Furthermore, C5aR has been shown to be crucial for arthritis development in K/B×N and antibody-induced arthritis (109, 129).

Since both FcγRs and the complement system appear necessary for the development of IC-driven inflammation, it has been proposed that FcγRs and the complement system may act in synergy in IC-triggered inflammation. In fact, this has been indicated in an IgG-mediated Arthus reaction in the skin and lung of FcRγ-chain deficient mice treated with a C5aR antagonist (130). It was found that FcRγ- and C5aR-mediated pathways were both necessary and only together were able to trigger full IC-mediated inflammation. Similarly, it was shown in an antibody-dependent model of autoimmune vitiligo, that only mice lacking both C3 and FcRγ were protected to the disease, whereas if one of the factors was present, the mice developed autoimmune vitiligo (131). It has also been reported that C5a is an early regulator of the expression of FcγR. Interaction of C5a with C5aR increases the expression of FcγRIII and decreases the expression of the inhibitory FcγRIIB (132, 133).

Present investigation

Aims

The involvement of FcγRs in the immune response has been extensively studied. However, the mechanism by which FcγRs are involved in the induction of IgG-mediated inflammation is not fully resolved.
In this thesis I have tried to answer the following questions regarding the role of FcγRs in experimental arthritis:

- Is expression of FcγRIII necessary for development of CIA?
- Which FcγRIII expressing cell is responsible for induction of CIA?
- Can arthritis be induced with single monoclonal anti-CII Abs and what impact do FcγRs and the different IgG subclasses have on disease induction?
- Do IgG Fc receptor polymorphism and C5 play a role in the development of CIA?

Experimental model

Mice

Mice deficient in FcRγ (38) (paper III), FcγRIIB (48) (papers II and III) and FcγRIII (45) (papers I, II, III and IV) were used. Since the mice were generated on an arthritis-resistant background, they were backcrossed for 5, 10 or 12 generations to the arthritis-susceptible DBA/1 background. As control animals to the deficient mice on the 5th generation, the corresponding wild type littermates were used. Mice backcrossed for 10 or 12 generations were compared with wild type DBA/1 mice.
To generate mice with the FcγRIII gene from DBA/1 mice or from SWR mice (paper IV), SWR mice were crossed with FcγRIII-deficient DBA/1 mice (Fig. 4). The offspring were intercrossed to generate FcγRIII-deficient SWR mice, which were crossed with either wild type DBA/1 or SWR mice. Finally, the offspring were intercrossed to generate homozygous mice with

the FcγRIII gene from either the DBA/1 mouse (F4.D +/+) or from the SWR mouse (F4.S +/+). Littermate controls lacking FcγRIII (F4.D -/- and F4.S -/-) were also generated.

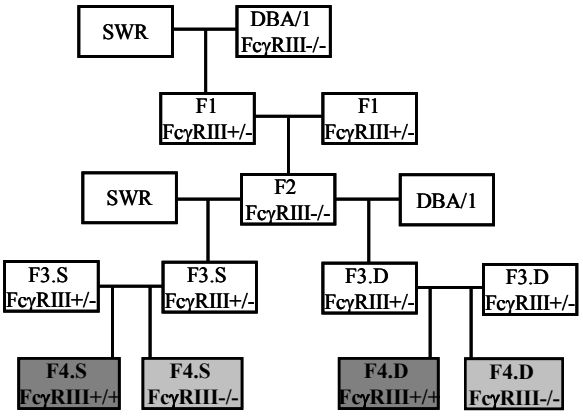

Figure 4. Breeding to generate mice with FcγRIII from the DBA/1 mouse (F4.D +/+) or from the SWR mouse (F4.S +/+).

Collagen-induced arthritis

Native CII was prepared from bovine nasal cartilage by pepsin digestion, as described previously (134), dissolved in 0.01 M HAc and emulsified 1:1 with CFA. Mice were injected intradermally at the base of the tail with 50 μg bovine CII (BCII) and observed three times a week for arthritis development starting 21 days after immunization (Fig. 5). The day of arthritis onset was recorded. The severity of arthritis was quantified according to a graded scale from 0 to 3 as follows: 0, normal; 1, swelling in one joint; 2, swelling in more than one but not in all joints; and 3, severe swelling of the entire paw and/or ankylosis. Each paw was graded and each mouse could achieve a maximum score of 12.

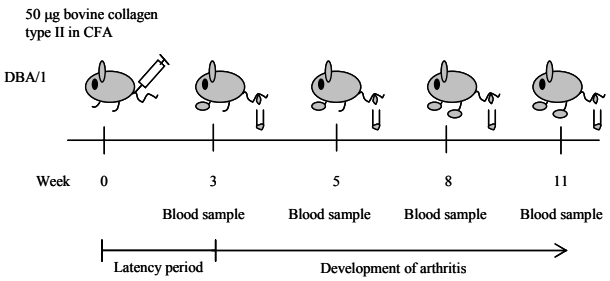

Figure 5. Collagen-induced arthritis

Enzyme-linked immunosorbent assay

BCII-specific ELISA

Blood samples were collected from the tail vein on several occasions after the BCII immunization. Sera were incubated on microtiter plates coated with native BCII in PBS. Specifically bound IgG was detected with sheep-anti mouse IgG conjugated to alkaline phosphatase, and visualized using p-nitrophenyl phosphate substrate. Polyclonal anti-BCII antibodies of known concentration, affinity purified from hyperimmunized mice, were used as standard on each microtiter plate.

