Autoantibodies and genetic variation in rheumatoid arthritis

aspects on susceptibility and disease course

Alf Kastbom

Linköping University
FACULTY OF HEALTH SCIENCES

AIR – Division of Rheumatology, Department of Molecular and Clinical Medicine, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden

"Prediction is very difficult, especially about the future"

Niels Bohr

Contents

Abstract

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation and subsequent destruction of synovial joints. Although its causes remain largely unknown, a substantial genetic contribution is known to exist. During the last decades the benefits of early aggressive treatment have become evident, and more potent therapeutic options have become available. These advances have increased the demands for rapid accurate diagnosis and prognostic markers of disease course and therapy response.

The 'rheumatoid factor' (RF) has long been used as a diagnostic and prognostic marker of RA. In this thesis, the utility of measuring antibodies to cyclic citrullinated peptides (CCP) was investigated. In a population-based arthritis incidence study, 69 very early arthritis patients (symptom duration < 3 months) were identified. The anti-CCP test, performed at baseline and related to diagnosis at the 2-year follow-up, had a diagnostic specificity for RA of 96% and a sensitivity of 44%, both of which were superior to RF. In a prospective cohort of 242 incident cases of RA (symptom duration < 1 year), 64% of the patients tested positive for anti-CCP at baseline (equal to RF). Despite receiving more active anti-rheumatic therapy, the anti-CCP-positive patients had a more aggressive disease course during 3 years as compared to those testing negative.

The 158VV genotype of Fcγ Receptor type IIIA (FcγRIIIA), which binds IgG with higher affinity than 158FF, was associated with an increased susceptibility to RA in men, but not in women. Previous studies report conflicting results, and none stratified according to gender. The 158V/F polymorphism of FcγRIIIA was not found to influence outcome of anti-tumour necrosis factor therapy in 282 RA patients, contradicting hints from previous studies. Genetic variation in proteins of the inflammasome, an interleukin-1 (IL-1) regulating intracellular protein complex, is associated with rare autoinflammatory conditions and possibly with Crohn's disease. In this first study on genetic variation of the inflammasome in RA, we describe a compound polymorphism of the genes CIAS1 and TUCAN that associates both with susceptibility to RA and to the severity of the disease. Hypothetically, these genes may identify a subgroup of RA patients that would benefit from anti-IL-1 therapy.

This thesis work emphasizes the benefits of testing for anti-CCP in the diagnosis and outcome prediction in early arthritis. FcγRIIIA genotype is likely to affect RA susceptibility and further work should apply a gender perspective. Inflammasome genetics may influence the risk of developing RA. Additional studies are warranted to settle whether it also identifies a subgroup of RA patients benefiting from IL-1 targeted therapy.

Abbreviations

ACPA	Anti-citrullinated protein antibodies
ACR	American College of Rheumatology
ADCC	Antibody-dependent cellular cytotoxicity
APC	Antigen-presenting cell
CCP	Cyclic citrullinated peptide
CD	Cluster of differentiation
CIA	Collagen-induced arthritis
CRP	C-reactive protein
CTLA-4	Cytotoxic T-lymphocyte antigen 4
DMARD	Disease modifying anti-rheumatic drug
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
F(ab')$_2$	Fraction antigen-binding
Fc	Fraction crystallizable
HLA	Human leukocyte antigen
IC	Immune complex
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibition motif
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
PGA	Physician's global assessment of disease activity
PTPN22	Protein tyrosine phosphatase non-receptor type 22
RF	Rheumatoid factor
SE	Shared epitope
SJC	Swollen joint count
SLE	Systemic lupus erythematosus
SNP	Single-nucleotide polymorphism
TJC	Tender joint count
TNF	Tumour necrosis factor alpha

List of original publications

This thesis is based on the following papers, which will be referred to by their Roman numerals (I-V):

I **Söderlin MK, Kastbom A, Kautiainen H, Leirisalo-Repo M, Strandberg G, and Skogh T.**
Antibodies against cyclic citrullinated peptide (CCP) and levels of cartilage oligomeric matrix protein (COMP) in very early arthritis: relation to diagnosis and disease activity.
Scand J Rheumatol 2004; 33:185-188

II **Kastbom A**, **Strandberg G, Lindroos A, and Skogh T.**
Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project).
Ann Rheum Dis 2004; 63:1085-1089

III **Kastbom A, Ahmadi A, Söderkvist P, and Skogh T.**
The 158V polymorphism of Fc gamma receptor type IIIA in early rheumatoid arthritis: increased susceptibility and severity in male patients (the Swedish TIRA project).
Rheumatology 2005; 44:1294-1298

