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FINDING GENES CONTROLLING ARTHRITIS IN MICE – FCγ RECEPTORS AND COMPLEMENT C5

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To my family

ABSTRACT

Autoimmune diseases are dependent on multifactorial factors including genes, environment and interactions between them. This thesis is focused on a chronic inflammatory disorder, Rheumatoid arthritis (RA). So far only very few genes and environmental factors have been identified reflecting the complexity of the autoimmune processes. Identification of new genes and the underlying mechanisms leading to disease progression is absolutely crucial for finding more specific therapies. Understanding the pathogenic events causing chronic joint destruction can be best done using animal models. The work in this thesis focused on well-defined mouse models of autoimmune diseases: collagen induced arthritis (CIA), collagen antibody induced arthritis (CAIA) and experimental autoimmune encephalomyelitis (EAE). We have used two different strategies to map the genes that influence arthritis development: congenic strains and heterogeneous stock inbred-outbred cross. Of particular interest in this thesis are gene regions previously identified in two-generation crosses between B10.Q and NOD strains; *Cia9* and *Cia2* loci that carry promising candidate genes such as FcγR cluster region and complement C5 respectively. To be able to elucidate the role of underlying genes in arthritis in both the loci, we generated sub-interval congenic lines (paper III and IV). We found that FcγRIIb and FcγRIII are most likely candidates for the original *Cia9* locus (paper III). Moreover, we were able to show that systemic rather than local production of complement C5 is crucial in arthritis development (paper IV). In order to identify additional loci controlling arthritis in mice we have used another genetic approach – using heterogeneous stock mice, a cross between eight different founders. At first, HS mice were screened for susceptibility to different animal models (paper II) and then CIA permissive $H2^q$ haplotype has been introduced and a large cohort of heterogeneous stock inbred-outbred cross was investigated for arthritis susceptibility (paper I). We found 18 new arthritis loci and fine mapped several already known loci including *Cia9* and *Cia2*. The last part of this thesis describes the prospect of using a thermo-responsive polymer as an adjuvant (paper V). These results suggest mapping of causative genes in a complex disease is multifaceted and a challenging task.

LIST OF PUBLICATIONS

I. **High resolution mapping of a complex disease, a model for rheumatoid arthritis, using heterogeneous stock mice**.
Ahlqvist E, Ekman D, Lindvall T, Popovic M, Förster M, Hultqvist M, Klaczkowska D, Teneva I, Johannesson M, Flint J, Valder W, Nandakumar KS, Holmdahl R.
Hum Mol Genet. 2011 Aug 1;20(15):3031-41

II. **Heterogeneous stock mice are susceptible to encephalomyelitis and antibody initiated arthritis but not to collagen- and G6PI-induced arthritis.**
Dorota Klaczkowska*, Bruno Raposo*, Kutty Selva Nandakumar
Scand J Immunol 2011, 73:46-52

III. **Polymorphisms in the FcγR gene cluster promote experimental arthritis: Differential IgG subclass pathogenicity.**
Dorota Klaczkowska, Rikard Holmdahl and Kutty Selva Nandakumar
Manuscript

IV. **Different genetic strategies confirm the importance of complement factor 5 in experimental arthritis**.
Dorota Klaczkowska, Anna Blom, Diana Ekman, Kutty Selva Nandakumar and Rikard Holmdahl
Manuscript

V. **A thermo-responsive polymer of *N*-isopropylacrylamide adjuvant operates independent of toll-like receptors: a strong influence by MHC class II and *Ncf1* genes on autoimmunity and arthritis.**
Akhilesh Kumar Shakya, Ashok Kumar, Dorota Klaczkowska, Malin Hultqvist, Kristin Hagenow, Rikard Holmdahl and Kutty Selva Nandakumar
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LIST OF ABBREVIATIONS

ACPA	Anti-citrullinated protein antibody
ACR	American College of Rheumatology
AADCC	Antibody-dependent cellular cytotoxicity
APC	Antigen presenting cells
CAIA	Collagen antibody induced arthritis
CIA	Collagen induced arthritis
CII	Collagen type II
CFA	Complete Freund's adjuvant
CNV	Copy number variation
CRP	C-reactive protein
CTLA-4	Cytotoxic T lymphocyte antigen – 4
DC	Dendritic cells
DMARD	Disease-modifying anti-rheumatic drug
EAE	Experimental autoimmune encephalomyelitis
EULAR	European League Against Rheumatism
ESR	Erythrocyte sedimentation rate
HLA	Human Leukocyte Antigen
HS	Heterogeneous stock
IFA	Incomplete Freund's adjuvant
LPS	Lipopolysaccharides
MHC	Major histocompatibility complex
NSAID	Non-steroidal anti-inflammatory drug
NADPH	Nicotinamide adenine dinucleotide phosphate
PRR	Pattern-recognition receptor
PTPN22	Protein tyrosine phosphatase non-receptor type 22
RF	Rheumatoid factor
RA	Rheumatoid arthritis
SE	Shared epitope
SLE	Systemic lupus erythematosus
T1D	Type 1 diabetes
TCR	T-cell receptor
TLR	Toll-like receptor
QTL	Quantitative trait locus

INTRODUCTION

Every day our body is challenged to protect its integrity from all dangers that can be seriously harmful. This is only possible due to the amazing mechanism we posses – immune system. It works on many phases to defend "us" against bacteria, microbes, viruses, toxins and parasites that are just waiting to invade us. In addition, immune system is also scanning the body to make sure that it's free from internal dangers like uncontrolled cell divisions. The aim of the immune system is to act fast since the main feature of pathogens is to spread immediately. Thus, immune system consists of specialized immune cells and molecules that evolved to build a complex network of defence mechanisms. Usually first line of the defence is a surface barrier (skin, mucous membranes) or already pre-formed molecules like bactericidal lyzozyme in the tears or other proteins circulating in the body, called complement components. The complement system is in charge to destroy or label the pathogen that can be recognized by specialized phagocytes like macrophages or neutrophils. This part of the defence is named as innate, since it is unspecific and recognizes the pathogens in a generic way through pattern-recognition receptors (PRRs). PRRs can recognize a number of evolutionarily conserved bacterial and viral molecules, including lipopolysaccharides (LPS), flagellin or double stranded RNA and activate phagocytosis of the microbes. Engulfment of pathogens by antigen presenting cells (APC) initiates a series of events leading to digestion, processing and finally presentation of the antigen to specialized group of cells. The other cells of innate system like mast cells, eosinophils and basophils can release various mediators that will help to fight against the invading pathogens and natural killer cells are designed to kill the infected cells.

Sometimes the propagation of pathogen is too pronounced and the innate mechanisms are not sufficient to defend against the infections, then the second line of the defence – the adaptive immune system is coming in to the picture. The adaptive immune system is composed of T cells and antibody-producing B cells. They are able to specifically recognize the pathogens due to their random rearrangements of receptor genes and thus generating an enormous amount of unique receptors that can bind various pathogens. B cells mature in the bone marrow and they are important players in the humoral immune response. T cells also originate from bone marrow but their development takes place in thymus. They are subjected to several checkpoints before they will be in the periphery. There are two steps, positive and negative selection are involved in this process. Positive selection is recognition of peptides with histocompatibility complex (MHC) by T cells and those that are having sufficient affinity for peptide-MHC complex are

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getting the survival signal and being selected. The negative selection consists of selection of only those T cells that do not have high affinity for self-antigens. Nevertheless, sometimes self-reactive cells are escaping to the periphery where they can be activated. This process is called autoimmunity. Approximately 3-5% of the population suffer from autoimmune diseases. They could be either organ-specific like multiple sclerosis (MS) and type I diabetes (TID), or systemic, like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Even though autoimmune diseases are quite common, still we know very little about the mechanisms leading to autoimmunity. We are however now beginning to understand the complexity of the genetics and molecular pathways behind several autoimmune diseases.

RHEUMATHOID ARTHRITIS

Rheumatoid arthritis (RA) is a clinical syndrome rather than a single disease consisting of distinct subsets (1) having different pathophysiological mechanisms (2) leading to disability by destruction of peripheral joints and bone erosions. RA is characterized by persistent synovial inflammation causing the swollenness and pain and is often accompanied with fatigue and stiffness of the joints. Usually it affects small joints of hands, feet and spine however larger joints like knees and shoulders can also be engaged. RA can result in extra-articular features like vasculitis, rheumatoid nodules or lung inflammation and co-morbidities such as heart failure, stroke or cancers (3). It is considered to be an autoimmune disorder as the presence of autoantibodies mainly rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) can be recognized. RA is a common disorder affecting 0.5–1.0 % of adults in developed countries. Women are three times more affected than man and the onset usually occurs at fifth decade of life and increases with the age (4). Prevalence of disease varies geographically so that it is more common in North America and Northern Europe compared to developing countries (4-6). However, etiology of RA is unknown but it is believed that different genetic and environmental factors are involved in disease development. RA is not a recent disease. Already, the evidence of RA can be found in skeletons dated 4000-1000 BCE. The first description of rheumatoid arthritis in modern medicine was made by French Dr. Augustin Jacob Landre-Beavuvais in 1800. Finally, 90 years later Archibald Garrod replaced the existing phrase "Rheumatic Gout" to new name "Rheumatoid Arthritis"(7, 8).

DIAGNOSIS AND TREATMENT

Since rheumatoid arthritis is not a single disease and symptoms are often similar to other inflammatory pathologies, patients are classified according to the specified selection of criteria. Recently, American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) developed the new criteria for RA diagnosis (9). New set of classification criteria for RA has focused on early stage of the disease (10) including joint evaluation, duration of symptoms, serologic tests (RF or ACPA) and acute-phase response (ESR- erythrocyte sedimentation rate, CRP- C reactive protein) (9). The main purpose of the new criteria is to increase sensitivity and specificity to diagnose the early stage of RA and to initiate the proper disease-modifying treatment at the earliest. Though, there is still no cure for RA, various pharmacological treatments are available to alleviate the sufferings of the patient.

These include NSAIDs (non-steroidal anti-inflammatory drugs), DMARDs (disease-modifying anti-rheumatic drugs), glucocorticoids and biological agents. Currently, the most commonly prescribed **DMARD** is methotrexate (MTX) but others like anti-malarial drug hydroxychloroquine (HCQ), sulfasalazine (SSZ) and leflunomide are also often used. They slow down the destructive process in bone and cartilage by suppressing the immune system, decreasing acute-phase markers, provide relief from pain and stiffness and generally improve long-term health (11). DMARDs have slower onset of action and the effect of treatment can be visualized after several weeks (MTX, SSZ) or even months (gold salt) after first administration and thus addition of non- steroidal anti-inflammatory drugs or short-term glucocorticoids during the flare-ups is used as an alternate. If response to the treatment is inadequate, DMARDs sometimes being combined (12) and given for example as a triple therapy consisting of methotrexate, hydroxychloroquine and sulfasalazine (13) or as a combination with anti-TNF (tumor necrosis factor-α) agents (14). **NSAIDs** like aspirin, diclofenac, and ibuprofen provide efficient symptomatic relief of pain and inflammation by inhibition of COX isoenzymes (COX-1 and COX-2) and thereby reducing the level of prostaglandins. However, they do not have major effect on disease progression or destruction in RA. Glucocorticoids (prednisone, prednisolone) are given either locally into inflamed joint or as oral/intramuscular administration that help to reduce synovitis by decreasing the joint swelling and allowed improvement of the treatment with another DMARDs (15). They are able to decrease the progression of bone erosion, especially in patients with early RA (16). Since many treatments have adverse effects, monitoring of the patient's blood counts and liver function tests is required (17). **Biological agents** are used when the therapy with DMARDs failed and arthritis progression is uncontrolled. They consist of either monoclonal antibodies or recombinant proteins that target immune molecules and cells. One of the first successfully used biological agents over the decade ago was TNF-α inhibitor (Infliximab), which revolutionized the rheumatology field. It is highly effective and inhibits leukocyte trafficking, acute-phase responses and reduces the destruction of the cartilage and bones. Another strategy of using monoclonal antibodies is to block IL-6 receptor (18) and it is mainly used in patients, who did not respond to anti-TNF-α treatment. In addition IL-15 (19), IL-17 (20) antagonists are under investigations and in the phase II clinical trials. Other therapies are directed toward depletion or inactivation of immune cells involved in the pathogenesis of RA. Anti-B cells therapy comprises of monoclonal antibodies (anti-CD20) that deplete B cells. T-cell target approach is focused on the termination of cell activation with a fusion protein between extracellular portion of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and Fc fragment of a human IgG1 (CTLA4Ig) (21).

ENVIROMENTAL FACTORS IN RHEUMATHOID ARTHRITIS

The aetiology of rheumatoid arthritis is still not clear. Most likely, environmental and genetic (including epigenetics) factors and their interactions are considered to be important triggers. It has been suggested that genetic factors can explain approximately 60% of the risk for development of RA (22, 23) and therefore environmental factors must be taken into account for disease outcome as well. It is most likely that there is more than one experimental factor that is involved in triggering RA development and those factors can interact in an additive manner (for ex. smoking plus infection). Cigarette **smoking** is one of the most potential and investigated risk factor for RA and it correlates in a dose dependent manner (24, 25). It has been shown that heavy smokers (41-50 packs/year) have thirteen folds increased risk to develop RA in both sexes (26). Interestingly, an interaction between shared epitopes (SE) HLA-DRB1 genes and smoking contributing to the development of sero-positive but not sero-negative RA has been found (27, 28). This phenomenon is mainly due to the citrullination of proteins that triggers specific autoimmunity in the patients carrying SE genes (29).