For subclass-specific BCII ELISA, a modification of the protocol described above was used. After incubation of the samples, plates were washed and incubated with biotinylated rat anti-mouse IgG1, IgG2a, IgG2b or IgG3. Bound Abs were detected with streptavidin conjugated with alkaline phosphatase and visualized using p-nitrophenyl phosphate substrate.

C5-specific ELISA

Blood samples were collected from the tail vein on several occasions after the BCII immunization. Sera were incubated on microtiter plates coated with C5 in PBS. After washing, plates were incubated with rabbit anti-C5. Bound antibodies were then detected using goat anti-rabbit IgG conjugated with alkaline phosphatase and visualized using p-nitrophenyl phosphate substrate.

Spleen proliferation assay

Spleen cells were isolated from FcγRIII +/+ and FcγRIII -/- mice 12 days after BCII/CFA immunization. The spleen cells were plated in 96-well microtiter plates and stimulated with BCII. The cells were incubated for 3 days and for the last 18 hours [^{3}H]thymidine was added to the cultures. [^{3}H]thymidine incorporation was measured using a β-scintillation counter.

Immunohistochemistry

The synovial membrane, together with the patella and surrounding connective tissue, were dissected from the knee joints of FcγRIIB -/- mice. Specimens were frozen and cryostat sections were cut. The sections were fixed and incubated with hydrogen peroxidase to block endogenous peroxidase activity. The presence of FcγRIII was detected using the monoclonal antibody 2.4G2 (rat IgG2b anti-mouse FcγRII/FcγRIII). Bound Abs were detected with biotinylated rabbit anti-rat IgG Ab and visualized using avidin-biotin-peroxidase complex and subsequently counterstained with Mayer's hematoxylin.

Histology

Paws were fixed in formaldehyde phosphate buffer, decalcified in Parengy decalcifying solution and embedded in paraffin. Sagital sections were cut and stained with hematoxylin and eosin.

Cell transfers

Transfer of spleenocytes and bone marrow cells

Single cell suspensions were prepared from donor spleens in PBS by pressing tissue through a mesh. Bone marrow cells were harvested in PBS from the femur of donor mice. Recipient mice (FcγRIII-deficient DBA/1 mice) were irradiated with 600 rad and 18×10^6 spleenocytes or 12×10^6 bone marrow cells in 250 µl of PBS were injected i.p. 24 h after irradiation. The recipient mice were immunized with BCII/CFA 3 days (spleenocytes) or 6 weeks (bone marrow cells) after transfer.

Transfer of in vitro cultured mast cells and monocytes

Bone marrow-derived mast cells (BMDMC) and bone marrow-derived monocytes (BMDM) were obtained by culturing bone marrow cells from DBA/1 mice *in vitro* with cell-specific growth factors (IL-3 for mast cells and M-CSF for monocytes) for 4 weeks or 8 days, respectively. The cells were then suspended in PBS and 6.4 - 10×10^6 BMDMC or BMDM were injected i.v. or i.p. into FcγRIII-deficient mice. Seven or 10 weeks after cell transfer, the recipient mice were immunized with BCII/CFA.

Transfer of macrophages

Peritoneal cells were harvested by washing the peritoneal cavity of donor mice with chilled HBSS, supplemented with heparin. The cells were centrifuged and washed with PBS and part of the cell suspension was further enriched for macrophages with magnetic activated cell sorting (MACS). Peritoneal cells were stained with PE-conjugated anti-CD11b (Mac-1) and washed with PBS supplemented with BSA and EDTA. The cells were subsequently incubated with magnetic beads directed against PE and then placed on a MACS column. The CD11b-positive fraction was retained in the column and eluted by releasing the magnet. The purified CD11b-positive cells were collected and resuspended in PBS and 1×10^6 cells were injected i.v. into FcγRIII-deficient mice. Likewise, $1 - 10 \times 10^6$ crude FcγRIII$^+$ or FcγRIII$^-$ peritoneal cells suspended in PBS were injected into FcγRIII-deficient mice. Three weeks after cell transfer, the recipient mice were immunized with BCII/CFA.

RNAse protection assay

Total RNA from peritoneal cells of normal or BCII/CFA-immunized DBA/1 mice was isolated. RNAse protection assay was performed using the mouse cytokine multi-probe template sets mCK-2 and mCK-3b from the RiboQuant system, according to the manufacture's instructions. Quantification of cytokine mRNA expression was determined using a phosphoimaging device and the levels of each gene transcript were quantified using Image Gauge V3.45.

Antibody-induced arthritis

In paper III, three monoclonal Abs, recognizing the same CII epitope (J1), were investigated; M284 (IgG1), M287 (IgG2a) and M2139 (IgG2b). In addition, CIIC1 (IgG2a), which recognizes a different epitope ($C1^1$) on the CII molecule, was used. Isotype-matched Abs recognizing unrelated antigens were used as controls. The purified monoclonal Abs were prepared in PBS and injected intraperitoneally (i.p.) or intravenously (i.v.) in a total volume of 150 – 250 µl. The Ab dose (3.6 – 9.0 mg) was administered in two injections on two consecutive days.

In paper IV, a cocktail of monoclonal anti-CII Abs was used. A total of 7.5 mg Ab per mouse were injected i.v. in a volume of 230 µl PBS on two consecutive days. Control mice received the same volume of vehicle (PBS). Five days after initial Ab transfer, 50 µg of LPS in PBS was given to each mouse, including controls.

The mice were inspected daily for arthritis development over 21 days. Grading of arthritis severity i.e. arthritic score was done according to the same protocol as in actively induced CIA.