IV **Kastbom A, Bratt J, Ernestam S, Lampa J, Padyukov L, Söderkvist P, and Skogh T.**
Fcγ receptor type IIIA genotype and response to tumor necrosis factor α-blocking agents in patients with rheumatoid arthritis.
Arthritis Rheum 2007; 52:448-452

V **Kastbom A, Verma D, Eriksson P, Skogh T, Wingren G, and Söderkvist P.**
Genetic variation in proteins of the cryopyrin inflammasome influences susceptibility and severity of rheumatoid arthritis (the Swedish TIRA project).
Submitted

Introduction

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation and subsequent destruction of synovial joints. The history of the disease dates back at least 3000 years, as skeletal remains from this era have shown lesions of typical RA appearance [1]. There have been controversies whether or not RA originates from America and was brought to Europe following the discovery by Columbus in 1492 [2]. Although recent findings have revealed RA-like deformities in combination with risk genes in an Italian female from the mid 16th century [3], distinct pre-Columbian findings outside America are lacking. In 1800, Landré-Beauvais was the first to describe RA as a disease entity, although it was then denoted 'asthenic gout' [4]. The designation 'rheumatoid arthritis' was presented by Garrod in 1859, and came into general use during the beginning of the 20th century.

The affected patient typically presents with several symmetrically swollen and painful joints, preferably of the hands and feet, fatigue and possibly slight fever. In many cases there is a family history of inflammatory joint disease. Even if the diagnosis is usually based on the 1987 American College of Rheumatology (ACR) classification criteria (Table 1) [5], these criteria were established for classification purposes, referring to prevalent RA cases, and were not primarily intended as a diagnostic tool. Although the sensitivity in early RA has shown to be modest [6, 7], the ACR criteria remain the golden standard in research.

RA is prevalent in all ethnic groups, affecting approximately 0.4-1.0 % of the population in the western world [8-10]. The incidence rate of RA in Scandinavia has been estimated to 24-29 per 100 000 persons and year, and shows a clear female preponderance [11-13]. Interestingly, the incidence rate of RA has been postulated to be declining [14-16], but this notion could possibly be due to changes regarding the definition of time of onset, diagnostic criteria, or disease severity [17]. However, if there is a true decrease in RA

occurrence, environmental factors and most intriguingly infectious agents should gain renewed interest regarding the causes of RA.

Table 1. *The American College of Rheumatology 1987 criteria for the classification of rheumatoid arthritis. Patients fulfilling ≥4/7 of the criteria are classified as having RA. Criteria 1-4 must have been present for at least 6 weeks.*

	Criterion	Comment
1.	Morning stiffness	At least 1 hour until maximal improvement
2.	Arthritis of 3 or more joint areas	Fluid or soft tissue swelling in ≥3 defined joint areas simultaneously
3.	Arthritis of hand joints	Not including distal interphalangeal joints
4.	Symmetric arthritis	Absolute symmetry is not required in phalangeal joints
5.	Rheumatoid nodules	Subcutaneous nodules
6.	Rheumatoid factor	Present at a serum level where >95% of control subjects are negative
7.	Radiographic changes	Erosions or periarticular decalcification on hand or wrist radiographs

Aetiology

Although the aetiology remains elusive, RA is believed to evolve in genetically susceptible individuals in whom environmental triggers are followed by immune reactions towards self-proteins. A remarkable diversity of environmental agents and lifestyle factors have been proposed to trigger the development of RA, but smoking remains the only one that consistently shows association with an increased risk of the disease [18-20]. Exposure to silica, mineral oil, and solvents have been suggested to constitute environmental risk factors [21-23], while educational level may be inversely correlated with the risk of RA [18, 24]. Infectious agents have at times been hypothesized to initiate the auto-reactive immune responses of RA by their resemblance to self-proteins expressed in the joint. For example, levels of antibodies against *Proteus mirabilis* are raised in RA patients compared to other arthritides and healthy controls [25]. Also, these antibodies were shown to mediate lysis of red blood cells coated with collagen IX [25]. However, whether or not eradication of *Proteus mirabilis* is beneficial to RA patients has not yet been investigated. Among viral infections, Epstein-Barr and parvovirus B19 have been the most extensively studied. Both

viral DNA and bacterial degradation products may be found in the synovial fluid of RA patients, but the association is seriously hampered by the poor specificity compared to patients with other arthritides or joint trauma [26, 27]. Also, and perhaps most importantly, no viruses or bacteria have systematically been isolated from RA joints in the absence of septic arthritis.

Family clustering implies a hereditary component of RA aetiology. However, it should be remembered that inheritance is not just a matter of genetics, but also lifestyle factors such as smoking habits may to a considerable extent be adopted from family members [19, 28]. Also, exposure to environmental risk factors could, hypothetically, show a high degree of concordance within families. However, a large and growing body of evidence points towards genetic variation as an essential basis for RA susceptibility.