During last few decades it was postulated that exposure to **infection** might also be important factor in RA. Both bacterial (*Mycobacterium tuberculosis*, *Escherichia coli, Klebsiella pneumoniae*) and viral agents (*Epstein-Barr virus*, *parvovirus B19, Cytomegalovirus*) have been associated with RA (30-32). Recent studies have highlighted the role of *Porphyromonas gingivalis* infection, which leads to periodontal diseases, as one of the possible mechanisms to trigger systemic inflammation (33, 34). *Porphyromonas gingivalis* expresses the enzyme peptidyl arginine deiminase (PAD) that may citrullinate human proteins and cause the loss of tolerance to self-antigens (35, 36). Therefore, **periodontitis** may have an impact on the progression of RA (34).

Moreover, recent Swedish study has shown that exposure to **silica** dust by working in stone crushing or rock drilling increased the risk for RA development threefold (37). Another major candidate is **mineral oil** that has been reported to increase the risk of RA especially in patients RF or ACPA positive (38). Besides, mineral oil has been shown to induce polyarthritis in rats (39). The protective effect of **Mediterranean diet** has been reported as a factor reducing the risk of development of RA (40, 41). Especially lifelong consumption of cooked vegetables (42), or oily fish and olive oil have a prominent effect on RA, hypothetically due to the high level of omega 3 lipids, which may act anti-inflammatory factors (43). On the

other hand, high-level **red meat** consumption, offal and meat fat increased the risk for the development of inflammatory polyarthritis (44). However, association between meat, iron or proteins intake and risk for RA has not been reported (45).

There are several reports available about decaffeinated **coffee** intake as one of the potential risk factors for RA, especially showing stronger evidence in sero-positive patients. Conversely, it is contradictory with another studies that were shown no correlation between decaffeinated/caffeinated coffee or tea consumption on disease progression (46-48). Interestingly, **alcohol** intake has shown to have a factorable effect on RA development in a Scandinavian cohort study (49).

In general, women are more predisposed to develop an autoimmune disease, including RA. This can be partially explained by the changes in the concentrations of female **hormones** like estrogen, testosterone, progesterone and prolactin. During pregnancy the disease onset is reduced by 70% and then increased during postpartum period (50, 51). Nevertheless, there is no consistent finding of the oral contraceptive use, breast-feeding or hormone replacement therapy on disease outcome (52). Thus, identification of environmental risk factors will lead to better understanding of disease mechanism and offer a chance to prevent the progression of arthritis in high-risk populations.

GENETIC FACTORS IN RHEUMATHOID ARTHRITIS

RA is a heterogeneous disease, where disease susceptibility can be explained by approximately 60% of genetic component (22, 23). Monozygotic twins have higher concordance rate (15%) compared to dizygotic twins (4%) (22). Identification of the genes that cause RA is not a trivial task and despite the enormous efforts, only few genes have been confirmed so far.

MAJOR HISTOCOMPATIBILITY COMPLEX

The first causative locus correlated with RA was identified by Stastny in 1978 and defined as Human Leukocyte Antigen (HLA)-DR that resides in the major histocompatibility complex (MHC) class II (53). The association between MHC and RA is so far the strongest link in RA and has been replicated independently in many populations. Subsequent analysis revealed that several alleles at HLA-DRB1 locus (DRB1*01, DRB1*04) are strongly associated with RA and those bear a common sequence or so called "shared epitope" (SE) (54). The sequence has been

mapped to the third hypervariable region of DRβ molecule, particularly to amino acids at position 70-74 (55). Since, the susceptibility epitope (QKRAA-glutamine-leucine-arginine-alanine-alanine, QRRAA or RRRAA) is located in the "wall" of the peptide-binding groove, it can influence the biding and presentation of the specific arthritogenic peptides by HLA-DR molecule to T cells and thus might trigger the autoimmune response. It has been shown that shared epitope can influence the severity of the RA and it is more pronounced in the patients carrying the homozygosity (56). Several another genes in the MHC locus have also been identified as additional risk factors independent of HLA-DRB1 allele (57). It is important to note that HLA genes account approximately for 30% of the heritable risk, which leave the room for identification of unknown non-HLA genes. Only less than ten years ago, due to development of advanced SNP genotyping technology (chips containing 1,000,000 SNPs), it was possible to genotype large patient's cohorts within feasible time and cost limits.

Recently, genome wide analysis studies (GWAS) reveal numerous genes located outside the MHC region. Among them, Protein tyrosine phosphatase non-receptor type 22 (**PTPN22**), Tumor necrosis factor receptor-associated factor 1, complement component 5 (**TRAF1-C5**), Peptidylarginine deiminases citrullinating enzyme 4 (**PADI4**), signal transducer and activator of transcription 4 (**STAT4**), Fcγ receptors (**FCGR**) are the most associated genes with RA and will be discussed in detailed below.

PTPN22

The gene PTPN22, also known as "Lyp", has been at first associated with T1D (type 1 diabetes) and shortly thereafter with RA (58). PTPN22 plays an important role in T-cell receptor signalling since Lyp together with Csk (regulatory kinase) down-regulate T cell activation (59, 60). Surprisingly, R620W missense polymorphism in PTPN22 causes gain of function activity and renders the Lyp more potent suppressor of early T cell development (61). This might cause failures during T cell selection process in the thymus as well as moderate the activity of T_{regs} (60, 62). Genetic risk factors have a tendency to overlap with different autoimmune disorders, hence the risk variant of PTPN22 has also been associated with systemic lupus erythematosus (SLE), Graves' disease and juvenile idiopathic arthritis (61) as well. Recently, it was shown that polymorphism in PTPN22 can altered the faction of B cells as well.

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TRAF1-C5

The TRAF1-C5 has been linked with RA in North American/Swedish genome-wide association studies as well as in a candidate gene approach investigated by Dutch group, which was confirmed by many others as well (63-66). C5 encodes the complement component 5 and its cleavage product C5a is a potent chemoattractant that can mobilize synovial and inflammatory cells and thus play the critical role in articular inflammation. In detail, this will be discussed in the section of "Genetic basis of arthritis". Tumor necrosis factor (TNF) receptor-associated factor 1 (TRAF1) plays a crucial role in cell proliferation and differentiation. The most strongly associated SNPs (rs3761847 in GWAS, rs10818488 in candidate gene approach) are located in an intergenic region of chromosome 9 between TRAF1 and C5 gene and increase the susceptibility and severity of anti-CCP positive RA (63). On the other hand, recently published data by Han et al., strongly suggest that only TRAF1 but not C5, is RA risk causative gene (67, 68). Furthermore, difference in RA susceptibility was 45 fold increased in patients carrying all three (HLA-SE, PTPN22, TRAF1) highest risk variants (68). This kind of analysis might establish individual prognosis for RA development leading to more accurate therapy. Another study has shown TRAF1-C5 association with SLE (69).

STAT4, CTLA4, CD40

Association of STAT4 with RA has been confirmed in both Caucasian and Asian populations (70-72). Additionally, the STAT4 risk haplotype was also associated with SLE (70). STAT4 is a key immune-regulator in cellular response to IL-12 and IL-23. Once it is activated via these cytokine receptors, it translocates to the nucleus and leads to transcription of IFN-γ. It plays role in the development of Th1 responses and expansion of Th17 cells (73). STAT4 is a potential therapeutic target, since its expression level is diminished after DMARDs treatment (74).

CTLA4, expressed on the surface of Th cells, belongs to the same family as CD28 and binds to CD80/86 on APC cells. Several studies have reported its role in multiple autoimmune disorders (75, 76). However, only modest association with RA has been found (77). Two important findings strengthen its influence in RA susceptibility. First, beneficial treatment of RA with antibodies neutralizing CTLA4 activity (78), and second that CTLA4 risk allele enhanced the development of ACPA-positive RA (79).

Role of CD40 has been confirmed and replicated in two independent studies (80, 81). It has been reported to be a genetic risk factor that might influence the RA severity and the common risk haplotype might be important for the rate of joint destruction in ACPA-positive RA patients (81).

PADI4

It is one of the isoenzymes that are involved in the process of posttranslational modification (citrullination) of arginine residues to citrulline, which subsequently may lead to the production of anti-CCP antibodies. First it was suggested to be associated with RA susceptibility in Japanese population, where causative SNPs affect the stability of the transcript (82) and soon after replicated by others (83). However, the conflicting results in European descent have been reported (84, 85).

FCGR

The FcγRs are receptors for the Fc region of immunoglobulin G (IgG). Since majority of RA patients are sero-positive (ACPA, RF) and IgG-immune complexes are present in their synovial fluid, it was proposed that FcγRs might have an important role in disease pathogenesis. The interaction between immune complexes and FcγRs activates macrophages within the joint leading to release of chemokines, cytokines and pro-inflammatory mediators that mediates cartilage breakdown(86). In humans three major FcγRs subclasses are present: high-affinity FcγRI (CD64) and two low-affinity FcγRII and FcγRIII (CD32 and CD16 respectively).

The low-affinity receptors are encoded by the cluster of five FCGR genes located on the chromosome 1q23.3: FCGR2A, FCGR3A, FCGR2C, FCGR3B and FCGR2B (from centromere to telomere). Several studies have implicated the common functional SNP in FCGR3A that confers phenylalanine (F) to valine (V) at position 158 in the immunoglobulin-binding domain, which as a functional impact. Polymorphism in FCGR3A gene affects the binding affinity of the receptor to IgG, in the way that V158 haplotype increases affinity to IgG more than F158 haplotype. Consequently, more potent capture of IgG immune complexes may influence efficient presentation of arthritogenic antigens. Moreover, the FCGR3A-158V homozygosity was associated with twofold increased risk for RA in UK, North-Indian and Pakistan populations (87) and may confer the risk for autoantibody positive RA (88, 89). However, conflicting results from other groups have been reported (90), indicating ethnicity

divergence. Interestingly, there is high sequence similarity between all the five low-affinity FCGRs genes, which is most likely due to segmental duplications. One of the good examples is 98% sequence homology between FCGR3B and FCGR3A gene (91). Fascinatingly, the copy number variable (CNV) regions in FCGR locus have been linked to SLE (92-94). CNV can modify the receptor expression and binding ability of immune complexes. To this extent, presence of variable copy number of FCGR3B gene influences the expression level of receptor on the cell surface. In the studies presented by Robinson et al., deletions of FCGR3B was associated with lower phagocytosis and therefore reduced clearance of immune complexes especially in ACPA positive patients. Since FcγRIIIb is mostly expressed on neutrophils, its role in RA pathology was further strengthened (95). Existence of copy number variations and single nucleotide polymorphisms in low- affinity FCGR locus reveal the genetic complexity and need for the development of new technology strategies.

ANIMAL MODELS OF AUTOIMMUNE DISEASES

Rheumatoid arthritis is a heterogeneous disease where complex genetic factors and environmental elements interact with each other and participate in disease pathogenesis and progression. Complexity of the disease including different ethnicity, lifestyle, and various treatments of investigated populations and ethical reasons confer limitations for finding new genes driving autoimmunity and the underlying mechanisms. Therefore utilization of animal models became crucial. Although there is no single animal model that can completely recapitulate the multifactorial human disease like RA, there are already several models for arthritis available that provide the opportunity to analyse different disease phenotypes. Usually, animal models for autoimmune diseases are conducted in rodents due to their smaller sizes, large number generations per year, relatively big number of offsprings (strain dependent) and the most importantly the possibility of genetic alterations (knock-in, knock-out, transgenic strains) and, the chance for controlled environment. Therefore animal models provide an excellent opportunity to progress/improve our knowledge about molecular mechanisms of candidate genes as well to find new, successful therapeutics. Currently, several spontaneous and induced animals models for autoimmune diseases are available. It is important to note that arthritis in rodents might be induced by inoculation with various joint-specific antigens/cartilage components such as type II collagen (CIA), cartilage oligomeric protein (COMP), proteoglycan (PG), type IX collagen, type XI collagen, as well as oils (adjuvants), microbial products or antibodies (96). Some of the models that are relevant to my thesis will be presented in more detail below. Other broadly used models for arthritis include spontaneous models such as TNF-α transgenic mouse, K/BxN mouse and SKG mouse. Briefly, SKG model is environmental dependent and is absent in germ-free animal house. However, it can be induced by injection of zymosan in a Dectin-1 dependent manner (97). Susceptibility of SKG mice to arthritis is due to a mutation in ZAP 70 gene that alters the thymic T-cell selection (98). TNF-α transgenic mouse over expressing human TNF -α develops chronic, progressive polyarthritis and the administration of monoclonal antibodies against human TNF-α completely abrogates the disease. This model is particularly useful for evaluating the efficiency of novel therapies in RA (99).