Sequencing

Total RNA was isolated from the mouse strains DBA/1, SWR, BALB/c, CBA, NOD, C57BL/6, C57BL/10, NZW, NZB, MRL/*lpr*, BXSB and from the F4.D +/+ and F4.S +/+ mice. cDNA was synthesized and amplification of FcγRIII cDNA was done with the forward primer 5´TCTCCTGAACCTCATCAGAC3´ and the reverse primer 5´AAGTCGTTGTGATTGAAGAA3´. The complete coding region of FcγRIII was sequenced on an ABI 3700 DNA Analyzer and the data were collected using Sequencher^{TM}4.1.

Results and discussion

Paper I. Expression of FcγRIII is required for development of collagen-induced arthritis.

We have previously shown that activating FcγRs are crucial for induction of arthritis since mice lacking FcRγ-chain are protected from CIA (107). However, the relative contribution of the different activating FcγRs has not been identified. In this study, we have used mice exclusively deficient in FcγRIII to elucidate the role of this receptor in CIA. We also investigated the expression of FcγRIII in the synovium of normal mice.

FcγRIII-deficient mice (FcγRIII -/-) and littermate controls (FcγRIII +/+), both on a DBA/1 background, were immunized with BCII/CFA and inspected for arthritis development. It was shown that the incidence and severity of CIA were considerably reduced in mice deficient in FcγRIII. Only 15 – 20 % of the FcγRIII -/- mice developed disease in contrast to an incidence of about 80 % in FcγRIII +/+ mice. In addition, the few FcγRIII -/- mice that did show signs of arthritis, developed only mild disease which did not progress. The Ab response to BCII was measured in the mice by BCII-specific ELISA and the results showed that deletion of FcγRIII does not influence the humoral response to BCII. FcγRIII -/- mice developed similar amounts of IgG anti-BCII Abs as FcγRIII +/+ mice. The cellular response, measured in a spleen proliferation assay to BCII, was also similar in both groups, indicating that an impaired T cell response in the FcγRIII -/- mice could not be the reason for protection from arthritis in these mice. Histopathology of the joints of FcγRIII +/+ mice showed synovial hyperplasia, infiltration of mononuclear cells and severe cartilage and bone erosion in contrast to joints of the few arthritic FcγRIII -/- mice, which displayed only mild synovial hyperplasia and synovial villie formation. FcγRIII -/- mice without any clinical signs of arthritis did not show any histopathological changes.

Since the synovium is the target for inflammatory reactions in RA we postulated that this tissue might express FcγRIII. To test this hypothesis we stained cryostat sections of normal mouse joints (FcγRIIB-deficient) with a FcγRII/III specific Ab. Strikingly, FcγRIII positive cells were observed in the synovial lining layer as well as in the subsynovial lining layer, whereas no staining could be observed with an isotype matched control Ab.

Considering that the arthritis pattern in mice lacking FcγRIII is similar to the one observed in mice deficient in FcRγ (both strains are equally protected against CIA), it is likely that the dominant activating receptor in CIA is FcγRIII and that other FcRγ-containing FcRs play minor roles. The fact that

mice lacking FcγRIIB, the other partner of the regulatory pair, consisting of FcγRIIB and FcγRIII, exhibit an enhanced disease (107) further increases the likelihood of a role for FcγRIII in this disease.

The observation that FcγRIII is expressed on synovial cells may imply that IC present in the joints trigger inflammation via binding to FcγRIII, which activates and recruits macrophages, granulocytes and mast cells to the joints. Since these cells also express FcγRIII, the inflammatory response may be amplified leading to the destruction of joints. Thus, agents that can block the interaction of IC with FcγRIII and inhibit the effector cell responses would certainly be attractive candidates for immunotherapy of RA.

Paper II. FcγRIII-expressing macrophages are essential for the development of collagen-induced arthritis.

In previous work we have demonstrated that FcγRIII is crucial for the induction of CIA (paper I) (109, 114). However, FcγRIII is expressed on several different cell populations including macrophages, mast cells, neutrophils and NK cells, and the relative contribution of these different cell types for arthritis development is not known. In this paper we have investigated which FcγRIII-positive (FcγRIII$^+$) cell population is necessary for induction of CIA, by transferring different FcγRIII$^+$ cell populations to FcγRIII-deficient mice and studying the induction of CIA in these mice.

In a first attempt to induce arthritis, we transferred FcγRIII$^+$ spleenocytes to FcγRIII-deficient mice and investigated the development of CIA in the recipient mice. Even though the mice developed anti-CII antibodies to substantial levels, only one out of 11 mice developed arthritis, which was very mild and restricted to the digits. Because of the expression of FcγRIII on synovial cells (paper I) we wanted to investigate the ability of bone marrow cells to induce arthritis, since they may include mesenchymal stem cells. However, this cell population was also unable to induce arthritis in FcγRIII-deficient mice, as none of the recipient mice developed CIA. Furthermore, mast cells have been shown to be crucial for the induction of the K/B×N model of arthritis (135), but the specific contribution of mast cells in CIA is not known. To study the involvement of these cells in CIA we transferred bone marrow derived mast cells to FcγRIII-deficient mice and studied the development of CIA. However, this cell population was also unable to induce CIA. We then transferred FcγRIII$^+$ mast cells from FcγRIIB-deficient mice to FcγRIII-deficient mice since FcγRIIB-deficient mast cells have shown enhanced sensitivity to IgG-mediated degranulation (136). However, no arthritis was induced when these cells were used. Further, the transfer of FcγRIII$^+$ bone marrow derived monocytes to FcγRIII-deficient mice failed to trigger CIA.