Given their high degree of matching regarding age and environmental factors, twins constitute an invaluable resource when estimating the influence of genetic *vs* environmental factors for disease susceptibility [29]. Further, when studying monozygotic twins, the genetic profile is completely matched. The first investigation on the concordance rate in monozygotic twins was performed in the late 60s, estimating the rate to be ~30% [30]. Later studies have found slightly lower rates, around 15%, and most importantly, that the concordance rate is consistently lower in dizygotic twins [31, 32], providing proof of concept that there is an increased risk of RA conferred by certain genes. Another marker of the genetic contribution is the ratio of the disease prevalence among affected siblings to the prevalence in the population from which they were recruited (λs), which in RA has been estimated to 3-15 [31, 33]. Using two nationwide twin cohorts from Finland and the UK, it has been calculated that genetic variation accounts for ~60% of RA susceptibility in these populations [34]. Furthermore, an investigation of the genetically homogenous Icelandic population found increased risk ratios also in second-degree relatives, but not among spouses [35]. Thus, it must be concluded that genetic determinants are important in RA aetiology.

The search for the genes conferring increased risk of RA has been intense and extensive. To this aim there are two main approaches; genetic linkage studies or candidate gene

investigations [36]. Linkage studies use RA families to investigate on which chromosomal regions disease associated genes are contained. Genetic markers with known positions in the genome are examined under the assumption that disease associated regions more often will be present in affected individuals as compared to their healthy relatives. Linkage studies may provide 'hot spots' of the genome, which then are to be further narrowed and characterized in search of the identity and functions of the culprit protein. The candidate gene approach, on the other hand, uses existing knowledge or hypotheses regarding the aetiopathogenesis to test if genetic variation in the protein of interest influences disease susceptibility. RA is a genetically complex trait, meaning that it is not subject to simple Mendelian inheritance, but most likely determined by several genes that individually confer only a small increase in susceptibility. This, in combination with the disease heterogeneity, imposes great challenges to efforts of characterizing the genetic basis of the disease. However, both genomic linkage scans and association studies have repeatedly shown that certain alleles of the Human Leukocyte Antigen (HLA) genes harbour an increased risk of RA [37]. It has been revealed that a specific amino acid sequence, RAA in positions 72-74 of the third hypervariable region of the DRB1 molecule, is common to the risk alleles and hence denoted 'shared epitope' (SE) [38]. The RAA sequence hypothetically augments the presentation of arthritogenic peptides to antigen presenting cells (APCs) and thereby elicits an immune response towards constituents of the synovial joints. Recently, it has been found that the amino acids flanking the RAA sequence modulate this increased risk of RA [39].

Apart from SE, a large number of genes have been suggested to increase RA susceptibility. From a pathogenetic point of view, genes encoding pro-inflammatory cytokines, in particular tumour necrosis factor alpha (TNF), are good candidates. However, in spite of numerous investigations, the findings have been inconclusive [40-44]. Also, molecules involved in antigen presentation and T-cell activation are putative candidates to cause RA. Hence, associations with RA have been shown both regarding the gene encoding MHC class II transactivator, which influences MHC expression [45, 46], and the negative T-cell regulator 'cytotoxic T-lymphocyte antigen 4' (CTLA-4) [47, 48]. However, neither of these associations have been consistently replicated [49-51]. In another T-cell regulator, namely the protein tyrosine phosphatase non-receptor type 22

(PTPN22), a polymorphism (C1858T) has repeatedly been associated with an increased risk of RA [52-58]. These findings are further supported by the fact that PTPN22 is located in a region (1p13) pointed out in genomic scans [59], and that the very same polymorphism is implicated in other autoimmune diseases like type I diabetes, systemic lupus erythematosus (SLE) and Graves' disease [58, 60-62]. Fc receptor polymorphisms have been suggested to influence susceptibility to RA and other autoimmune diseases, although results are conflicting. This is further reviewed below. In conclusion, only HLA-DRB1 and PTPN22 may so far be regarded as reliable genetic determinants of RA susceptibility.