COLLAGEN-INDUCED ARTHRITIS

Collagen-induced arthritis (CIA) is the most commonly used animal model that in many features resemble RA (100) and thus a preferred pre-clinical model for rheumatoid arthritis. It is induced by an intradermal immunization with collagen type II (CII) emulsified together with adjuvant, at the base of the tail. Clinical signs of disease typically occur approximately three to five weeks after initial immunization and histologically are characterized by infiltration of inflammatory cells, synovial hyperplasia, bone and cartilage erosion. The clinical picture of mice with arthritis is represented by swollen and reddish paws. CIA can be induced in many species including mice, rats and non-human primates (101, 102). Collagen is one the most abundant protein in the body and so far 28 types of collagen have been described. However 90% of collagens in the body are type I. Collagen type II is the major component of the joint cartilage and jelly-like substance that fills the eyeball (*vitreous humour*). It is synthesised by chondrocytes and composed of three $\alpha 1$(II)-chains that form triple helix. During CII biosynthesis posttranslational modification like hydroxylation and glycosylation occur (103). Similar to humans, the MHC encoding genes are important for CIA development. All the susceptible strains are predisposed to CIA due to the expression of MHC class II haplotypes H-2^q or H-2^r. By using the transgenic mice, the susceptible gene within the MHC region (called A^q) has been identified (104). The A^q molecule presents the immuno-dominant T cell epitope of CII (256-270) glycopeptide that confers susceptibility to CIA (105). CIA can be induced in predisposed strains with collagen of different origins, H-2^q haplotype strains are susceptible to autologous (mouse) or heterologous (rat, chick, bovine, human) type II collagen immunization (106, 107). The CIA induced by homologous collagen causes more chronic arthritic disease (107). Furthermore, H-2^r and H-2^b haplotype bearing mice develop arthritis upon immunization only with bovine collagen. The same sequence of immunodominant CII epitope (CII256-270) is shared between rats, human, chicken, and bovine species but vary with one amino acid at position 266 (aspartic acid instead of glutamic acid) in mouse that alters peptide recognition and affinity binding (108). This finding indicates why heterologous CII peptides induce more severe disease than mouse CII. In paper II we have used CII of three different origins: chicken, rat and bovine for immunization of the heterogeneous stock mice. It is important to stress that several other non-MHC related loci controlling CIA susceptibility in mice have also been found, such as *Ncf1*, C5 and FcγR cluster of genes.

Discovery of the non-MHC gene, *Ncf1* regulating arthritis has shown the advantage of utilization of animal models in the detection of the genes with a small effect. *Ncf1* gene encodes the p47phox protein, a subunit of phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidative complex. At first, it was positionally cloned using pristine-induced arthritis model in rats (109). DA rats showed lower production of reactive oxygen species (ROS) and developed a more severe arthritis compared to E3 strain. Similar results were replicated in mouse strain with a spontaneously occurred *Ncf1* gene mutation (110). Recently, the causative SNP that impairs the function of *Ncf1* gene in DA rats has been mapped (111). Thus, reduced ROS production increases the severity of arthritis followed by an elevated titre of antibodies and the production of pro-inflammatory cytokines. In the paper V we have investigated whether the influence of mutated *Ncf1* gene on arthritis severity is independent of classical adjuvant.

COLLAGEN ANTIBODY INDUCED ARTHRITIS

As mentioned before, RA is associated with autoantibody production against self-proteins such as CII, citrullinated proteins (ACPA) or IgG (RF). The role of autoantibodies in disease pathology was highlighted by the fact that serum taken from arthritic mouse that has been immunized with CII or from a RA patient can induce arthritis in non-immunized recipient (112, 113). Moreover, arthritis can be also be induced in naïve mouse by transfer of a cocktail or with a single mAb pre-formed anti-collagen monoclonal antibodies, so called CAIA (collagen antibody induced arthritis) (114-116). The first clinical sings of arthritis in antibody transfer model appears within 24-48h after the antibody administration and they resemble acute phase of CIA. In contrast to CIA, CAIA model is transient and usually mice after one month became normal again. To enhance the severity and incidence of CAIA, single injection of lipopolysaccharide (LPS) is used. Disease susceptibility is MHC independent (112, 114). Moreover, CAIA can be induced in the absence of either T cells or B cells indicating no influence from adaptive immune system. On the other hand T and B cell knockout mice develop less severe arthritis, which implies regulatory effect of these cells (117). Requirement of neutrophils and macrophages is essential for the disease development (118) as well as complement proteins and FcγRs. CAIA model is highly reproducible and provide valuable information about the effectors phase of arthritis without involving the priming pathway. This model reflects the contribution of humoral immunity in the development of arthritis. Monoclonal antibodies used in this thesis were previously characterized and they are presented on the Figure 1.

Isotypes			Name of mAbs	
γ1			M284	
γ2b	CB313, CB28	UL-1	M2139	CIIC2
γ2a	CIIC1, CB26		M287	

Epitopes:	C1: 358-369	U1: 494-504	J1: 551-564	D3: 687-698
Sequence:	GARGLTGROGDA	LVGPRGERGFP	MPGERGAAGIAGPK	ARGAQGPPGATGFP

Figure 1. Monoclonal antibodies used in arthritis experiments for this thesis

GLUCOSE-6-PHOSPHATE ISOMERASE INDUCED ARTHRITIS

Glucose-6-phosphate isomerase (G6PI) is an enzyme of the Krebs cycle and is ubiquitously express in the body. More than 15 years ago, by chance Kouskoff et al (119) have discovered a new model of spontaneous arthritis, so called K/BxN model. It was found in the cross of bovine ribonuclease specific T- cell receptor transgenic C57BL/6 mouse with the $H2^{g7}$ carrying NOD mouse. The offsprings developed severe and destructive arthritis with the production of high titter of G6PI-specific antibodies (120). Since then research has turned towards understanding the role of G6PI in arthritis development. However, it was still mysterious why response to a universal glycolytic enzyme is so joint specific. Further studies revealed that arthritis might be induced by injection of serum from arthritic mice or by immunization with human G6PI protein as well as with human $G6PI_{325-339}$ peptide (121-123). This model is dependent on the presence of T-cells, B-cells, and complement components as well as FcγR effector cells. In paper II, we have investigated if heterogeneous stock mice are sensitive for arthritis induced by recombinant G6PI.

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Experimental autoimmune encephalomyelitis (EAE) is largely used an experimental animal model that mimics human multiple sclerosis (MS). EAE can be induced in many species including rat, mouse, guinea pig, rabbit and monkeys. EAE can be induced actively by immunization with the emulsion consisting of one of the following antigens: purified myelin, spinal cord homogenate (SCH – **paper II**), myelin proteins or peptides: myelin basic protein

(MBP), proteolipid protein (PLP), oligodendrocyte protein (MOG – **paper II**) and adjuvant usually CFA and followed with injections of two doses of pertussis toxin that allows to open the brain-blood barrier. The passive induction of EAE may be achieved with an adoptive transfer of encephalitogenic T cells. EAE is a T-cell mediated autoimmune disorder since B cell knockout mice can develop severe EAE. However the role of B cells in EAE pathogenesis is elusive due to their dualism. B cells produce autoantibody binding to myelin antigens leading to destruction of the nervous system but they could also play a regulatory role during the recovery phase. First symptoms of the disease appear few days after the immunization and they are recognized by tail weakness following by successive paralysis of the body starting from hind limbs, front limbs leading to tetra paralysis that may eventually cause death.
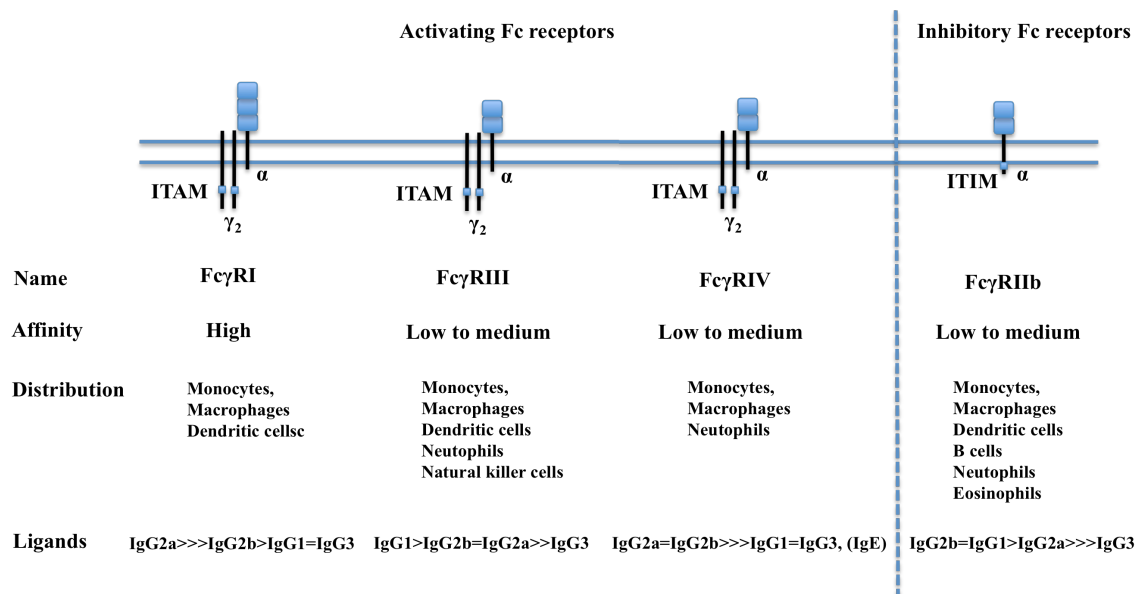
FCγ RECEPTORS

So far four FcγRs have been described in mouse genome: FcγRI (CD64), FcγRIIb (CD32) and FcγRIII (CD16) and the recently discovered FcγRIV that can be divided into two major classes: activating (FcγRI, FcγRIII, FcγRIV) and inhibitory (FcγRIIb). On the basis of sequence similarity in extracellular domain of mouse and human receptors, FcγRI are orthologous, as the same applied for FcγRIIb, whereas FcγRIII seems to be an orthologue with human FcγRIIA, while FcγRIV was proposed to be an orthologue with human FcγRIIIA. Both human and mouse FcγRs are the result of duplications and diversification and are characterized by high sequence homology. Similar to human, mouse FcγRs are present on chromosome 1 (except the FcγRI in chromosome 3) and are located in close distance to each other, the so-called a cluster region in proximity to the family of the FcR-like genes. The work in this thesis is focused on the mouse low-affinity receptors (FcγRIIb, FcγRIII, FcγRIV) and they will be described in greater detail.

STRUCTURE OF FCγRS

All FcγRs are glycoproteins. Majority of FcγRs (FcγRII, FcγRIII, FcγRIV) have two C2-type extracellular Ig domains whereas FcγRI consist one additional domain, which was claimed to drive the high-affinity binding of this receptor (124). The extracellular part of Fc receptors shows high sequence similarities but they differ in their transmembrane and intracellular domains, which are responsible for intracellular signalling. Only one of the two extracellular domains of FcγR is in contact with Fc part of IgG (125) and thus the interaction of FcγR-IgG is in 1:1 stoichiometry (126). FcγRIIb contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) whereas FcγRIII and FcγRIV contain immunoreceptor tyrosine-based activatory motif (ITAM) in the intracellular domain. The activating FcγRs are dimmers consist of a ligand-binding α-chain and the common γ-chain (carrying ITAM motif) while inhibitory FcγRIIb is a single chain receptor (127). The common γ-chain plays an important role not only in passing on the activating signals but also as an essential mediator in the expression of activating FcγRs and thus FcγRI, FcγRIII and FcγRIV are dependent on this receptor subunit expression for activation. Deletion of the common γ-chain leads to loss of function of all the activating receptors. Moreover loss of function in other unrelated Fc receptor proteins have also been described (128). Studies using γ-chain knockout animals revealed impaired antibody–mediated effector mechanisms, like phagocytosis of immune complexes (ICs) and ADCC.

SPECIFICITY, AFFINITY AND DISTRIBUTION

The harmonious expression of inhibitory and activating receptors on the same cell is important for the creation a balanced immune response. Furthermore, recent studies have highlighted the divergent roles of FcγRs depending on their affinity to IgG subclasses. With the exception of the FcγRI that binds with a high affinity (10^7-10^9 M^{-1}) both monomeric IgG as well as IgG molecules in the form of immune complexes, the remaining activating FcγRIII and FcγRIV and inhibitory FcγRIIb receptors have lower binding affinities (10^6-10^7 M^{-1}) and bind mainly ICs (129). Despite containing the low affinity receptors to IgG, the cell, which expresses numerous FcγRs are in fact has high avidity to IgG-opsonised pathogen. There are four different IgG subclasses in mice: IgG1, IgG2a, IgG2b, IgG3 that have diverse binding affinities for the activating and inhibitory FcγRs. Better understanding of the efficacy of the IgG subclasses in mediating the immune response was made by determining the A/I ratio, the ratio of affinities of a given IgG subclasses for activating FcγRs to inhibitory FcγRIIb (130). For example IgG1 has the lowest A/I ratio of 0.1, indicating that function of this isotype is severely regulated by FcγRIIb. The FcγRs are widely expressed on the haematopoietic system.

	Activating Fc receptors			Inhibitory Fc receptors
	ITAM — α — γ₂	ITAM — α — γ₂	ITAM — α — γ₂	ITIM — α
Name	FcγRI	FcγRIII	FcγRIV	FcγRIIb
Affinity	High	Low to medium	Low to medium	Low to medium
Distribution	Monocytes, Macrophages Dendritic cellsc	Monocytes, Macrophages Dendritic cells Neutophils Natural killer cells	Monocytes, Macrophages Neutrophils	Monocytes, Macrophages Dendritic cells B cells Neutophils Eosinophils
Ligands	IgG2a>>>IgG2b>IgG1=IgG3	IgG1>IgG2b=IgG2a>>IgG3	IgG2a=IgG2b>>>IgG1=IgG3, (IgE)	IgG2b=IgG1>IgG2a>>>IgG3

Figure 2. Mouse Fc receptors for IgG, expression and ligands (based on (130-133)).

Macrophages and monocytes express both inhibitory and activating receptors, whereas DCs express mainly FcγRI, FcγRIV, and FcγRIIb, whereas neutrophils dominantly express FcγRIII

and FcγRIV and inhibitory FcγRIIb. The are two unique cell types that exclusively express just one type of FcγRs, they are NK cells that express activating FcγRIII and B cells that express FcγRIIb. The summary of this paragraph is presented in Figure 2.