As the transfer of the different FcγRIII$^+$ cells has so far not been successful in inducing arthritis, we next isolated peritoneal cells containing FcγRIII$^+$ macrophages and transferred them to FcγRIII-deficient mice. The results show that when 1-10 × 10^6 peritoneal cells were injected, 50 % of the mice developed a severe progressive disease, with marked swelling and erythema in both front and hind paws. As controls, FcγRIII$^-$ crude peritoneal cells were transferred to FcγRIII-deficient mice. In this group only one out of six mice developed a very mild disease affecting the digits. The naïve peritoneum consists mainly of macrophages, but B cells and a small population of mast cells are also present. To distinguish which FcγRIII$^+$ cell population in the peritoneum is responsible for arthritis induction, we purified CD11b$^+$ cells from the peritoneum with MACS. CD11b is mainly expressed on macrophages and 1 × 10^6 of these cells were injected into FcγRIII-deficient mice. The recipient mice were immunized with BCII/CFA and studied for arthritis development. Indeed, these cells could induce arthritis in the FcγRIII-deficient mice, as 50 % of the mice developed a progressive disease.

Since the peritoneal cells were arthritogenic in the FcγRIII-deficient mice, we wanted to determine the cytokine profile in these cells. Using RNAse protection assay we investigated the expression of several different cytokines in cells from normal and arthritic mice. The results show that cells from DBA/1 mice with CIA exhibited a significant up-regulation of TNFα and IL-12p35 mRNA compared to peritoneal cells from normal DBA/1 mice.

In this study, we transferred several different FcγRIII$^+$ cell populations to FcγRIII-deficient mice in order to find the FcγRIII-expressing effector cell in CIA and we show that the only cell population capable of triggering arthritis are peritoneal cells. Both crude peritoneal cells and CD11b$^+$ peritoneal cells were capable of inducing arthritis, indicating that CD11b$^+$ cells are the most likely FcγRIII-expressing effector cells in CIA. Of the cell populations in the peritoneum, CD11b is expressed on macrophages and a subset of B cells. However, B cells do not express FcγRIII, so the most likely FcγRIII$^+$ cell responsible for induction of CIA is the macrophage. We confirm that the induction of arthritis in FcγRIII-deficient mice is attributed to expression of FcγRIII since transfer with FcγRIII$^-$ crude peritoneal cells did not result in development of arthritis. Considering that spleenocytes do contain macrophages to some extent, it was surprising that these cells were not able to induce arthritis. However, only about 15 % of the spleenocytes express FcγRIII, whereas 50 % of the peritoneal cells are positive for FcγRIII. This might explain the absence of arthritis after spleenocyte transfer and the ability of peritoneal cells to induce arthritis. Another explanation could be that only certain subpopulations of macrophages are important for the induction of CIA. Moreover, highly differentiated macrophages may also be a characteristic of the effector cells in CIA, as bone marrow-derived monocytes

lacked arthritogenic capacity although they expressed FcγRIII. The reason for the difference in arthritis induction using bone marrow derived mast cells in our study and the K×B/N model of arthritis is not known, but one explanation could be that different autoantigens (CII versus glucose-6-phosphate isomerase) are targets in the different arthritis models, which may lead to triggering of different effector cells.

The up-regulation of mRNA of the macrophage-derived cytokines TNFα and IL-12p35 in peritoneal cells from arthritic mice further strengthen the view that these cells are important mediators in inflammation since these cytokines are known to contribute to the inflammatory response by recruiting and activating inflammatory cells and differentiation of T helper type I cells. In conclusion, $CD11b^+$ macrophages are the most likely $FcγRIII^+$ cells responsible for induction of CIA.

Paper III. Induction of arthritis by single monoclonal IgG anti-collagen type II antibodies and enhancement of arthritis in mice lacking inhibitory FcγRIIB.

Passively transferred polyclonal IgG anti-CII Abs from CII-immunized DBA/1 mice have been shown to induce arthritis in healthy recipients (90-92). However, single monoclonal anti-CII Abs have only been shown to induce synovitis, but not macroscopic arthritis (137). Only the combination of several monoclonal anti-CII Abs, particularly in combination with LPS, which is not arthritogenic in itself, has been shown to provoke severe arthritis (138-141). In this paper we investigated whether single monoclonal anti-CII Abs can induce arthritis in naïve arthritis-susceptible DBA/1 mice without a subsequent injection of LPS. We also studied whether there is an IgG subclass dependency for the arthritogenic response. Furthermore, we investigated the role of FcγRs in antibody-induced arthritis by comparing arthritis development in DBA/1 mice with mice deficient in the different FcγRs.

The potential of single mAbs to induce arthritis was tested by injecting monoclonal anti-CII Abs of different subclasses into DBA/1 mice and studying the mice for arthritis development. The results demonstrate that single monoclonal anti-CII Abs of all subclasses tested were able to induce arthritis. Clinical symptoms of arthritis were seen in 20-25 % of the mice with the low Ab doses (3.6 – 4.5 mg) while 50 % of the mice became arthritic when injected with high doses (9 mg) of the IgG1 and IgG2b Abs, indicating that although all subclasses were able to provoke arthritis, some subclasses were more efficient. Abs of the IgG1 and IgG2b subclasses were more arthritogenic than IgG2a Abs. The route of administration did not affect the outcome of arthritis; both i.v. and i.p. administration of the Abs could induce

arthritis. The arthritis was persistent and histopathology of the joints demonstrated synovial hyperplasia, pannus formation and cartilage and bone destruction. Mice that had been injected with isotype-matched control Abs did not develop arthritis.