Pathophysiology

Although not yet fully understood, the pathophysiology of RA is in certain aspects better described than its aetiology. The most prominent cellular players of the synovial inflammation are macrophages, T cells, synovial fibroblasts and B cells, which act in concert in promoting synovial hyperplasia and degradation of cartilage and bone. It is believed that APCs in the synovial membrane present autoantigens to T cells, which then initiate an antigen-driven immune response characterized by macrophage infiltration and altered cytokine production. In response to this, synovial fibroblasts are activated, adhere to cartilage constituents, and produce degrading proteinases such as matrix metalloproteinases (MMPs) [63]. Parallel to this, B cells encounter both the autoantigen(s) and stimulating T cells, which promote their differentiation into plasma cells with the ability to produce autoantibodies. Although the pathogenic implication of autoantibodies in RA remains elusive, they may form immune complexes able to mediate tissue damage by complement activation or Fc receptor ligation [64]. Indications of an ongoing adaptive immune response in RA joints are provided by the abundant presence of CD4+ T cells in combination with the HLA-DRB1 association. Also, ectopic lymphoid tissue forming germinal centres may be found in the synovium [65], indicating antigen recognition by B cells. Subsequently, the rheumatoid synovium becomes hyperplasic, and grows in a malignancy-like manner over the cartilage and joint-near structures, causing irreversible structural damage.

Immunological communication is conducted by cytokines, and imbalances in the cytokine network are seen in several autoimmune diseases [66]. Among the growing number of cytokines identified, TNF and interleukin 1 (IL-1) have attracted most interest in RA. TNF was originally identified in 1975, by its potential to induce necrosis of tumour cells similar to that of endotoxins [67]. Its role in inflammation, and particularly in RA, begun to unfold a decade later by the findings that TNF facilitates degradation of cartilage and bone [68], and promotes infiltration of inflammatory cells [69, 70], while the synthesis of collagen and peptidoglycans by chondrocytes is inhibited [71, 72]. The first trial in RA patients, using a monoclonal chimeric anti-TNF antibody, was a success [73]. The pro-inflammatory effects of IL-1 are also well documented (reviewed in [74]). In RA, levels of IL-1 are greatly increased in arthritic joints as compared to a non-affected contralateral joint of the same patient [75]. Also, IL-1 has been suggested to be a powerful mediator especially of cartilage and bone degradation [76]. There are two different forms of IL-1 receptor agonists, IL-1α and IL-1β, both of which are predominantly synthesized by macrophages upon stimulation by endotoxins, cytokines or immune complexes. IL-1β is the major secreted form, while IL-1α is expressed on the cell membrane involved in cell-cell interactions.

Autoantibodies in RA

Ever since the description of the rheumatoid factor (RF) in 1940 [77], autoantibodies have been subjected to much interest, and a great deal of debate regarding their roles in RA pathogenesis, diagnosis, and prognosis. It should be remembered that the presence of autoantibodies, *i.e.* antibodies toward self-antigens, is not necessarily equivalent to autoimmune disease or other immuno-pathologies. On the contrary, antibodies recognizing self-antigens are frequently found in healthy individuals, and may even be physiologically important in immune homeostasis and in clearance of aging cells [78, 79]. This fact emphasizes the need for strict methodology when determining cut-off levels in autoantibody analyses.

RF is a family of autoantibodies that recognizes the 'fraction crystallisable' (Fc) part of IgG molecules (Figure 1), and exists as IgA-, IgG-, and IgM-isotypes.

Figure 1. *Schematic illustration of antibody structure and the rheumatoid factor, here recognizing the Fc parts of two adjacent IgG antibodies bound to epitopes on a particle surface.*

The most commonly used method of RF analysis, the latex agglutination test, mainly detects IgM-RF, but enzyme-linked immunosorbent assays (ELISAs) have been developed to allow isotype-specific RF detection. The prevalence of RF in RA patients is estimated to 70-80%, being more common among patients with severe disease and/or extra-articular manifestations [80, 81]. However, its specificity for RA is hampered by a prevalence of more than 10% in conditions that may mimic RA in early phases [82]. Furthermore, the prevalence of RF seems to increase with age, since 17% of Finnish non-RA subjects aged 78-88 years tested positive at a cut-off level defined by the 95th percentile of healthy subjects aged 19-60 [83]. With these points taken into consideration, there clearly are some caveats of the RF test in diagnosing RA, although it remains one of the seven ACR classification criteria [5]. In patients testing RF positive at diagnosis, the disease course and mortality rates have consistently been found less favourable [80, 84-88]. IgA-RF has been proposed to be superior in predicting aggressive disease [89, 90], but this has been contradicted by others [91].

In 1964, Nienhuis and Mandema discovered a highly RA-specific autoantibody that showed a perinuclear staining pattern on human buccal mucosal cells, hence denoted anti-perinuclear factor (APF) [92]. However, the diagnostic sensitivity turned out to be low, and APF never came in clinical use. More than ten years later, anti-keratin antibodies (AKA) detected by indirect immunofluorescence microscopy on rat oesophagus sections, also showed promisingly high specificity for RA [93]. Unfortunately, the sensitivity was

relatively low (58%) and the analysis is laborious and difficult to standardize. Subsequent work revealed that filaggrin actually was the target antigen of AKA [94], and that a post-translational modification of the antigen was required, namely the conversion of arginine to citrulline [95] (Figure 2).