NATURAL POLYMORPHISM IN MOUSE FCγRS GENES

Functional imbalances in the activating and inhibitory Fc receptors due to genetic variations predispose to the development of autoimmune disorders. Several autoimmune-prone inbred mouse strains such as MRL, NZB, BXSB, 129, NOD carry the polymorphism in promotor region of FcγRIIb that reduces the expression level of the receptor on B cells and macrophages (134, 135). Moreover, the polymorphism in FcγRIIb resulting in two variants of *Ly17.1* and *Ly17.2* has been described. However there is no evidence that this coding region polymorphism influences the receptor functions (136). In addition FcγRIII exists in three different haplotypes: FcγRIII:V, FcγRIII:H and FcγRIII:T. It was shown that mice that carry the FcγRIII:H haplotype are more susceptible to arthritis than mice with FcγRIII:V haplotype (137). Comparison of FcγR polymorphisms occurring in NOD and B10.Q strains are presented in Table 1.

Receptor	NOD	B10.Q
FcγRIIb	Ly 17.1 Deletions in the promotor region	Ly17.2
FcγRIII	H:haplo	T:haplo
FcγRIV	Ile57	Thr57

Table 1. The summary of known polymorphisms in NOD and B10.Q strains (133, 137, 143-145).

Additionally, by using knockout approaches, it is possible to elucidate the contribution of each of the FcγR in autoimmune disorders. For example, FcγRIIb deficiency renders normally resistant H-2^b strain of mice susceptible to CIA (138). Moreover, FcγRIIb knockout mice showed increased susceptibility to CAIA (115) and they are predisposed to develop spontaneous hypergammaglobulinemia, production of anti-chromatin antibodies(139) and impaired removal of immune-complexes resulting in severe glomerulonephritis mimicking human SLE(140). On the other hand mice that are deficient in common γ-chain or in FcγRIII

are highly resistant to CIA, even though anti-CII Ab production is comparatively normal in these mice(141, 142).

FEEDBACK REGULATION

The inhibitory FcγRIIb is the most abundantly expressed FcγR. It is present on all leukocytes, except T cells and NK cells (Figure 2) and exists in two different forms: FcγRIIb-1 and FcγRIIb-2. The FcγRIIb-1 is solely expressed by B cells whereas FcγRIIb-2 is found on remaining cell populations and has the ability to induce endocytosis or phagocytosis via receptor cross-linking. Thus, FcγRIIb plays an important role as the negative regulator in innate and adaptive immune responses. FcγRIIb expression level seems to be setting the threshold level for B cell activation. Additionally, it acts as the checkpoint during later peripheral B cell development, including the exclusion of low-affinity autoreactive B cells (146), preventing the expansion of IgG positive auto-reactive plasma cells. Furthermore, it has been suggested that FcγRIIb can also regulate the plasma cell survival and apoptosis. The FcγRIIb is highly expressed on terminally differentiated plasma cells, but BCR expression is very low or absent. Apoptotic signal of plasma cell is triggered by FcγRIIb upon cross-linking with the immune complexes. Interestingly, autoimmune-prone mice, which have significantly reduced level of FcγRIIb, are protected from apoptosis. Hence, the failure in plasma cell survival may influence their higher abundance (147, 148). As mentioned before, the FcγRIIb is expressed widely on innate immune effector cells (mast cells, granulocytes, macrophages) and controls their antibody-mediated responses such as phagocytosis, antibody-dependent cellular cytotoxicity (ADCC) and release of pro-inflammatory mediators. However, its negative regulation is strictly dependent on the IgG isotype bound to the inhibitory receptor and is determined by already described A/I ratio. Another crucial function of inhibitory receptor is the regulation of DC activation and maturation. Several groups have already described the role of immune complexes bound to DC as an enhancing factor in antigen presentation process (149, 150). In addition, expression of FcγRIIB on DC regulates immune-complex mediated maturation, which was exemplified by spontaneous maturation of DCs derived from FcγRIIb deficient mice (151).
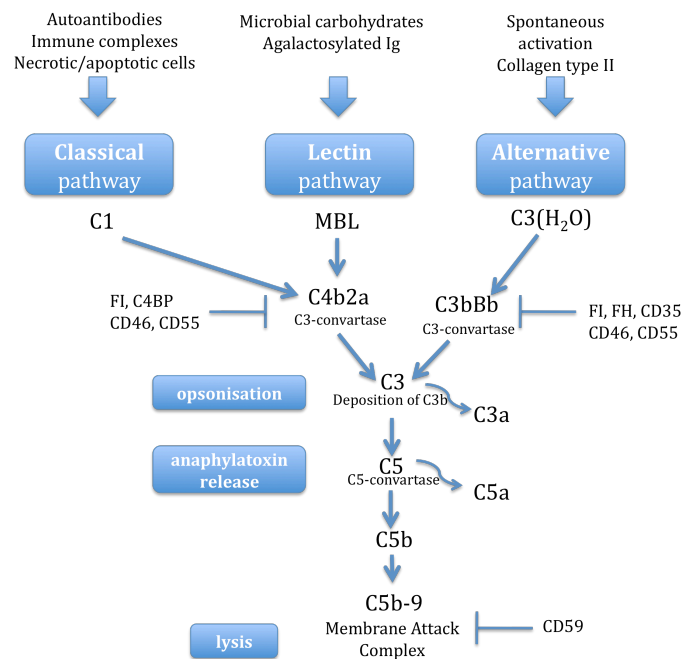
COMPLEMENT SYSTEM

Complement system is a key component of innate immunity contributing to inflammation but it also plays an immuno-regulatory role in the modulation of adaptive immune responses (152, 153). Complement can be activated by three different pathways: classical, mannose-binding lectin and alternative that are depicted on Figure 3. Complement system consists more than 30 plasma and cell surface-associated proteins (154), which are constitutively synthesized in the liver and secreted into the circulation. However, pro-inflammatory cytokines like IL-6, TNF-α or IFN-γ stimulate the production of complement components in the macrophages(155, 156). Activation of the complement proteins occurs through the catalytic cascade where the activation of one protein leads to the proteolytic cleavage of the next resulting in the amplification of the signal. Once the cascade is activated, complement components can be involved in opsonizing the invading pathogens and tagging them for phagocytosis (C3b, C4b), attracting the leukocytes (C3a, C5a) or direct killing the invaders through the assembly of membrane attack complex (MAC, C5b-C9). Moreover, it has a crucial role in the clearance of immune-complexes (C3b and C4b). Thus, lack of C3 protein may lead to accumulation of immune complexes in the tissue, which subsequently can cause inflammation. C3a and C5a are very potent chemo-attractants that can recruit neutrophils, phagocytes and eosinophils to the site of inflammation, stimulate the production of pro-inflammatory molecules and enhance vascular permeability (157-159). Furthermore, anaphylatoxins like C5a and C3a play a crucial role in de-granulation of the mast cells, eosinophils and basophils. Since the activation of complement cascade is rapid in action, it needs to be tightly controlled; therefore various plasma soluble/membrane-bound complement inhibitors are present in the host to protect the tissue from destruction (160).

ROLE OF COMPLEMENT IN RA

Contribution of complement to the development of inflammatory diseases such as acute respiratory distress syndrome (163), sepsis (164), glomerulonephritis (165), asthma (166) and rheumatoid arthritis (167) have been described. The common feature of these diseases is accumulation of neutrophils that have been attracted by the anaphylatoxin, C5a. Activation of neutrophils triggered by binding of C5a to C5aR cause the release of reactive oxygen species (ROS) that subsequently may lead to tissue damage. Moreover, it was shown by Shushakova et al (168) that C5a can modulate the expression level of both FcγRIII and FcγRIIb and thus enhance the autoimmune response. As mentioned before, RA is characterized by elevated levels

of immune complexes (169) that may potentially activate the complement proteins. Indeed, there are ample evidences of complement activation in synovial fluid of patients with RA. Furthermore, increased consumption of C3 and C4 (170) as well as high concentrations of anaphylatoxins C3a and C5a in synovial fluid of RA patients have been reported (171, 172). It is not only the increased activation of complement components but also their deficiency is associated with more pronounce inflammation (160, 173). Hence, damage of the tissue caused by complement than the complement activation itself is the key factor in the manifestation of many diseases.

Figure 3. Simplified scheme of activation pathways of complement system and its inhibitors (Based on (161, 162)).

To date, it is still under debate, which pathway of complement activation is more relevant in RA. The possibility of alternative pathway as the main trigger could be explained by the elevated concentrations of Bb fragment present in the synovial fluid of RA patients. On the other hand, classical pathway may be triggered through the binding of various immune complexes present in the serum and synovial fluids of RA patients (174). However, the contribution of the lectin pathway to RA is not conclusive (175, 176).

ROLE OF COMPLEMENT IN ANIMAL MODELS

The role of complement in arthritis can be determined by utilization of genetically modified animals. It was shown that C3 or factor B knockout mice are partially resistant to CIA (177),

which indicate that both the classical and alternative pathways are likely to be involved in arthritis. However, Banda et al (178) have shown that only alternative but not classical pathway has a major impact on CIA development. Thus, importance of both pathways in CIA is not yet clear. Furthermore, C3 and factor B deficient mice developed less severe arthritis with delayed onset after injection with anti-collagen monoclonal antibodies (179). In relation to C5 protein, deficiency or administration of neutralizing antibodies prevent CIA and ameliorates already established disease (180, 181). In paper IV we have shown that Cia2 congenic line that is deficient in C5 is resistant to CAIA and CIA development. The Cia2 locus has been also identified in the F2 cross between DBA/1 and SWR/J strain (182). The deficiency in SWR/J and NOD strain is cause by 2bp deletion in exon near the 5'end of the Hc gene that results in the frame-shift and early termination causing C5 deficiency (183). Furthermore, the importance of complement components was linked with spontaneous K/BxN arthritis model. Utilization of different crossing of K/BxN and knockout mice for various components revealed that this model is dependent on alternative pathway and the tissue destruction is not due to MAC assembly (184). Moreover, C3 depletion by cobra venom factor results in diminish antibody response and therefore complement system has been shown to have regulatory function on humoral immune response.

SYNTHESIS OF COMPLEMENTS COMPONENTS

The most of complement components are synthesized by hepatocytes. However, it has been shown by Brodeur et al (174) that synthesis of complement factors can occur also within rheumatoid synovium. In fact all the normal tissues are able to produce at least one component of the complement system (185). All different cell types have been shown to synthesize complement components including fibroblast, phagocytes, endothelial cells, alveolar type II epithelial cells and adipocytes (186, 187). It was demonstrated that cells of synovial membrane (particularly fibroblast, phagocytes, endothelium) could produce complement components C2, C3, C4 and C5. In another study, using *in situ* hybridization method, increased expression of mRNA for factor B, C3, C3aR and C5aR in synovial tissue of RA patients was reported (188). This data suggests that during inflammatory conditions like RA, complement components are rather produced locally in the synovium than derived from plasma. However, our findings using bone marrow transfer experiment showed that liver could be the main source of complement C5 even under inflammatory conditions (**paper IV**).

IDENTIFICATION OF GENES

Identification of genes involved in the disease pathogenesis is tedious and complex both in humans as well as in animal models. One of the advantages of using animal models is the possibility to identify genes with small effects compared to mapping them in human studies. Moreover the animal's genome can be modulated. Therefore animal models provide the excellent tool to gain the knowledge about mechanism and pathways leading to disease development. Identification of the genes consists of two steps. Initially, two or more strains that differ genetically in respect to the investigated trait are being crossed and linkage analysis that correlates the complex phenotypes to specific regions of chromosomes, quantitative trait loci (QTL) is being applied. The next step aimed to narrow down the chromosomal region to the smallest size as much as possible, ideally containing only one gene, which is called positional cloning. Since this is almost impossible to achieve (for example FcγRs cluster region in **paper III**), one needs to find a specific phenotype for the gene such as functional assays or knockout animals (189).

INBRED STRAINS

First inbred strain, DBA was created by C.C Little in early twentieth century. Inbred strains are made by sister-brother mating for more than 20 generations resulting the mice being as much alike as possible and carrying around >99% of homozygosity at all the loci. Although there are more that 450 inbred stains available, only those well characterized are used in experiments. Each inbred strain has unique sets of characteristics that differ from all other inbred strains. They are often selected for their specific phenotypes, for example C57BL/6 has increased preferences for narcotics and alcohol, whereas 129 strain is widely used in transgenic technology (190-192). Therefore inbred strains provide valuable tools for developing animal models for human diseases.

INTERCROSSES AND BACKCROSSES

The most common way to achieve the identification of the disease-causative genes is to cross two inbred strains that represent diverse phenotypes. The first cross generates F_1 offspring where all the animals are genetically similar and heterozygous for all the loci. Then, F_1 individuals can be either intercrossed (F_2) or backcrossed with one of the parental inbred strain (N_2). Now, the genome is shuffled and chromosomes have mosaic structures containing the

fragments inherited from both the parental strains. The F_2 generation provides the information of disease fashion (recessive, additive, dominant) whereas N_2 generation is more powerful in detection of dominant genes (189). Both animals of F_2 or N_2 generations carry unique genotypes due to recombination. For that reason, all animals need to be phenotyped as well as genotyped. Several types of markers can be used, including single nucleotide polymorphisms (SNPs) or simple sequence repeats (SSRs or microsatellites). Usually, QTL detection is performed by genome scan with 100 markers (193). Two-generation crosses have been used to detect most of the CIA loci. The Cia2 and Cia9 loci earlier investigated and further studied in this thesis were identified in crosses between arthritis resistant strain – NOD and the intermediate susceptible strain – B10.Q (194).