To investigate the role of FcγRs in the outcome of arthritis, we first compared the induction of Ab-mediated arthritis in DBA/1 mice with FcRγ-deficient DBA/1 mice (lacking functional FcγRI, FcγRIII and FcεRI). It was shown that none of the monoclonal anti-CII Abs tested could trigger arthritis in mice lacking the FcRγ-chain. The role of FcγRIII alone in Ab-mediated arthritis was investigated by injecting monoclonal anti-CII Abs into mice deficient in FcγRIII. It was shown that arthritis induced with monoclonal anti-CII Abs of the subclasses IgG1 and IgG2b is prevented in DBA/1 mice lacking FcγRIII, whereas monoclonal IgG2a anti-CII Abs are able to induce arthritis in these mice to the same extent as in wild type DBA/1 mice, although with a delayed disease onset. When the arthritogenic response was investigated in mice deficient in FcγRIIB, an enhanced disease was observed with the IgG1 and IgG2b anti-CII Abs. Thus, almost 100 % of the FcγRIIB-deficient mice developed arthritis with the low Ab dose. Histology of the joints of FcγRIIB-deficient mice displayed an aggressive joint inflammation with massive infiltration of inflammatory cells, pannus formation and cartilage and bone destruction. However, no significant difference regarding arthritis development was observed when monoclonal IgG2a anti-CII antibodies were injected into FcγRIIB-deficient mice.

Earlier studies of the passive transfer of arthritis with single mAbs have failed to trigger clinical symptoms of arthritis and a requirement of two or more Abs or simultaneous injection of LPS has shown to be necessary. Here, we demonstrate for the first time that single mAbs can induce arthritis, without the need for LPS. Although M284, M287 and M2139 recognized the same CII epitope (J1) with the same affinity, the Abs displayed different arthritogenic properties. Thus, the subclasses IgG1 (M284) and IgG2b (M2139) were more arthritogenic than IgG2a (M287). This is consistent with the findings from the K/B×N arthritis model, where IgG1 is the dominating pathogenic subclass (142). One explanation for the more severe arthritis observed with IgG1 anti-CII mAbs may be that these Abs preferentially bind to FcγRIII. The mild arthritis seen with IgG2a anti-CII Abs may be explained by the fact that these Abs preferentially bind to FcγRI, which may limit the engagement of FcγRIII, leading to a less arthritogenic response. FcγRI has not been shown to be involved in development of arthritis, only in cartilage destruction (112, 114). Furthermore, the IgG1 and IgG2b subclasses also showed a more severe arthritis in the FcγRIIB-deficient mice, indicating that activating FcγRIII is regulated by the inhibitory receptor, FcγRIIB. This was not seen with IgG2a anti-CII Abs, which may indicate

that only antibodies interacting with FcγRIII are regulated by FcγRIIB. In conclusion, the results from this paper show that arthritis can be induced with single monoclonal anti-CII antibodies although the severity of disease is dependent on the IgG subclass. In addition, arthritis induction is dependent on the activating of FcγRs and in particular, FcγRIII.

Paper IV. IgG Fc receptor polymorphisms and C5 influence susceptibility to collagen-induced arthritis.

SWR mice do not develop CIA despite carrying the arthritis-susceptible H-2^q haplotype and production of high amounts of IgG anti-CII Abs after CII-immunization (143). However, these anti-CII Abs can induce arthritis when transferred to DBA/1 mice (144). The SWR mouse lacks the complement component C5 (145) and it has a 50 % deletion of the Vβ-chain of the T-cell receptor (146). Both of these defects have been suggested to be the cause for the resistance to CIA (147-149), although this is still a controversy, since no firm conclusions regarding the contribution of the two defects to arthritis resistance have been drawn. As polymorphic forms have been described for FcγRIIIA and FcγRIIB in humans and have been linked to RA (119, 120) and SLE (19, 121), we were interested to study if possible IgG Fc receptor polymorphisms could be responsible for the arthritis resistance seen in the SWR mouse. In addition, we have re-investigated the role of C5 in arthritis induction.

To investigate if a functional polymorphism of FcγRIII exists, we generated mice with the FcγRIII gene from the DBA/1 mouse (F4.D +/+) or from the SWR mouse (F4.S +/+). Littermate controls lacking FcγRIII (F4.D -/- and F4.S -/-) were also generated. The mice were immunized with BCII/CFA and the development of arthritis was observed. It was shown that only F4.D +/+ mice developed a severe progressive arthritis, with marked swelling and erythema in both front and hind paws. In contrast, F4.S +/+ mice were relatively resistant to CIA, showing a very mild and non-progressive arthritis restricted to the digits. In addition, resistance or low incidence of arthritis was observed in mice lacking FcγRIII (F4.D -/- and F4.S -/-). The humoral response, measured as Ab response to BCII, did not differ between the F4.D +/+ and F4.S +/+ mice. However, F4.D +/+ mice exhibited an increased level of IgG1 anti-BCII Abs compared to F4.S +/+ mice. To investigate if this increased IgG1 anti-CII level was the reason for the observed difference in arthritis development between the two strains, we induced arthritis by passive transfer of a cocktail of monoclonal anti-CII antibodies (of IgG1, IgG2a and IgG2b subclasses) to F4.D +/+ and F4.S +/+ mice and examined them for arthritis development. The same pattern as that in actively induced arthritis was observed. All F4.D +/+ mice developed a severe progressive

disease whereas F4.S +/+ mice were highly resistant. Only one F4.S +/+ mouse became arthritic and the disease was very mild. This indicated that the difference in IgG1 anti-BCII Ab level observed in actively induced CIA did not contribute to the difference in arthritis development between the two strains.