Figure 2. Citrullination is a reaction where an arginine residue is converted to a citrulline residue. Peptididylarginine deiminase and calcium ions are required for the reaction to occur.

These achievements led to the development of synthetic antigens, cyclic citrullinated peptides (CCP), which could be used in an ELISA with superior performance compared to the 'older relatives' APF and AKA [82]. Peptidylarginine deimination of proteins, *i.e.* citrullination, are dependent on peptidylarginine deiminases (PADs), of which five isotypes have been described. PAD2 is expressed in macrophages, while polymorphonuclear leucocytes harbour PAD4 [96]. PAD activation demands a calcium concentration well above that of the enzyme's normal milieu, possibly pointing towards a physiological role in end stage-differentiation or death of cells, *i.e.* when calcium homeostasis is disrupted [96, 97]. Although cytoplasmic proteins of importance to cell structure are believed to be the primary target of PADs, the most abundant citrullinated protein structures in RA joints were found to be α– and β-chains of fibrinogen [98].

Anti-citrullinated protein antibodies (ACPA), as measured by the anti-CCP test, have proven to be highly RA-specific, *i.e.* ≥99% compared to healthy controls, and ≥95% compared to other arthritides [99, 100]. This remarkable specificity implies an aetiopathogenetic connection, especially considering the findings that the presence of ACPA predicts the development of RA not only in patients with early arthritis [101, 102], but also prior to the appearance of clinical symptoms [103, 104]. Furthermore, genetic variation in PAD4 was reported to associate with RA susceptibility and occurrence of

ACPA in a Japanese population [105], but subsequent studies on Caucasians showed conflicting results [57, 106, 107]. Instead, it seems that the development of ACPA is strongly related to carriage of the shared epitope [108-110], which efficiently presents citrullinated antigens [111]. The simultaneous presence of ACPA and SE strongly predicts the future onset of RA in a population-based setting, as elegantly shown by Berglin and colleagues [112]. Furthermore, the 1858T variant of PTPN22 in combination with ACPA showed 100% specificity for developing RA [54]. Neither citrullination nor the synovial presence of citrullinated proteins is an RA-specific phenomenon [113], but rather seems to be related to inflammation *per se* [114]. Local ACPA production in RA synovium has been suggested, since synovial fluid levels are higher than those in serum of paired samples [113]. Although citrullinated proteins also occur in the joints of arthritic mice [115], murine arthritis in native mice has not been found to be associated with ACPA formation [116].

The clinical utility of ACPA tests, of which the anti-CCP test is by far the most commonly used, has been thoroughly investigated in recent years. The first generation anti-CCP test, using filaggrin-derived antigens, was modified and re-launched as the anti-CCP2 test, showing increased sensitivity and similar specificity for RA [99]. The anti-CCP2 assay has shown a sensitivity of 50-60% in early RA [99, 117], and ~80% in established disease [117, 118]. Apart from its close relation to present or future RA diagnosis, a positive anti-CCP2 test is also predictive of the development and progression of joint damage [81, 87, 110, 119, 120]. Although this ability is, at least, in line with that of RF, to date it has not been found to predict extra-articular manifestations to the same extent [81, 121]. Comparisons between the anti-CCP2 test and RF regarding the ability to predict mortality are sparse, but may be in favour of the latter [86]. However, this needs further investigation.

Apart from ACPA and RF, a broad variety of autoantibodies have been detected in RA sera, recognizing for instance glucose-6-phosphate isomerase, type II collagen, and endoplasmic reticulum chaperone binding protein (reviewed in [122]). However, these autoantibodies either lack sensitivity or specificity and their importance in pathogenesis and/or clinical utility remain to be proven.

Disease course and pharmacotherapy

The disease course of RA varies considerably, both in terms of intra-individual symptom fluctuations and the inter-individual differences seen between mild unobtrusive disease, and severe forms with disabling joint destruction and organ manifestations such as pericarditis, pleuritis, and vasculitis. In its more aggressive forms, RA is associated with severe disability and premature death, the latter mainly due to ischaemic heart disease [88, 123], but after instituting efficient anti-rheumatic medication, *e.g.* anti-TNF therapy, this risk may possibly be reduced [124, 125].