CONGENIC STRAINS

Since, identified QTL contains hundreds of genes and it is unlikely to pinpoint the disease-causative gene at this stage. Therefore, the next step is to isolate the QTL into congenic strain. This is obtained by backcrossing of the desired QTL to the background strain for more that 10 generations resulting in 99.9% purity of the recipient genome. Congenic strain has either a chromosome region from disease-resistant strain that replace the same region from a susceptible strain (Cia2 and Cia9 congenic strains described in **papers III and IV**) or reciprocally when the disease-susceptible region is introduced in to the resistant background. This process is time consuming and it can be shortened by speed congenic approaches, that is based on marker-assisted genotyping of each offspring and selecting those individuals with the least contaminating fragments from the donor genome. The number of backcrossing is strictly dependent on the number of screened individuals and the number of markers used for genotyping. With speed congenic technique, it is possible to shorten the time to six backcross generations. Each backcross generation needs to be phenotyped to confirm the presence of investigated QTL. Once the congenic strain is "ready", the locus can then be further dissected into smaller sub-congenic lines and characterized. Therefore testing the sub-congenic lines for disease susceptibility will help to reduce the size of the causative interval and hopefully pinpoint the gene. The strategy of generation of sub-congenic lines used in *Cia9* project and testing mice with several smaller overlapping gene regions/intervals made us possible to exclude big parts of the original locus.

HETEROGENOUS STOCK MICE

Heterogeneous stock cross is an advanced intercross of several inbred strains that have been bred for large number of generations and were kept in pseudo-random breeding scheme to achieve high density of recombinations in each individual. In the present investigation, we used Northport HS stock mice that was generated by crossing eight inbred progenitor strains: A/J, AKR/J, BALB/CJ, LP/J, CBA/2J, C3H/HeJ, C57BL/6J and DBA/2J for more than 55 generations, which resulted in mosaic structure of each chromosome. The average distance between the recombinations is less than 2 cM (195), which allows for high resolution mapping of small-effect QTLs (195-197). However, the high level of recombination requires high number of markers that need to be very closely spaced for genotyping and the use of a robust statistical method (HAPPY). In order to use the HS cross for genome wide locus identification, large number of animals are required due to increased number of alleles and higher variability in the HS population. In paper II, HS mice were screened for susceptibility to autoimmune disease models like arthritis (CAIA, CIA, GPI-induced arthritis) and EAE induced by spinal cord homogenate of different origins or MOG peptide. It is clear that to be able to induce CIA in HS stock, arthritis prone MHC H2^q haplotype needs to be introduced. Therefore, HS stock animals were crossed with C57Bl/10Q (B10.Q) mice. In paper I, we used more than 1700 HS x BQ mice to study CIA development. All the mice were phenotyped, genotyped and analyzed in detail. As a result, we were able to find 18 new loci as well as fine mapped several already known loci like *Cia9* (**paper III**) and *Cia2* (**paper IV**).

"SMART POLYMERS" AS ADJUVANTS

As described above, most of animal models for autoimmune diseases use either adjuvant alone or in the formulation with an antigen to induce disease. Usually, the antigens are emulsified in mineral oil with or without mycobacterium, called as CFA (complete Freund adjuvant) or IFA (incomplete Freund adjuvant) respectively. The pathogen-associated molecular patterns (PAMPs) present on the mycobacterium or other bacterium derivatives can be recognized by pathogen-recognition receptors (PRPs) like Toll-like receptors (TLRs) expressed on the antigen-presenting cells (APC) (198). Addition of an adjuvant in an immunization emulgate enhances, modulates, generates long-lasting antigen depots and sustains the immune response against the antigen. However, they have strong ability to distort the immunological mechanisms and thus the real response to the self-antigen can be misinterpreted. In additions, some of the adjuvants contain toxic compounds. Therefore searching for adjuvants that are able to modulate immune response in a more controlled way and allow us to study the actual immune responses are in great need. Recently the interest of synthetic functional polymers has been highlighted. These polymers posses a unique feature to respond to a change in environment like temperature, pH, electric or magnetic field. They are called "stimulus responsive" or "smart polymers" (199).
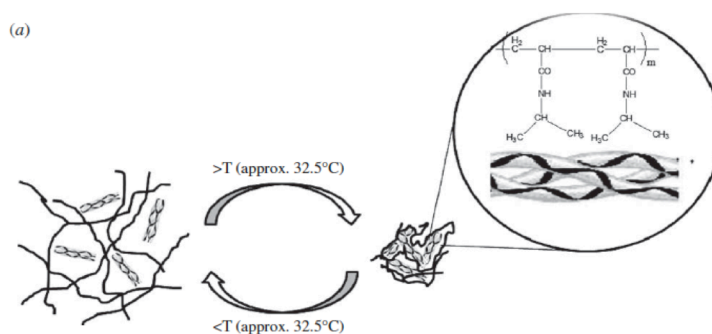
Figure 4. Schematic picture of interactions between the thermo-responsive PNiPAAm and CII (Adapted from Shakya et al (200)).

Smart polymers undergo reversible changes in microstructure from hydrophilic to a hydrophobic state. In the macroscopic view, it will be visible, as a precipitate formation from the solution. For example, one of the thermo-responsive polymers; poly(N-isopropylacrylamide) PNiPAAm, has a lower critical solution temperature (LCST) of precipitation around 32.5°C in solution and changes reversibly from hydrophilic below the

critical temperature and hydrophobic above it (Figure4). PNiPAAm is a biocompatible and biodegradable polymer. This all attributes of thermo-responsive PNiPAAm have suggested a possibility of their use for the formation of colloidal suspension with an antigen. Indeed, Shakya *et al.* (200) has shown that PNiPAAm can act as a polymeric adjuvant in collagen-induced arthritis and lead to arthritis development with the induction of CII-specific antibody response in all the immunized mice. Mice that developed arthritis after immunization with PNiPAAm-CII have shown a massive infiltration of the immune cells in the joints and significant damages of cartilage and bone structure. Moreover, PNiPAAm-Ova immunization did not skew the cytokines response towards any one of T-helper cell populations, suggesting the possibility to apply PNiPAAm as a general adjuvant and study the actual immune response to the antigen (200). Recently, another synthetic polymer poly(lactide-co-glycolide) PLGA, has been used to study the sustained-release of steroidal treatment in adjuvant induced arthritis (201). In the **paper V,** we have further characterized the arthritis induced by CII mixed with the new polymeric PNiPAAm adjuvant, including cytokine profile, genetic background, TLRs and *Ncf1* gene influence.

PRESENT INVESTIGATIONS

The papers included in this thesis have focus on identification of QTLs, the gene's polymorphisms and mechanisms that lead to arthritis development. We have used different genetic approaches to achieve this complex task.

PAPER I

Background - In paper I, we have used the heterogeneous stock mice, an advanced intercross lines between eight inbreed strains of mice. Since HS mice were crossed for many generations, which resulted in grained-mosaic structure of each chromosome, it was possible to map CIA QTLs with high resolution.

Method – To be able to induce CIA in HS cohort, we used the variant of the inbred-outbred cross between HS mice and B10.Q mice. Homozygous $H2^q$ mice were used to set up 81 breeding cages that produced 1764 F_3 mice. We have used these mice in CIA experiment.

Results – We identified 26 loci controlling arthritis development involving arthritis incidence, severity and time of onset. Among them 18 loci are new. In addition, we were able to fine-map several already known loci, including *Cia2* and *Cia9*. Several, of the novel loci contain small number of candidate genes and therefore are good starting point for positional cloning efforts. Interestingly, for the first time, using both mixed model and single-marker association analysis, we could map the *Cia2* locus to *Hc* gene, since all the identified SNPs are within the gene. This finding indicates the precision of HS strategy. However, we could not exclude the neighbouring gene *Traf1* despite the high resolution gene regions in the HS mice. Nevertheless, we could still confirm the contribution of *Cia9* locus in arthritis with the peak marker situated within the Fc-receptor cluster.

Conclusions – The results obtained in this paper suggest that HS strategy can provide an excellent opportunity to fine map already known disease loci and also to find novel QTLs in complex diseases, such as arthritis.

PAPER II

Background – The heterogeneous stock mice have proven to be useful in the identification of new and fine mapping of already known QTLs. Therefore, in paper II we investigated ordinary heterogeneous stock mice for their susceptibility in animal models of different autoimmune diseases like arthritis and encephalomyelitis.

Method – We screened the Northport HS mice for three models of arthritis: CIA induced with collagen of different origin (bovine, rat, chicken), CAIA induced by 4 monoclonal antibodies and G6PI induced arthritis. We investigated EAE susceptibility induced either with mouse spinal cord homogenate (SCH), rat SCH or MOG.

Results – We found that HS mice are resistant to arthritis induced with rat and bovine collagen as well with G6PI protein. However, very low arthritis incidence after immunization with chicken collagen was observed (2 out of 68 mice). Regarding CAIA model, HS mice developed arthritis with a high frequency (50%). In addition, we showed that HS mice are prone to EAE induced by all three antigens (mSCH, rSCH and MOG).

Conclusions – From this study we can conclude that HS mice could be useful for identification of new QTLs involved in encephalomyelitis as well as at the effector phase of arthritis.

PAPER III

Background – Results from previous gene segregation experiments between B10.Q (intermediate susceptibility to arthritis) and NOD.Q mouse strains (arthritis resistant) identified two loci contributing to CIA, one disease-promoting locus (*Cia9*) on chromosome 1 and another disease protective locus (*Cia2*) on chromosome 2. Later, these gene regions were confirmed for their influence on arthritis using backcross experiments. NOD allele at *Cia9* locus promotes arthritis and the linkage peaked at the location of the FcγR region. In this study, we postulated that within the *Cia9* locus, Fc gamma receptor cluster might be the causative gene region involved in arthritis development.

Method – To be able to identify the underlining genes, the big (165-175Mbp) *Cia9* gene region has been split up into smaller sub-congenic regions in various mouse strains to be able to localize the genes. Three different overlapping sub-congenic lines were created: *Cia9c*, *Cia9b* and *Cia9i*. All sub-congenic mice were tested for susceptibility to arthritis phenotypes using CIA and CAIA arthritis models.

Results – *Cia9c* congenic line devoid of FcγR cluster genes but contains SLAM locus (173-175Mbp) showed no influence in arthritis development. Similarly, *Cia9b* congenic line, which cover region (165-172.6 Mbp) above of FcγR cluster, is also not susceptible to either CIA or CAIA. Interestingly, *Cia9i* fragment (170.9-173.4 Mbp), which encloses three Fc gamma receptors showed a significant difference in CIA severity and incidence. Similarly, mice having *Cia9i* fragment are also highly susceptible to CAIA induced by four monoclonal antibody

cocktail (M2139+CIIC1+UL1+CIIC2). Interestingly, we demonstrated that *Cia9i* strain showed differential IgG subclass dependency in arthritis development.

Conclusions – This paper demonstrates the importance of dissecting QTL to reveal genetic effect of complex traits. This study supports our hypothesis that augmented susceptibility to arthritis of *Cia9i* mice can be due to one or both of FcγRIIb and FcγRIII genes. However more functional studies are needed to identify the causative gene.

PAPER IV

Background – In paper IV we focussed on the arthritis protective *Cia2* locus that was found in chromosome 2 in the B10.Q x NOD.Q cross. The promising candidate in *Cia2* locus is complement component C5 encoding *Hc* gene as the NOD allele is deficient for C5 due to the 2 bp deletion in an exon near the 5´end. We generated sub-congenic mice having *Cia2* locus in the B10.Q genetic background. In the present study, we investigated the source of complement C5 production in arthritis. It is known that liver produces most of complement proteins including C5 but under inflammatory conditions macrophages are also involved in C5 secretion.

Method – In this paper, we used two different genetic strategies: congenic approach and heterogeneous inbred-outbred cross. *Cia2* sub-congenic fragments were tested for CIA and CAIA susceptibility. Additionally in order to address the importance of C5 source in arthritis development, we performed bone marrow transfer experiments using *Cia2* congenic animals. We transferred bone marrow from C5 deficient congenic animals into B10.Q and also reciprocal transfers. We have investigated the activation of two complement components pathways by evaluating C3b deposition.

Results –We also have shown that congenic mice, which lack C5 are resistant to arthritis induced by CAIA or CIA. *Cia2* locus completely suppressed arthritis development in a dominant fashion. Moreover, hemolytic activity is significantly diminished in C5 deficient mice compared to littermate controls despite sufficient classical and alternative pathway of complement activation. Moreover, only B10.Q mice, which received the bone marrow from B10.Q or from C5 deficient mice developed arthritis. The reciprocal transfer revealed resistance. Further, the analysis of heterogeneous stock cross confirmed the fine mapped *Cia2* locus in arthritis development.

Conclusions – This studies supports the common dogma that hepatocytes are the main C5 producers even under inflammatory conditions.

PAPER V

Background – Poly(*N*-isopropylacrylamide) (PNiPAAm) is biodegradable and biocompatible, thermo-responsive polymer. Previously it was shown that PNiPAAm could be used as an adjuvant in several immunological applications, including collagen induced arthritis in mice. Therefore, in paper V, we further characterized and evaluated its properties using several different mouse strains.

Method – PNiPAAm was synthesized through free radical polymerization. For CIA induction we immunized mice with an equal mixture of collagen type II and PNiPAAm (ratio 1:1). Different mouse strains bearing various MHC haplotypes (H-$2^{q,d,p \text{ or } b}$) as well as *Ncf1* mutated were immunized and re-evaluated for susceptibility to arthritis using this new polymeric adjuvant. We also investigated the influence of Toll-like receptors using a panel of TLR-KO mice. Since all the TLR-KO animals are in B6 background that are not susceptible to CII-PNiPAAm arthritis, we investigated the anti-CII antibody response. Additionally, we investigated arthritis susceptibility in CII specific T cell, Vβ12 transgenic mice.