Since the results of experiments involving passively and actively induced CIA indicated that a possible FcγRIII polymorphism could exist that might influence susceptibility to arthritis, we decided to sequence the complete coding region of FcγRIII in SWR mice, DBA/1 mice and in nine common mouse strains. The result demonstrates that FcγRIII is expressed as three distinct haplotypes in mice. The SWR mouse exhibits one haplotype (FcγRIII:V) while C57BL/6 and C57BL/10 mice exhibit a second haplotype (FcγRIII:T). The rest of the strains tested, DBA/1, NZB, NZW, NOD, BXSB, MRL/lpr, BALB/C and CBA mice all show a common third haplotype (FcγRIII:H). The SWR mouse and the DBA/1 mouse differ at two amino acid positions, one located in the first immunoglobulin domain and the other in the intracellular part of the receptor. The FcγRIII haplotypes of our crossings were also determined and it was shown, as expected, that F4.S +/+ mice exhibit the FcγRIII:V haplotype and F4.D +/+ mice display the FcγRIII:H haplotype.

FcγRIIB are known to be expressed as two haplotypes, Ly-17.1 and Ly-17.2, but it is not known which haplotypes SWR and DBA/1 mice carry. Since the genes for FcγRIII and FcγRIIB are located close to each other on chromosome 1 and act in concert during inflammation, we also investigated if FcγRIIB differed between the SWR and DBA/1 strains. It was shown that SWR mice carry the Ly-17.1 haplotype whereas DBA/1 mice carry the Ly-17.2 haplotype. We also determined the haplotypes of the crossings F4.D +/+ and F4.S +/+ and it was shown that the FcγRIIB gene was inherited from the wild type mice, as F4.D +/+ exhibit Ly-17.2 whereas F4.S +/+ exhibit Ly-17.1.

Furthermore, considering that SWR are known to be deficient in C5 we wanted to investigate the contribution of this deficiency to arthritis development. First, we supplemented SWR mice with C5 by injecting C5-containing sera. The SWR mice were immunized with BCII/CFA and studied for arthritis development. It was shown that 50 % of the SWR mice supplemented with C5 developed CIA, whereas none of the untreated SWR mice developed disease. To further elucidate the role of C5 for induction of arthritis, we treated F4.D +/+ mice with anti-C5 Abs before arthritis onset. The results showed that the arthritis was totally blocked in treated mice, whereas untreated F4.D +/+ mice developed arthritis to the same extent as in previous

experiments. These results indicate that C5 plays an important role in induction of CIA.

Several studies have focused on the reason for the resistance to arthritis observed in the SWR mouse. However, no firm conclusions have been drawn from these studies. In this paper, we demonstrate for the first time that a polymorphism in the FcγRIII gene may be responsible for the resistance to CIA seen in the SWR mouse. SWR and DBA/1 differ in FcγRIII by 2 amino acids, one located in the first immunoglobulin domain and one in the intracellular part of the receptor. The polymorphism in the first immunoglobulin domain could alter the ligand binding affinity leading to a difference in arthritis development. The second amino acid difference, in the intracellular part of the receptor could alter the signaling efficiency of the receptor. Thus, even though the α-chain of FcγRIII does not contain any signaling motif, the amino acid substitutions could be involved in the interaction between the α-chain and FcRγ, which might lead to impaired receptor function. Considering that FcγRIII and FcγRIIB appear to act in concert, determining the magnitude of the effector cell responses in CIA, the expression of the different haplotypes of FcγRIIB in SWR and DBA/1 mice may also lead to differences in arthritis development.

Furthermore, it has been suggested that C5 and FcγR may act in synergy in antibody-mediated inflammation (130, 131). It has also been demonstrated that C5a increases the expression of FcγRIII and decreases the expression of FcγRIIB (132, 133). Our findings are in agreement with the concept of a synergy between C5 and FcγRIII for extensive inflammation, with an additional dependency on certain haplotypes of FcγRIII. Thus, we show that F4.S +/+ mice that express the FcγRIII:V haplotype in combination with C5 only develop a mild arthritis, whereas F4.D +/+ mice expressing C5 and the FcγRIII:H haplotype develop a severe arthritis. This suggests that C5 can initiate arthritis but without help from the arthritis-susceptible FcγRIII:H haplotype, full-blown arthritis can not be triggered. In conclusion, these data indicate a significant role for C5 in the induction of CIA and that polymorphic forms of FcγRIII and FcγRIIB influence the severity of the disease.

Conclusions

The following conclusions can be drawn on the role of FcγR in experimental arthritis:

- Expression of FcγRIII is crucial for induction both of actively and passively induced CIA.

- Macrophages are the most likely FcγRIII expressing cell responsible for induction of CIA.
- Single monoclonal anti-CII Abs can induce a severe progressive arthritis in arthritis-susceptible DBA/1 mice and the subclasses IgG1 and IgG2b are more arthritogenic than IgG2a.
- FcγRIII is expressed as three distinct haplotypes in mice and haplotypes of FcγRIII and FcγRIIB determine the severity of CIA.
- C5 is important for the onset of CIA.

Reflections

In recent years several studies have focused on antibodies and autoimmune diseases. It is evident that antibodies and ICs are of major importance in these diseases. However, the mechanism by which ICs contribute to disease is not completely understood, although both FcγRs and the complement system have been shown to be important (107, 109, 126, 127). In this thesis, I have investigated the role of FcγRs in the development of CIA.