Momentary disease activity can be assessed, both for clinical and research purposes, by a 28-joint disease activity score (DAS28) comprising swollen joint count (SJC), tender joint count (TJC), erythrocyte sedimentation rate (ESR), and the patient's assessment of general health [126]. DAS28>5.1 is classified as high disease activity, 3.2-5.1 as moderate, and <3.2 as low disease activity. Patients are regarded to be in remission at DAS28<2.6 [127]. Functional status may be assessed by the Stanford health assessment questionnaire (HAQ) [128], of which a Swedish version have been developed and validated [129]. Therapeutic response may be assessed in two ways; the response criteria of the European League against Rheumatism (EULAR) are based on DAS28, where 'good response' means a DAS28 improvement of ≥1.2 with an outcome value of <3.2, and 'moderate response' is either DAS28 improvement of ≥1.2, or improvement >0.6 and outcome value <5.1 [130]. The ACR response criteria measure only percentage change, regardless of the numerical outcome value, with the levels set at 20%, 50%, and 70%. SJC and TJC must independently improve by the corresponding percentage, together with three out of five of: HAQ score, C-reactive protein (CRP)/ESR, the patient's assessment of pain or assessment of disease activity, and the physician's assessment of disease activity [131].

The strategy of RA pharmacotherapy has been dramatically reformed during the last 15 years by switching from the expectant and wary 'pyramid approach' (starting with symptomatic therapy and followed by the addition of increasingly potent disease-modifying drugs (DMARDs) as the disease progressed) to early instituted potent DMARD medication. The new and more aggressive strategy, aiming at remission, has

been shown to improve disease activity and the progression of joint destruction for several years after diagnosis [132, 133]. Further improvements were achieved in the late 1990s by the introduction of TNF inhibitors. All anti-TNF substances available today; adalimumab, etanercept, and infliximab, have shown to be highly efficacious in lowering disease activity and slowing joint destruction [134-136]. Today, early DMARD therapy is almost always instituted, predominantly as methotrexate in monotherapy at a dose of 20-25mg/week [137]. In the case of sustained disease activity after 8 weeks or presence of unfavourable prognostic factors, methotrexate is often supplemented with other DMARDs, *e.g.* sulphasalazine and hydroxychloroquine [138]. If conventional DMARD therapy fails to bring disease activity under control, TNF inhibitors become an alternative [139]. The addition of low-dose glucocorticoids to DMARDs is currently experiencing a revival as it has been shown to improve radiographic progression and remission rate in early RA patients, without negatively affecting bone loss [140, 141]. However, long-term effects on metabolic factors remain to be elucidated.

Although great achievements regarding therapeutic options and strategies have been made in recent years, the ability to predict disease course and response to therapy has not improved accordingly. For instance, if RA patients in need of TNF-blocking therapy could be identified early, joint damage could probably be prevented. Further, all anti-rheumatic therapies are associated with a risk of potentially serious side-effects; for DMARDs mostly haematological or gastrointestinal [137], and for TNF inhibitors infectious or immunological [142-144]. Thus, for each patient this risk must be balanced against the potential benefit in order to optimize the anti-rheumatic therapy. To this cause, predictors of disease outcome and therapeutic response are warranted.

Fc receptors

Fc receptors (FcRs) recognize the constant part of immunoglobulins (Ig) and mediate many of the cellular effects elicited by antigen-antibody recognition. Immunoglobulins are bound to FcRs in a class-specific manner, *i.e.* IgG is recognized by Fcγ receptors (FcγRs), IgA by FcαRs, and IgE by FcεRs (reviewed in [145]). FcRs are expressed on the surface of a wide variety of haematopoietic cells, and also exist as soluble molecules in the circulation. In the context of allergy and autoimmune diseases, FcεRs and FcγRs are the most thoroughly studied. In this thesis, cellular FcγRs are focused.

Fcγ Receptors

Apart from the neonatal Fc receptor (FcRn), which transfers maternal IgG across the placenta to the foetus [146], three classes of human FcγRs have been identified [147]. As depicted in Table 2, these are further divided into subclasses, which differ in cellular expression and affinity.

Table 2. *Human receptors for IgG overviewed regarding affinity, distribution and function.*

Receptor	Affinity	Cell	Function
FcRn	IgG1, IgG2, IgG3, IgG4. Low affinity	Epithelial cells, endothelial cells	IgG transfer /homeostasis
FcγRI (CD64)	IgG1, IgG3, CRP. High Fcγ affinity	Macrophages, dendritic cells, neutrophils, eosinophils	Phagocytosis, immune complex endocytosis, cell activation
FcγRIIa (CD32)	IgG1, IgG2, IgG3, CRP. Low Fcγ affinity	Macrophages, dendritic cells, neutrophils, eosinophils, platelets	Phagocytosis, immune complex endocytosis, cell activation
FcγRIIb (CD32)	IgG1. Low Fcγ affinity	B cells, macrophages, mast cells, dendritic cells	Cell inhibition
FcγRIIIa (CD16)	IgG1, IgG3, CRP. Low Fcγ affinity	Macrophages, NK-cells, T cells, synoviocytes	Phagocytosis, immune complex endocytosis, cell activation, apoptosis
FcγRIIIb (CD16)	IgG1, IgG3, CRP. Low Fcγ affinity	Neutrophils	Phagocytosis, cell activation