Results – Immunization with CII and PNiPAAm induced serum cytokine production that indicated no major deviation toward any of the three major T helper cell populations. The new arthritis mouse model is toll-like receptor independent, since all immunized TLR KO mice developed an anti-CII response. We observed that mice with $H2^q$ haplotype were highly susceptible to arthritis compared to strains carrying other MHC haplotypes. Moreover, our results emphasized the requirement for a strong adjuvant for induction of arthritis in B6 mice. Additionally, we showed that CIA severity in *Ncf1* mutated mice is independent of any classical adjuvant and arthritis induction in Vβ12 transgenic mice could be eosinophil-independent.

Conclusions – The biocompatible and biodegradable PNiPAAm offers an unique opportunity to study the actual immune response to a self-protein, which is not biased towards any particular cytokine profile, and it's TLR-independent.

CONCLUDING REMARKS

So far, genetic studies have led to identify a number of QTLs that influence the development and pathogenesis of arthritis. However the precise identification of causative genes and mechanisms behind them seem to be more complicated. In the present thesis we have used two different genetics strategies that could help to resolve the complex task of finding genes. Utilization of two-generation crosses strategy very often results in large genomic fragments containing huge number of genes and then isolation of identified QTL into congenic lines is tedious and time-consuming process. However, if it is successful will definitely lead to better understanding of underlying mechanisms and therefore may be helpful in designing new therapies. Present work has focused on two loci *Cia2* and *Cia9* that have been found in two-generation cross strategy. By using the several sub-congenic lines for the original *Cia9* locus, we were able to exclude significant part of the genetic fragment and narrow down the locus to few genes. In this project, we investigated the role of polymorphism in FcγR cluster genes and its influence on arthritis development. We demonstrated that disease severity in this congenic is driven by polymorphism in FcγRIII and/or FcγRIIb but not FcγRIV.

Additionally, congenic strain approach helped us to investigate the role of key factors in innate immunity – complement component 5 in arthritis development. We found hepatocyte-derived C5 is the main source even under inflammatory conditions. Moreover both the *Cia2* and *Cia9* loci were also confirmed in the heterogeneous stock mice using genome- wide scan, which indicates the usefulness of this genetic approach to find disease-causing QTLs. The last part of this thesis described and characterized the new model of arthritis using synthetic thermo-responsive polymer as an adjuvant, which allowed to study the actual immune responses to a self-protein. I share the belief of using animal models to gain valuable fundamental knowledge, which is most important to unravel the molecular pathways and mechanisms leading to pathology. With recent advancement in technologies, development of new therapies is very promising.

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REFERENCES

1. van der Helm-van Mil AH, Huizinga TW. Advances in the genetics of rheumatoid arthritis point to subclassification into distinct disease subsets. Arthritis Res Ther. 2008;10(2):205.

2. van Oosterhout M, Bajema I, Levarht EW, Toes RE, Huizinga TW, van Laar JM. Differences in synovial tissue infiltrates between anti-cyclic citrullinated peptide-positive rheumatoid arthritis and anti-cyclic citrullinated peptide-negative rheumatoid arthritis. Arthritis Rheum. 2008;58(1):53-60.

3. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet.376(9746):1094-108.

4. Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. Semin Arthritis Rheum. 2006;36(3):182-8.

5. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev. 2005;4(3):130-6.

6. Silman AJ, Ollier W, Holligan S, Birrell F, Adebajo A, Asuzu MC, et al. Absence of rheumatoid arthritis in a rural Nigerian population. J Rheumatol. 1993;20(4):618-22.

7. Storey GD. Alfred Baring Garrod (1819-1907). Rheumatology (Oxford). 2001;40(10):1189-90.

8. Entezami P, Fox DA, Clapham PJ, Chung KC. Historical perspective on the etiology of rheumatoid arthritis. Hand Clin.27(1):1-10.

9. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum.62(9):2569-81.

10. van der Linden MP, Knevel R, Huizinga TW, van der Helm-van Mil AH. Classification of rheumatoid arthritis: comparison of the 1987 American College of Rheumatology criteria and the 2010 American College of Rheumatology/European League Against Rheumatism criteria. Arthritis Rheum.63(1):37-42.

11. Smolen J, Aletaha D. The burden of rheumatoid arthritis and access to treatment: a medical overview. Eur J Health Econ. 2008;8 Suppl 2:S39-47.

12. Choy EH, Smith C, Dore CJ, Scott DL. A meta-analysis of the efficacy and toxicity of combining disease-modifying anti-rheumatic drugs in rheumatoid arthritis based on patient withdrawal. Rheumatology (Oxford). 2005;44(11):1414-21.

13. O'Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ, et al. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. N Engl J Med. 1996;334(20):1287-91.

14. van Vollenhoven RF, Ernestam S, Geborek P, Petersson IF, Coster L, Waltbrand E, et al. Addition of infliximab compared with addition of sulfasalazine and hydroxychloroquine to methotrexate in patients with early rheumatoid arthritis (Swefot trial): 1-year results of a randomised trial. Lancet. 2009;374(9688):459-66.

15. Bijlsma JW, Hoes JN, Van Everdingen AA, Verstappen SM, Jacobs JW. Are glucocorticoids DMARDs? Ann N Y Acad Sci. 2006;1069:268-74.

16. Bijlsma JW, Van Everdingen AA, Huisman M, De Nijs RN, Jacobs JW. Glucocorticoids in rheumatoid arthritis: effects on erosions and bone. Ann N Y Acad Sci. 2002;966:82-90.

17. Chakravarty K, McDonald H, Pullar T, Taggart A, Chalmers R, Oliver S, et al. BSR/BHPR guideline for disease-modifying anti-rheumatic drug (DMARD) therapy in consultation with the British Association of Dermatologists. Rheumatology (Oxford). 2008;47(6):924-5.

18. Nishimoto N, Yoshizaki K, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: a multicenter, double-blind, placebo-controlled trial. Arthritis Rheum. 2004;50(6):1761-9.

19. McInnes IB, Gracie JA. Interleukin-15: a new cytokine target for the treatment of inflammatory diseases. Curr Opin Pharmacol. 2004;4(4):392-7.

20. Colmegna I, Ohata BR, Menard HA. Current understanding of rheumatoid arthritis therapy. Clin Pharmacol Ther.91(4):607-20.

21. Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N Engl J Med. 2003;349(20):1907-15.

22. Silman AJ, MacGregor AJ, Thomson W, Holligan S, Carthy D, Farhan A, et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br J Rheumatol. 1993;32(10):903-7.

23. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum. 2000;43(1):30-7.

24. Masdottir B, Jonsson T, Manfredsdottir V, Vikingsson A, Brekkan A, Valdimarsson H. Smoking, rheumatoid factor isotypes and severity of rheumatoid arthritis. Rheumatology (Oxford). 2000;39(11):1202-5.

25. Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. Ann Rheum Dis. 2003;62(9):835-41.

26. Hutchinson D, Shepstone L, Moots R, Lear JT, Lynch MP. Heavy cigarette smoking is strongly associated with rheumatoid arthritis (RA), particularly in patients without a family history of RA. Ann Rheum Dis. 2001;60(3):223-7.

27. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum. 2004;50(10):3085-92.

28. Linn-Rasker SP, van der Helm-van Mil AH, van Gaalen FA, Kloppenburg M, de Vries RR, le Cessie S, et al. Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles. Ann Rheum Dis. 2006;65(3):366-71.

29. Klareskog L, Stolt P, Lundberg K, Kallberg H, Bengtsson C, Grunewald J, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum. 2006;54(1):38-46.

30. Pierer M, Rothe K, Quandt D, Schulz A, Rossol M, Scholz R, et al. Anti-cytomegalovirus seropositivity in rheumatoid arthritis is associated with more severe joint destruction and more frequent joint surgery. Arthritis Rheum.

31. Molina V, Shoenfeld Y. Infection, vaccines and other environmental triggers of autoimmunity. Autoimmunity. 2005;38(3):235-45.

32. Oliver JE, Silman AJ. Risk factors for the development of rheumatoid arthritis. Scand J Rheumatol. 2006;35(3):169-74.

33. Wegner N, Wait R, Sroka A, Eick S, Nguyen KA, Lundberg K, et al. Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. Arthritis Rheum.62(9):2662-72.

34. Farquharson D, Butcher JP, Culshaw S. Periodontitis, Porphyromonas, and the pathogenesis of rheumatoid arthritis. Mucosal Immunol.5(2):112-20.

35. Hitchon CA, Chandad F, Ferucci ED, Willemze A, Ioan-Facsinay A, van der Woude D, et al. Antibodies to porphyromonas gingivalis are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives. J Rheumatol.37(6):1105-12.

36. Rutger Persson G. Rheumatoid arthritis and periodontitis - inflammatory and infectious connections. Review of the literature. J Oral Microbiol.4.

37. Stolt P, Kallberg H, Lundberg I, Sjogren B, Klareskog L, Alfredsson L. Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. Ann Rheum Dis. 2005;64(4):582-6.

38. Sverdrup B, Kallberg H, Bengtsson C, Lundberg I, Padyukov L, Alfredsson L, et al. Association between occupational exposure to mineral oil and rheumatoid arthritis: results from the Swedish EIRA case-control study. Arthritis Res Ther. 2005;7(6):R1296-303.

39. Lorentzen JC, Klareskog L. Susceptibility of DA rats to arthritis induced with adjuvant oil or rat collagen is determined by genes both within and outside the major histocompatibility complex. Scand J Immunol. 1996;44(6):592-8.

40. Skoldstam L, Hagfors L, Johansson G. An experimental study of a Mediterranean diet intervention for patients with rheumatoid arthritis. Ann Rheum Dis. 2003;62(3):208-14.

41. Pattison DJ, Symmons DP, Young A. Does diet have a role in the aetiology of rheumatoid arthritis? Proc Nutr Soc. 2004;63(1):137-43.

42. Linos A, Kaklamani VG, Kaklamani E, Koumantaki Y, Giziaki E, Papazoglou S, et al. Dietary factors in relation to rheumatoid arthritis: a role for olive oil and cooked vegetables? Am J Clin Nutr. 1999;70(6):1077-82.

43. Cleland LG, James MJ, Proudman SM. The role of fish oils in the treatment of rheumatoid arthritis. Drugs. 2003;63(9):845-53.

44. Pattison DJ, Symmons DP, Lunt M, Welch A, Luben R, Bingham SA, et al. Dietary risk factors for the development of inflammatory polyarthritis: evidence for a role of high level of red meat consumption. Arthritis Rheum. 2004;50(12):3804-12.

45. Benito-Garcia E, Feskanich D, Hu FB, Mandl LA, Karlson EW. Protein, iron, and meat consumption and risk for rheumatoid arthritis: a prospective cohort study. Arthritis Res Ther. 2007;9(1):R16.

46. Hutchinson D, Moots R. Coffee consumption, RF, and the risk of RA. Ann Rheum Dis. 2001;60(5):540-1.

47. Mikuls TR, Cerhan JR, Criswell LA, Merlino L, Mudano AS, Burma M, et al. Coffee, tea, and caffeine consumption and risk of rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis Rheum. 2002;46(1):83-91.

48. Karlson EW, Mandl LA, Aweh GN, Grodstein F. Coffee consumption and risk of rheumatoid arthritis. Arthritis Rheum. 2003;48(11):3055-60.

49. Kallberg H, Jacobsen S, Bengtsson C, Pedersen M, Padyukov L, Garred P, et al. Alcohol consumption is associated with decreased risk of rheumatoid arthritis: results from two Scandinavian case-control studies. Ann Rheum Dis. 2009;68(2):222-7.

50. Silman A, Kay A, Brennan P. Timing of pregnancy in relation to the onset of rheumatoid arthritis. Arthritis Rheum. 1992;35(2):152-5.

51. Ostensen M, Villiger PM. The remission of rheumatoid arthritis during pregnancy. Semin Immunopathol. 2007;29(2):185-91.

52. Pikwer M, Bergstrom U, Nilsson JA, Jacobsson L, Berglund G, Turesson C. Breast feeding, but not use of oral contraceptives, is associated with a reduced risk of rheumatoid arthritis. Ann Rheum Dis. 2009;68(4):526-30.

53. Stastny P. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med. 1978;298(16):869-71.

54. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. 1987;30(11):1205-13.

55. Nepom GT, Byers P, Seyfried C, Healey LA, Wilske KR, Stage D, et al. HLA genes associated with rheumatoid arthritis. Identification of susceptibility alleles using specific oligonucleotide probes. Arthritis Rheum. 1989;32(1):15-21.

56. Weyand CM, Hicok KC, Conn DL, Goronzy JJ. The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. Ann Intern Med. 1992;117(10):801-6.

57. Lee HS, Lee AT, Criswell LA, Seldin MF, Amos CI, Carulli JP, et al. Several regions in the major histocompatibility complex confer risk for anti-CCP-antibody positive rheumatoid arthritis, independent of the DRB1 locus. Mol Med. 2008;14(5-6):293-300.

58. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet. 2004;75(2):330-7.

59. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet. 2004;36(4):337-8.

60. Gregersen PK. Gaining insight into PTPN22 and autoimmunity. Nat Genet. 2005;37(12):1300-2.

61. Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P, et al. Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. Nat Genet. 2005;37(12):1317-9.

62. Rieck M, Arechiga A, Onengut-Gumuscu S, Greenbaum C, Concannon P, Buckner JH. Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. J Immunol. 2007;179(7):4704-10.

63. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. PLoS Med. 2007;4(9):e278.

64. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. N Engl J Med. 2007;357(12):1199-209.

65. Nishimoto K, Kochi Y, Ikari K, Yamamoto K, Suzuki A, Shimane K, et al. Association study of TRAF1-C5 polymorphisms with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in Japanese. Ann Rheum Dis.69(2):368-73.