When an antigen enters the body it may be bound by antigen presenting cells and presented to T cells. Antigen-specific B cells can, with help from the T cells, become activated and start to produce antigen-specific antibodies. These antibodies can then bind to the antigen and form an IC. Usually, these ICs are taken care of by inflammatory cells and the production of antigen-specific Abs is inhibited after the infection is cleared. But if the antigen is not an intruder, but self-tissue like the cartilage, the immune cells may start to attack the body's own tissue and the production of antigen-specific Abs may continue, as the antigen is always present in the body. If ICs can not be cleared from the tissue they will interact with the complement system and the FcγRs and induce inflammation. The relationship between these two systems in triggering inflammation is perhaps on the verge of being elucidated. It has been shown that C5 are important in early inflammation (132) and that the complement system and in particular C5 works up-stream of FcγRs during IC-mediated inflammation (133). Furthermore, activation of C5 leads to production of C5a, an activation product of C5. C5a binds to C5aR and this interaction has been shown to up-regulate the expression of FcγRIII and to down-regulate the expression of FcγRIIB and thereby reduce the threshold for FcγR activation on these cells (132, 133). In this thesis, I have shown that FcγRIII is crucial for the induction of arthritis. However, a background level of arthritis development is observed in FcγRIII-deficient mice, which might be caused by the presence of C5. The same background level of arthritis is seen in C5-deficient mice during development of CIA (127), which might be caused by the presence of FcγRIII. This indicates that the complement system and FcγRs are not independent pathways but rather work together to give rise to full-blown CIA.

My hypothesis is that in autoimmune arthritis, when an IC is formed in the joint, the first thing that happens is that the classical pathway of the com-

plement system becomes activated (Fig. 6). Activation of the complement system leads to activation of inflammatory cells, preferentially synovial macrophages in the joint, carrying FcγRIII. Complement activation also induces the release of the anaphylatoxin, C5a, which binds to C5aR on macrophages. This interaction will increase the expression of FcγRIII and decrease the expression of FcγRIIB and thereby disturb the net balance of activation and inhibition of FcγRs on the cell. Thus, the FcγRIII activation of the effector cells will be enhanced, causing the release of inflammatory mediators such as TNFα. Both inflammatory mediators and the anaphylatoxins C5a and C3a will recruit more inflammatory cells, such as mast cells, NK cells and neutrophils, from the blood to the tissue. These cells also express FcγRIII and so the inflammation may then well be enhanced, leading to joint damage. Furthermore, we have shown that not only is FcγRIII important for arthritis induction, but also that the expression of certain haplotypes of FcγRIII is necessary for full-blown arthritis to occur. In a scenario in which a mouse expresses the less arthritis-susceptible haplotype FcγRIII:V, the joint damage is not as severe as in one that carries the arthritis-susceptible haplotype FcγRIII:H. This may be due to impaired binding of IgG or impaired signaling through the receptor. Another explanation could be that the nucleotide substitutions leading to the different haplotypes of FcγRIII interfere with binding of transcriptions factors activated by the C5a-C5aR interaction. This could result in lack of increased expression of FcγRIII and the inhibition of inflammation.

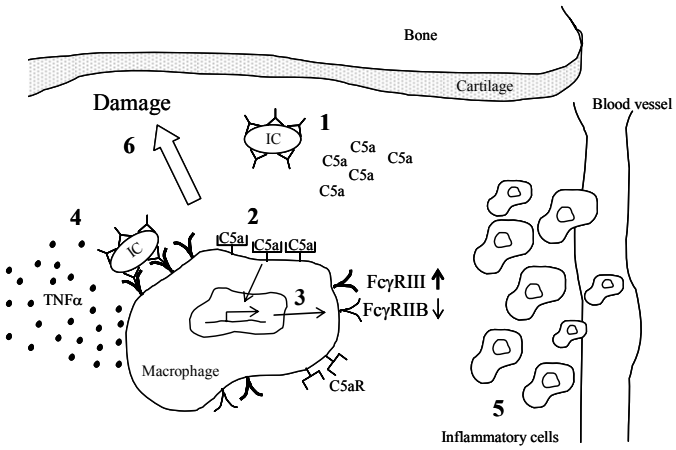

Figure 6. Hypothesis for the induction of IC-mediated arthritis. 1) ICs stimulate activation of the classical pathway of the complement system and C5 and the split product C5a are produced. 2) C5a binds to its receptor, C5aR. 3) This interaction causes increased expression of FcγRIII and decreased expression of FcγRIIB. 4) ICs bind to FcγRIII and activate the cell, leading to release of chemotactic factors and inflammatory cytokines, such as TNFα. 5) Cytokines and chemotactic factors recruit inflammatory cells from the blood. 6) Action of the soluble mediators lead to joint damage.

In antibody-mediated arthritis we demonstrated that FcγRIII is important for the induction of arthritis, since the most arthritogenic subclass, IgG1, preferentially binds to FcγRIII. That IgG2a is not very arthritogenic might seem surprising since this subclass has always been regarded as the most important in arthritis induction, as CIA has been thought to be a Th1-dependent disease. Our results, demonstrating that macrophages during CIA produce IL-12, strengthen this view since this cytokine drives the development of Th1 cells. In addition, these cells also had increased expression of TNFα, which is also regarded as a Th1-mediated cytokine. However, it has been shown that Th1 cells are important in early disease and that the response shifts to a clear Th2 phenotype during the later phases (88). Our results, in antibody-mediated arthritis, are in agreement with this study, as only the late Th2 phase of arthritis is studied in this form of arthritis. Thus, our observation that the Th2 subclass IgG1 is more arthritogenic than IgG2a, further supports the concept that Th2 is important in the late stage of disease.