Furthermore, a fourth class of FcγR (FcγRIV) was recently described in mice. Although not expressed by natural killer (NK) cells, FcγRIV appears to be closely related to the human FcγRIIIA [147]. Interestingly, most of the FcγRs display affinity for CRP, an interaction which is probably important in the clearance of CRP-opsonized pathogens or cell debris [148, 149]. Ligation of FcγRs can mediate a broad range of cell responses, such as phagocytosis, immune complex (IC) elimination, antibody-dependent cellular cytotoxicity (ADCC), and cytokine production. All FcγRs, except FcγIIB, promote cell activation. In FcγRI, FcγRIIA and FcγRIIIA, phosphorylation of an immunoreceptor tyrosine-based activation motif (ITAM) is crucial to initiate the intracellular cascade of kinases, the subtypes of which differ according to cell type. The inhibitory FcγRIIB contains an immunoreceptor tyrosine-based inhibitory motif (ITIM), which, in the case of cross-linking with an ITAM-bearing receptor, will restrain the activating signals by dephosphorylation [150, 151].

The role of FcγRs in arthritis has been elegantly investigated in murine knock-out models of arthritis. Mice lacking the γ-chain, *i.e.* a subunit common to FcγRI and FcγRIII, were shown to develop collagen-induced arthritis (CIA) to much lesser extent than control mice, even though levels of anti-collagen antibodies were similar [152]. A subsequent study, where FcγRIII was specifically deleted, concluded that this receptor is most likely of greater importance than FcγRI [153]. Regarding the inhibitory FcγRIIB, its absence has repeatedly been shown to increase susceptibility and severity of CIA [152, 154]. Findings in the murine K/B x N serum transfer model of arthritis are in concordance with those obtained in CIA models [155], further strengthening the role of FcγRs in antibody-mediated models of arthritis.

In human autoimmune diseases, the importance of FcγRs has been highlighted in studies showing that genetic variations in the genes encoding FcγRs, clustered at chromosome 1q, associate with susceptibility and severity of SLE and idiopathic thrombocytopenic purpura [156-160]. In RA, the influences of single-nucleotide polymorphisms (SNPs) in FcγRIIA, IIB, IIIA, and IIIB have been investigated in several genetically distinct populations. Regarding FcγRIIB, two studies (in addition to Paper III) have investigated the 232-I/T functional polymorphism without finding any influence on RA susceptibility

[161, 162]. However, Radstake *et al* reported that this polymorphism may not only alter FcγRIIB expression on APCs in RA patients, but also tune the rate of radiologic joint damage [162]. Furthermore, increased expression of FcγRIIB has been associated with decreased TNF production in RA monocytes [163]. Due to the remarkable homology between FcγRIIA and FcγRIIB, specific detection of the latter has been hampered by the lack of available monoclonal antibodies. As this issue now seems to have been resolved [162], the functions of FcγRIIB should be further clarified during the years to come. An extreme sequence homology to FcγRIIIB could also be the reason for the conflicting results regarding the FcγRIIIA-158V/F polymorphism in RA. This SNP, which confers a valine to phenylalanine substitution at position 158, was first described in 1997 [160]. The valine allele was shown to mediate higher affinity IgG binding, higher intracellular calcium concentration, and more pronounced apoptosis promotion. The first investigation on the FcγRIIIA-158V/F polymorphism in RA reported an increased risk of developing RA in Spanish individuals homozygous for the low-affinity phenylalanine allele, especially if SE was present [164]. A few months later, Morgan *et al* reported that the high-affinity allele, FcγRIIIA-158V, conferred increased susceptibility to RA, in particular nodular disease, in UK Caucasians and in a North Indian/Pakistani population [165]. This association was also found in another UK population of more than 800 patients [166], but could not be confirmed by Milicic *et al* [167]. A small Norwegian study could not disclose any influence of FcγRIIIA-158V/F on RA susceptibility or severity [168], which was concordant to the results of a Dutch study on 95 RA families [169]. From Japan, it has been reported that FcγRIIIA-158V is associated with RA [161] and that, in a subpopulation positive for anti-glucose-6-isomerase antibodies (which are arthritogenic in the K/B x N murine model of RA), the low-affinity variant 158F is protective against RA [170].