66. Zhu J, Zhang D, Wu F, He F, Liu X, Wu L, et al. Single nucleotide polymorphisms at the TRAF1/C5 locus are associated with rheumatoid arthritis in a Han Chinese population. BMC Med Genet.12:53.

67. Han TU, Bang SY, Kang C, Bae SC. TRAF1 polymorphisms associated with rheumatoid arthritis susceptibility in Asians and in Caucasians. Arthritis Rheum. 2009;60(9):2577-84.

68. Chang M, Rowland CM, Garcia VE, Schrodi SJ, Catanese JJ, van der Helm-van Mil AH, et al. A large-scale rheumatoid arthritis genetic study identifies association at chromosome 9q33.2. PLoS Genet. 2008;4(6):e1000107.

69. Kurreeman FA, Goulielmos GN, Alizadeh BZ, Rueda B, Houwing-Duistermaat J, Sanchez E, et al. The TRAF1-C5 region on chromosome 9q33 is associated with multiple autoimmune diseases. Ann Rheum Dis.69(4):696-9.

70. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med. 2007;357(10):977-86.

71. Lee HS, Remmers EF, Le JM, Kastner DL, Bae SC, Gregersen PK. Association of STAT4 with rheumatoid arthritis in the Korean population. Mol Med. 2007;13(9-10):455-60.

72. Kobayashi S, Ikari K, Kaneko H, Kochi Y, Yamamoto K, Shimane K, et al. Association of STAT4 with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in the Japanese population. Arthritis Rheum. 2008;58(7):1940-6.

73. Kaplan MH. STAT4: a critical regulator of inflammation in vivo. Immunol Res. 2005;31(3):231-42.

74. Walker JG, Ahern MJ, Coleman M, Weedon H, Papangelis V, Beroukas D, et al. Changes in synovial tissue Jak-STAT expression in rheumatoid arthritis in response to successful DMARD treatment. Ann Rheum Dis. 2006;65(12):1558-64.

75. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661-78.

76. Gough SC, Walker LS, Sansom DM. CTLA4 gene polymorphism and autoimmunity. Immunol Rev. 2005;204:102-15.

77. Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW, et al. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. Am J Hum Genet. 2005;77(6):1044-60.

78. Nogid A, Pham DQ. Role of abatacept in the management of rheumatoid arthritis. Clin Ther. 2006;28(11):1764-78.

79. Daha NA, Kurreeman FA, Marques RB, Stoeken-Rijsbergen G, Verduijn W, Huizinga TW, et al. Confirmation of STAT4, IL2/IL21, and CTLA4 polymorphisms in rheumatoid arthritis. Arthritis Rheum. 2009;60(5):1255-60.

80. Raychaudhuri S, Remmers EF, Lee AT, Hackett R, Guiducci C, Burtt NP, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat Genet. 2008;40(10):1216-23.

81. van der Linden MP, Feitsma AL, le Cessie S, Kern M, Olsson LM, Raychaudhuri S, et al. Association of a single-nucleotide polymorphism in CD40 with the rate of joint destruction in rheumatoid arthritis. Arthritis Rheum. 2009;60(8):2242-7.

82. Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet. 2003;34(4):395-402.

83. Ikari K, Kuwahara M, Nakamura T, Momohara S, Hara M, Yamanaka H, et al. Association between PADI4 and rheumatoid arthritis: a replication study. Arthritis Rheum. 2005;52(10):3054-7.

84. Iwamoto T, Ikari K, Nakamura T, Kuwahara M, Toyama Y, Tomatsu T, et al. Association between PADI4 and rheumatoid arthritis: a meta-analysis. Rheumatology (Oxford). 2006;45(7):804-7.

85. Burr ML, Naseem H, Hinks A, Eyre S, Gibbons LJ, Bowes J, et al. PADI4 genotype is not associated with rheumatoid arthritis in a large UK Caucasian population. Ann Rheum Dis.69(4):666-70.

86. Blom AB, Radstake TR, Holthuysen AE, Sloetjes AW, Pesman GJ, Sweep FG, et al. Increased expression of Fcgamma receptors II and III on macrophages of rheumatoid arthritis patients results in higher production of tumor necrosis factor alpha and matrix metalloproteinase. Arthritis Rheum. 2003;48(4):1002-14.

87. Morgan AW, Griffiths B, Ponchel F, Montague BM, Ali M, Gardner PP, et al. Fcgamma receptor type IIIA is associated with rheumatoid arthritis in two distinct ethnic groups. Arthritis Rheum. 2000;43(10):2328-34.

88. Thabet MM, Huizinga TW, Marques RB, Stoeken-Rijsbergen G, Bakker AM, Kurreeman FA, et al. Contribution of Fcgamma receptor IIIA gene 158V/F polymorphism and copy number variation to the risk of ACPA-positive rheumatoid arthritis. Ann Rheum Dis. 2009;68(11):1775-80.

89. Robinson JI, Barrett JH, Taylor JC, Naven M, Corscadden D, Barton A, et al. Dissection of the FCGR3A association with RA: increased association in men and with autoantibody positive disease. Ann Rheum Dis.69(6):1054-7.

90. Radstake TR, Petit E, Pierlot C, van de Putte LB, Cornelis F, Barrera P. Role of Fcgamma receptors IIA, IIIA, and IIIB in susceptibility to rheumatoid arthritis. J Rheumatol. 2003;30(5):926-33.

91. Ravetch JV, Perussia B. Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. J Exp Med. 1989;170(2):481-97.

92. Morris DL, Roberts AL, Witherden AS, Tarzi R, Barros P, Whittaker JC, et al. Evidence for both copy number and allelic (NA1/NA2) risk at the FCGR3B locus in systemic lupus erythematosus. Eur J Hum Genet.18(9):1027-31.

93. Aitman TJ, Dong R, Vyse TJ, Norsworthy PJ, Johnson MD, Smith J, et al. Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. Nature. 2006;439(7078):851-5.

94. Willcocks LC, Lyons PA, Clatworthy MR, Robinson JI, Yang W, Newland SA, et al. Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. J Exp Med. 2008;205(7):1573-82.

95. Robinson JI, Carr IM, Cooper DL, Rashid LH, Martin SG, Emery P, et al. Confirmation of association of FCGR3B but not FCGR3A copy number with susceptibility to autoantibody positive rheumatoid arthritis. Hum Mutat. 2012;33(4):741-9.

96. Boissier MC, Chiocchia G, Ronziere MC, Herbage D, Fournier C. Arthritogenicity of minor cartilage collagens (types IX and XI) in mice. Arthritis Rheum. 1990;33(1):1-8.

97. Yoshitomi H, Sakaguchi N, Kobayashi K, Brown GD, Tagami T, Sakihama T, et al. A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. J Exp Med. 2005;201(6):949-60.

98. Sakaguchi N, Takahashi T, Hata H, Nomura T, Tagami T, Yamazaki S, et al. Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. Nature. 2003;426(6965):454-60.

99. Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, et al. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J. 1991;10(13):4025-31.

100. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen an experimental model of arthritis. J Exp Med. 1977;146(3):857-68.

101. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. Nature. 1980;283(5748):666-8.

102. Cathcart ES, Hayes KC, Gonnerman WA, Lazzari AA, Franzblau C. Experimental arthritis in a nonhuman primate. I. Induction by bovine type II collagen. Lab Invest. 1986;54(1):26-31.

103. Rosenbloom J, Blumenkrantz N, Prockop DJ. Sequential hydroxylation of lysine and glycosylation of hydroxylysine during the biosynthesis of collagen in isolated cartilage. Biochem Biophys Res Commun. 1968;31(5):792-7.

104. Brunsberg U, Gustafsson K, Jansson L, Michaelsson E, Ahrlund-Richter L, Pettersson S, et al. Expression of a transgenic class II Ab gene confers susceptibility to collagen-induced arthritis. Eur J Immunol. 1994;24(7):1698-702.

105. Kjellen P, Brunsberg U, Broddefalk J, Hansen B, Vestberg M, Ivarsson I, et al. The structural basis of MHC control of collagen-induced arthritis; binding of the immunodominant type II collagen 256-270 glycopeptide to H-2Aq and H-2Ap molecules. Eur J Immunol. 1998;28(2):755-67.

106. Holmdahl R, Jansson L, Andersson M, Larsson E. Immunogenetics of type II collagen autoimmunity and susceptibility to collagen arthritis. Immunology. 1988;65(2):305-10.

107. Holmdahl R, Jansson L, Larsson E, Rubin K, Klareskog L. Homologous type II collagen induces chronic and progressive arthritis in mice. Arthritis Rheum. 1986;29(1):106-13.

108. Michaelsson E, Andersson M, Engstrom A, Holmdahl R. Identification of an immunodominant type-II collagen peptide recognized by T cells in H-2q mice: self tolerance at the level of determinant selection. Eur J Immunol. 1992;22(7):1819-25.

109. Olofsson P, Holmberg J, Tordsson J, Lu S, Akerstrom B, Holmdahl R. Positional identification of Ncf1 as a gene that regulates arthritis severity in rats. Nat Genet. 2003;33(1):25-32.

110. Hultqvist M, Olofsson P, Holmberg J, Backstrom BT, Tordsson J, Holmdahl R. Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the Ncf1 gene. Proc Natl Acad Sci U S A. 2004;101(34):12646-51.

111. Hultqvist M, Sareila O, Vilhardt F, Norin U, Olsson LM, Olofsson P, et al. Positioning of a polymorphic quantitative trait nucleotide in the Ncf1 gene controlling oxidative burst response and arthritis severity in rats. Antioxid Redox Signal. 2011;14(12):2373-83.

112. Stuart JM, Dixon FJ. Serum transfer of collagen-induced arthritis in mice. J Exp Med. 1983;158(2):378-92.

113. Wooley PH, Luthra HS, Singh SK, Huse AR, Stuart JM, David CS. Passive transfer of arthritis to mice by injection of human anti-type II collagen antibody. Mayo Clin Proc. 1984;59(11):737-43.

114. Nandakumar KS, Svensson L, Holmdahl R. Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes. Am J Pathol. 2003;163(5):1827-37.

115. Nandakumar KS, Andren M, Martinsson P, Bajtner E, Hellstrom S, Holmdahl R, et al. Induction of arthritis by single monoclonal IgG anti-collagen type II antibodies and enhancement of arthritis in mice lacking inhibitory FcgammaRIIB. Eur J Immunol. 2003;33(8):2269-77.

116. Terato K, Hasty KA, Reife RA, Cremer MA, Kang AH, Stuart JM. Induction of arthritis with monoclonal antibodies to collagen. J Immunol. 1992;148(7):2103-8.

117. Nandakumar KS, Backlund J, Vestberg M, Holmdahl R. Collagen type II (CII)-specific antibodies induce arthritis in the absence of T or B cells but the arthritis progression is enhanced by CII-reactive T cells. Arthritis Res Ther. 2004;6(6):R544-50.

118. Watson WC, Brown PS, Pitcock JA, Townes AS. Passive transfer studies with type II collagen antibody in B10.D2/old and new line and C57Bl/6 normal and beige (Chediak-Higashi) strains: evidence of important roles for C5 and multiple inflammatory cell types in the development of erosive arthritis. Arthritis Rheum. 1987;30(4):460-5.

119. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. Cell. 1996;87(5):811-22.

120. Matsumoto I, Staub A, Benoist C, Mathis D. Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. Science. 1999;286(5445):1732-5.

121. Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, et al. From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. Immunity. 1999;10(4):451-61.

122. Bockermann R, Schubert D, Kamradt T, Holmdahl R. Induction of a B-cell-dependent chronic arthritis with glucose-6-phosphate isomerase. Arthritis Res Ther. 2005;7(6):R1316-24.

123. Iwanami K, Matsumoto I, Tanaka Y, Inoue A, Goto D, Ito S, et al. Arthritogenic T cell epitope in glucose-6-phosphate isomerase-induced arthritis. Arthritis Res Ther. 2008;10(6):R130.

124. Allen JM, Seed B. Isolation and expression of functional high-affinity Fc receptor complementary DNAs. Science. 1989;243(4889):378-81.

125. Radaev S, Sun P. Recognition of immunoglobulins by Fcgamma receptors. Mol Immunol. 2002;38(14):1073-83.

126. Kato K, Fridman WH, Arata Y, Sautes-Fridman C. A conformational change in the Fc precludes the binding of two Fcgamma receptor molecules to one IgG. Immunol Today. 2000;21(7):310-2.

127. Latour S, Fridman WH, Daeron M. Identification, molecular cloning, biologic properties, and tissue distribution of a novel isoform of murine low-affinity IgG receptor homologous to human Fc gamma RIIB1. J Immunol. 1996;157(1):189-97.

128. Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol. 2001;19:197-223.

129. Ravetch JV, Clynes RA. Divergent roles for Fc receptors and complement in vivo. Annu Rev Immunol. 1998;16:421-32.

130. Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. FcgammaRIV: a novel FcR with distinct IgG subclass specificity. Immunity. 2005;23(1):41-51.

131. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol. 2008;8(1):34-47.

132. Willcocks LC, Smith KG, Clatworthy MR. Low-affinity Fcgamma receptors, autoimmunity and infection. Expert Rev Mol Med. 2009;11:e24.

133. Hirano M, Davis RS, Fine WD, Nakamura S, Shimizu K, Yagi H, et al. IgEb immune complexes activate macrophages through FcgammaRIV binding. Nat Immunol. 2007;8(7):762-71.

134. Xiu Y, Nakamura K, Abe M, Li N, Wen XS, Jiang Y, et al. Transcriptional regulation of Fcgr2b gene by polymorphic promoter region and its contribution to humoral immune responses. J Immunol. 2002;169(8):4340-6.