The results reported in this thesis show that FcγRIII plays a crucial role in the induction of arthritis, both when arthritis was induced actively and passively. One appealing possibility from these data is that FcγRs may be used as therapeutic targets in the treatment of RA in the future. This could inhibit the interaction of IC with cell-surface bound FcγRs and lead to less activation of inflammatory cells and hence less joint inflammation.

Populärvetenskaplig sammanfattning

Kronisk ledgångsreumatism (reumatoid artrit, RA) drabbar ca 1 % av befolkningen och räknas som en av de stora folksjukdomarna. Kvinnor drabbas av sjukdomen i betydligt större utsträckning än män, ca 2.5 gånger så ofta. RA drabbar i första hand de små lederna i kroppen, som finger- och tåleder, men kan även angripa större leder. Orsaken till sjukdomen är idag inte känd, men man vet att både genetiska faktorer och miljöfaktorer spelar roll vid sjukdomsförloppet.

RA är en autoimmun sjukdom vilket betyder att vita blodkroppar och antikroppar, går till attack mot kroppens egna vävnader. Antikroppar är proteiner som bildas av celler i immunsystemet och som normalt finns i vävnader och i blod. Deras funktion är att cirkulera i kroppen och leta upp och fånga inkräktare som t.ex. bakterier och virus. Antikroppar finns i 5 olika varianter i kroppen. Den vanligaste varianten är IgG, vilken också är den sorts antikropp som jag har arbetat med i denna avhandling. I nästan alla friska människor finns vita blodkroppar som producerar antikroppar med förmågan att attackera kroppens egen vävnad, men normalt kontrolleras dessa celler av speciella mekanismer i kroppen så att de inte gör någon skada. Men i vissa fall fungerar inte denna kontrollmekanism. Antikropparna aktiverar då vissa av immunsystemets celler genom att binda till mottagarmolekyler (receptorer) på ytan av cellerna.

I min avhandling har jag studerat receptorer för IgG (FcγR) och undersökt deras betydelse för utvecklandet av en RA-liknande sjukdom hos möss, kollagen-inducerad artrit. I möss finns tre olika receptorer som binder IgG, de kallas för FcγRI, FcγRIIB och FcγRIII. Kollagen-inducerad artrit framkallas genom att injicera kollagen typ II (en av beståndsdelarna i brosk) i möss. Genom att använda denna modell har vi studerat huruvida möss som saknar FcγRIII kan utveckla artrit (delarbete I). Det visade sig att om möss saknar FcγRIII är de nästan helt skyddade från att utveckla kollagen-inducerad artrit. I arbetet visar vi även att FcγRIII finns uttryckt på celler i leden där inflammationen sker.

När vi i delarbete I tagit reda på att FcγRIII är nödvändig för utvecklandet av kollagen-inducerad artrit, så var vi även intresserade av att ta reda på vilken av de celler som uttrycker FcγRIII, som är ansvarig för utvecklandet av

sjukdomen. FcγRIII finns på olika typer av immunceller såsom makrofager, mast celler, NK celler och neutrofiler. För att studera detta så har vi tagit celler från möss som uttrycker FcγRIII och överfört dem till mössen som saknar FcγRIII och sedan studerat om mottagarmössen kan utveckla kollagen-inducerad artrit (delarbete II). Vi undersökte flera olika celltyper och vi fann att makrofagen är den celltyp som är viktigast för utvecklandet av sjukdomen. Vi visar även att dessa makrofager under sjukdomsförloppet producerar speciella signalmolekyler, så kallade cytokiner, av sorten TNFα och IL-12p35, som är väldigt viktiga för utvecklandet av artrit.

När mössen utvecklar kollagen-inducerad artrit så produceras antikroppar mot kollagen och dessa antikroppar är sjukdomsframkallande. I delarbete III har vi studerat vilka av FcγR som är viktiga för denna antikroppsinducerade artrit. Här använde vi möss som saknar FcγRIII, FcγRIIB och möss som saknar både FcγRI och FcγRIII. Resultaten visade att möss som saknar FcγRIII eller både FcγRI och FcγRIII är skyddade från sjukdomen, medan möss som saknar FcγRII får en kraftigare sjukdom. IgG finns i fyra olika varianter i möss, IgG1, IgG2a, IgG2b och IgG3. Vi har även visat att använder man antikroppar av typerna IgG1 och IG2b så blir mössen sjukare än om man använder antikroppar av typen IgG2a.

I delarbete IV har vi studerat en musstam som heter SWR och som inte utvecklar kollagen-inducerad artrit. Vi har undersökt vad som skiljer denna musstam från DBA/1 möss, som är mottaglig för kollagen-inducerad artrit. Vi visar att dessa två stammar skiljer sig både i avseende på FcγRII och på FcγRIII. De har olika varianter av dessa receptorer. Skillnaderna på receptorerna kan bidra till de olika mottagligheterna för kollagen-inducerad artrit som kan ses hos SWR och DBA/1 möss. En förklaring kan vara att de olika varianterna av receptorerna binder IgG i olika grad. Vi visar även att en annan komponent, C5, är viktig i inflammation och arbetar tillsammans med FcγRIII under utvecklandet av kollagen-inducerad artrit.

Sammanfattningsvis så har jag i denna avhandling visat att FcγR spelar en stor roll vid utvecklandet av kollagen-inducerad artrit. I framtiden kan detta leda till nya behandlingar mot RA. För om man kan förhindra att antikropparna binder till FcγR så kan man även förhindra att immunsystemets celler aktiveras och på så sätt förhindra sjukdomen eller i alla fall lindra den.

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