135. Luan JJ, Monteiro RC, Sautes C, Fluteau G, Eloy L, Fridman WH, et al. Defective Fc gamma RII gene expression in macrophages of NOD mice: genetic linkage with up-regulation of IgG1 and IgG2b in serum. J Immunol. 1996;157(10):4707-16.

136. Hibbs ML, Hogarth PM, Harris RA, McKenzie IF. Gm-3.2, a new granulocyte/macrophage alloantigen. Immunogenetics. 1985;21(1):61-70.

137. Andren M, Johanneson B, Alarcon-Riquelme ME, Kleinau S. IgG Fc receptor polymorphisms and association with autoimmune disease. Eur J Immunol. 2005;35(10):3020-9.

138. Yuasa T, Kubo S, Yoshino T, Ujike A, Matsumura K, Ono M, et al. Deletion of fcgamma receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. J Exp Med. 1999;189(1):187-94.

139. Fukuyama H, Nimmerjahn F, Ravetch JV. The inhibitory Fcgamma receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G+ anti-DNA plasma cells. Nat Immunol. 2005;6(1):99-106.

140. Bolland S, Ravetch JV. Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. Immunity. 2000;13(2):277-85.

141. Kleinau S, Martinsson P, Heyman B. Induction and suppression of collagen-induced arthritis is dependent on distinct fcgamma receptors. J Exp Med. 2000;191(9):1611-6.

142. Diaz de Stahl T, Andren M, Martinsson P, Verbeek JS, Kleinau S. Expression of FcgammaRIII is required for development of collagen-induced arthritis. Eur J Immunol. 2002;32(10):2915-22.

143. Hibbs ML, Hogarth PM, McKenzie IF. The mouse Ly-17 locus identifies a polymorphism of the Fc receptor. Immunogenetics. 1985;22(4):335-48.

144. Muta T, Kurosaki T, Misulovin Z, Sanchez M, Nussenzweig MC, Ravetch JV. A 13-amino-acid motif in the cytoplasmic domain of Fc gamma RIIB modulates B-cell receptor signalling. Nature. 1994;369(6478):340.

145. Slingsby JH, Hogarth MB, Walport MJ, Morley BJ. Polymorphism in the Ly-17 alloantigenic system of the mouse FcgRII gene. Immunogenetics. 1997;46(4):361-2.

146. Pearse RN, Kawabe T, Bolland S, Guinamard R, Kurosaki T, Ravetch JV. SHIP recruitment attenuates Fc gamma RIIB-induced B cell apoptosis. Immunity. 1999;10(6):753-60.
147. Xiang Z, Cutler AJ, Brownlie RJ, Fairfax K, Lawlor KE, Severinson E, et al. FcgammaRIIb controls bone marrow plasma cell persistence and apoptosis. Nat Immunol. 2007;8(4):419-29.
148. Ravetch JV, Nussenzweig M. Killing some to make way for others. Nat Immunol. 2007;8(4):337-9.
149. Groh V, Li YQ, Cioca D, Hunder NN, Wang W, Riddell SR, et al. Efficient cross-priming of tumor antigen-specific T cells by dendritic cells sensitized with diverse anti-MICA opsonized tumor cells. Proc Natl Acad Sci U S A. 2005;102(18):6461-6.
150. Rafiq K, Bergtold A, Clynes R. Immune complex-mediated antigen presentation induces tumor immunity. J Clin Invest. 2002;110(1):71-9.
151. Kalergis AM, Ravetch JV. Inducing tumor immunity through the selective engagement of activating Fcgamma receptors on dendritic cells. J Exp Med. 2002;195(12):1653-9.
152. Rus H, Cudrici C, Niculescu F. The role of the complement system in innate immunity. Immunol Res. 2005;33(2):103-12.
153. Morgan BP, Marchbank KJ, Longhi MP, Harris CL, Gallimore AM. Complement: central to innate immunity and bridging to adaptive responses. Immunol Lett. 2005;97(2):171-9.
154. Walport MJ. Complement. First of two parts. N Engl J Med. 2001;344(14):1058-66.
155. Gadjeva M, Verschoor A, Brockman MA, Jezak H, Shen LM, Knipe DM, et al. Macrophage-derived complement component C4 can restore humoral immunity in C4-deficient mice. J Immunol. 2002;169(10):5489-95.
156. Verschoor A, Brockman MA, Gadjeva M, Knipe DM, Carroll MC. Myeloid C3 determines induction of humoral responses to peripheral herpes simplex virus infection. J Immunol. 2003;171(10):5363-71.
157. Nauta AJ, Roos A, Daha MR. A regulatory role for complement in innate immunity and autoimmunity. Int Arch Allergy Immunol. 2004;134(4):310-23.
158. Riedemann NC, Guo RF, Ward PA. A key role of C5a/C5aR activation for the development of sepsis. J Leukoc Biol. 2003;74(6):966-70.
159. Liu ZM, Zhu SM, Qin XJ, Cheng ZD, Liu MY, Zhang HM, et al. Silencing of C5a receptor gene with siRNA for protection from Gram-negative bacterial lipopolysaccharide-induced vascular permeability. Mol Immunol. 2010;47(6):1325-33.
160. Mizuno M. A review of current knowledge of the complement system and the therapeutic opportunities in inflammatory arthritis. Curr Med Chem. 2006;13(14):1707-17.
161. Okroj M, Heinegard D, Holmdahl R, Blom AM. Rheumatoid arthritis and the complement system. Ann Med. 2007;39(7):517-30.
162. Ballanti E, Perricone C, di Muzio G, Kroegler B, Chimenti MS, Graceffa D, et al. Role of the complement system in rheumatoid arthritis and psoriatic arthritis: relationship with anti-TNF inhibitors. Autoimmun Rev. 2011;10(10):617-23.
163. Robbins RA, Russ WD, Rasmussen JK, Clayton MM. Activation of the complement system in the adult respiratory distress syndrome. Am Rev Respir Dis. 1987;135(3):651-8.
164. Ward PA. The dark side of C5a in sepsis. Nat Rev Immunol. 2004;4(2):133-42.
165. Welch TR. Complement in glomerulonephritis. Nat Genet. 2002;31(4):333-4.
166. Hawlisch H, Wills-Karp M, Karp CL, Kohl J. The anaphylatoxins bridge innate and adaptive immune responses in allergic asthma. Mol Immunol. 2004;41(2-3):123-31.
167. Linton SM, Morgan BP. Complement activation and inhibition in experimental models of arthritis. Mol Immunol. 1999;36(13-14):905-14.
168. Shushakova N, Skokowa J, Schulman J, Baumann U, Zwirner J, Schmidt RE, et al. C5a anaphylatoxin is a major regulator of activating versus inhibitory FcgammaRs in immune complex-induced lung disease. J Clin Invest. 2002;110(12):1823-30.

169. Zubler RH, Nydegger U, Perrin LH, Fehr K, McCormick J, Lambert PH, et al. Circulating and intra-articular immune complexes in patients with rheumatoid arthritis. Correlation of 125I-Clq binding activity with clinical and biological features of the disease. J Clin Invest. 1976;57(5):1308-19.

170. Morgan BP, Daniels RH, Williams BD. Measurement of terminal complement complexes in rheumatoid arthritis. Clin Exp Immunol. 1988;73(3):473-8.

171. Moxley G, Ruddy S. Elevated C3 anaphylatoxin levels in synovial fluids from patients with rheumatoid arthritis. Arthritis Rheum. 1985;28(10):1089-95.

172. Jose PJ, Moss IK, Maini RN, Williams TJ. Measurement of the chemotactic complement fragment C5a in rheumatoid synovial fluids by radioimmunoassay: role of C5a in the acute inflammatory phase. Ann Rheum Dis. 1990;49(10):747-52.

173. D'Cruz D, Taylor J, Ahmed T, Asherson R, Khamashta M, Hughes GR. Complement factor 2 deficiency: a clinical and serological family study. Ann Rheum Dis. 1992;51(11):1254-6.

174. Brodeur JP, Ruddy S, Schwartz LB, Moxley G. Synovial fluid levels of complement SC5b-9 and fragment Bb are elevated in patients with rheumatoid arthritis. Arthritis Rheum. 1991;34(12):1531-7.

175. Sato R, Matsushita M, Miyata M, Sato Y, Kasukawa R, Fujita T. Substances reactive with mannose-binding protein (MBP) in sera of patients with rheumatoid arthritis. Fukushima J Med Sci. 1997;43(2):99-111.

176. Kilpatrick DC. Mannan-binding lectin and its role in innate immunity. Transfus Med. 2002;12(6):335-52.

177. Hietala MA, Jonsson IM, Tarkowski A, Kleinau S, Pekna M. Complement deficiency ameliorates collagen-induced arthritis in mice. J Immunol. 2002;169(1):454-9.

178. Banda NK, Thurman JM, Kraus D, Wood A, Carroll MC, Arend WP, et al. Alternative complement pathway activation is essential for inflammation and joint destruction in the passive transfer model of collagen-induced arthritis. J Immunol. 2006;177(3):1904-12.

179. Hietala MA, Nandakumar **KS**, Persson L, Fahlen S, Holmdahl R, Pekna M. Complement activation by both classical and alternative pathways is critical for the effector phase of arthritis. Eur J Immunol. 2004;34(4):1208-16.

180. Wang Y, Rollins SA, Madri JA, Matis LA. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. Proc Natl Acad Sci U S A. 1995;92(19):8955-9.

181. Wang Y, Kristan J, Hao L, Lenkoski CS, Shen Y, Matis LA. A role for complement in antibody-mediated inflammation: C5-deficient DBA/1 mice are resistant to collagen-induced arthritis. J Immunol. 2000;164(8):4340-7.

182. Spinella DG, Jeffers JR, Reife RA, Stuart JM. The role of C5 and T-cell receptor Vb genes in susceptibility to collagen-induced arthritis. Immunogenetics. 1991;34(1):23-7.

183. Wetsel RA, Fleischer DT, Haviland DL. Deficiency of the murine fifth complement component (C5). A 2-base pair gene deletion in a 5'-exon. J Biol Chem. 1990;265(5):2435-40.

184. Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, et al. Arthritis critically dependent on innate immune system players. Immunity. 2002;16(2):157-68.

185. Ruddy S, Colten HR. Rheumatoid arthritis. Biosynthesis of complement proteins by synovial tissues. N Engl J Med. 1974;290(23):1284-8.

186. Krey PR, Scheinberg MA, Cohen AS. Fine structural analysis of rabbit synovial cells. II. Fine structure and rosette-forming cells of explant and monolayer cultures. Arthritis Rheum. 1976;19(3):581-92.

187. Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR, et al. Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science. 1987;237(4813):402-5.

188. Neumann E, Barnum SR, Tarner IH, Echols J, Fleck M, Judex M, et al. Local production of complement proteins in rheumatoid arthritis synovium. Arthritis Rheum. 2002;46(4):934-45.

189. Darvasi A. Experimental strategies for the genetic dissection of complex traits in animal models. Nat Genet. 1998;18(1):19-24.

190. Peirce JL, Derr R, Shendure J, Kolata T, Silver LM. A major influence of sex-specific loci on alcohol preference in C57Bl/6 and DBA/2 inbred mice. Mamm Genome. 1998;9(12):942-8.

191. Taketo M, Schroeder AC, Mobraaten LE, Gunning KB, Hanten G, Fox RR, et al. FVB/N: an inbred mouse strain preferable for transgenic analyses. Proc Natl Acad Sci U S A. 1991;88(6):2065-9.

192. Beck JA, Lloyd S, Hafezparast M, Lennon-Pierce M, Eppig JT, Festing MF, et al. Genealogies of mouse inbred strains. Nat Genet. 2000;24(1):23-5.

193. Glazier AM, Nadeau JH, Aitman TJ. Finding genes that underlie complex traits. Science. 2002;298(5602):2345-9.

194. Johansson AC, Sundler M, Kjellen P, Johannesson M, Cook A, Lindqvist AK, et al. Genetic control of collagen-induced arthritis in a cross with NOD and C57BL/10 mice is dependent on gene regions encoding complement factor 5 and FcgammaRIIb and is not associated with loci controlling diabetes. Eur J Immunol. 2001;31(6):1847-56.

195. Mott R, Talbot CJ, Turri MG, Collins AC, Flint J. A method for fine mapping quantitative trait loci in outbred animal stocks. Proc Natl Acad Sci U S A. 2000;97(23):12649-54.

196. Talbot CJ, Nicod A, Cherny SS, Fulker DW, Collins AC, Flint J. High-resolution mapping of quantitative trait loci in outbred mice. Nat Genet. 1999;21(3):305-8.

197. Valdar W, Solberg LC, Gauguier D, Burnett S, Klenerman P, Cookson WO, et al. Genome-wide genetic association of complex traits in heterogeneous stock mice. Nat Genet. 2006;38(8):879-87.

198. Kawai T, Akira S. TLR signaling. Semin Immunol. 2007;19(1):24-32.

199. Galaev IY, Mattiasson B. 'Smart' polymers and what they could do in biotechnology and medicine. Trends Biotechnol. 1999;17(8):335-40.

200. Shakya AK, Kumar A, Nandakumar KS. Adjuvant properties of a biocompatible thermo-responsive polymer of N-isopropylacrylamide in autoimmunity and arthritis. J R Soc Interface. 2011;8(65):1748-59.

201. Khaled KA, Sarhan HA, Ibrahim MA, Ali AH, Naguib YW. Prednisolone-loaded PLGA microspheres. in vitro characterization and in vivo application in adjuvant-induced arthritis in mice. AAPS PharmSciTech. 2010;11(2):859-69.