Investigations on FcγR expression and function in RA patients are complicated by the possible effects of ongoing therapy [171]. However, several interesting observations have been reported regarding FcγRIIIA. For instance, the expression pattern of FcγRIIIA in human tissues is intriguingly alike that of commonly described extra-articular manifestations of RA [172]. Further, in contrast to FcγRIIA which also is activating, the proportion of monocytic cells expressing FcγRIIIA is increased in synovial fluid as compared to peripheral blood cells [173]. The relative importance of the different

activating FcγRs was investigated by Abrahams *et al*, using monoclonal antibodies as receptor stimuli. It was shown that FcγRIIIA was by far the most important FcγR to induce TNF release from adhered monocytes [174], but the fact that F(ab')$_2$ fragments of the monoclonals were unable to induce a cytokine response, and that F(ab')$_2$ pre-incubation abrogated the stimulatory effect, raises questions as to whether the stimulatory effect of the intact antibody was instead due to Fc- FcγR interaction.

Taken together, both murine models and human studies have shown that FcγRs play important roles in RA aetiopathogenesis. However, their complex relationship remains incompletely understood.

The inflammasome

The term 'inflammasome' was coined by Tschopp and colleagues in 2002, describing a cytosolic protein complex with the ability to activate the cysteine protease caspase-1 [175], and thereby regulate the formation of bioactive IL-1β and IL-18. Several types of inflammasomes have subsequently been identified, and this family of protein complexes is suggested to be intracellular homologues to the surface-bound pathogen-recognition receptors, *e.g.* toll-like receptors. Hence, being members of innate immunity, the inflammasomes may respond to intrinsic danger signals or so called pathogen-associated molecular patterns, *i.e.* phylogenetically conserved microbial molecular signatures, by eliciting a host defence (reviewed in [176]).

The cryopyrin inflammasome

In 2004, the cryopyrin inflammasome was characterized, comprising cryopyrin (also known as NALP3), apoptosis-associated speck-like protein (ASC) and cardinal (tumour-up-regulated CARD-containing antagonist of caspase-9; also known as CARD-8 or TUCAN) [177]. It was also shown that the assembly of these proteins activates caspase-1 and thereby promotes the cleavage of the inactive cytoplasmic 31kDa peptide pro-IL-1β into its bioactive form, IL-1β (Figure 3).

Figure 3. Schematic illustration of the cryopyrin inflammasome where cryopyrin, ASC, and cardinal assemble by their pyrin (PYD) and caspase-reqruitment domains (CARD). This enables two caspase-1 molecules to be brought in proximity, promoting their activation and ability to cleave pro-IL-1β.

The expression pattern of cryopyrin is not yet completely outlined, but its presence seems to be most prominent in immune cells and non-keratinized epithelia of oropharynx, oesophagus and ectocervix [178, 179], which agrees with a possible role in the 'first line of defence'. The gene encoding cryopyrin, cold-induced autoinflammatory syndrome 1 (CIAS1), is best known for the pronounced association between its polymorphisms and rare autoinflammatory conditions such as Muckle-Wells syndrome (MWS), familial cold autoinflammatory syndrome (FCAS), and neonatal-onset multisystem inflammatory disease (NOMID) [180]. The findings that these genetic variants promote increased IL-1β production [177, 181], and that these patients often experience dramatic improvement upon IL-1 blocking therapy [182-184], provide important links between the cryopyrin inflammasome, IL-1β, and inflammatory disease. Characterization of stimuli that may trigger the cryopyrin inflammasome is ongoing. So far, it has been shown that viral RNA [185], urate crystals [186], and the bacterial degradation product muramyl dipeptide [187] are potent inducers of cryopyrin inflammasome activity.

Although IL-1 has obvious implications in RA pathology, the cryopyrin inflammasome has been sparsely investigated in this context. Increased levels of cryopyrin have been demonstrated in RA synovium compared to osteoarthritis [179], but the IL-1 antagonist anakinra has, due to lower clinical efficacy in RA, been far from the success of TNF inhibitors [139, 188]. For example, it was recently reported from a Dutch RA cohort that only 14% of patients remained on anakinra therapy after 2 years [189].

Aims

The general aim of this thesis work was to shed light on aetiopathogenetic connections as well as diagnostic and prognostic potential of anti-citrullinated protein antibodies and certain genetic variations in relation to RA.

Specific aims were to:

- Elucidate the diagnostic and prognostic properties of anti-CCP2 in early RA (paper I and II).

- Evaluate the influence of the FcγRIIIA-158V/F polymorphism on RA susceptibility and severity (paper III).

- Disclose the possible influence of the FcγRIIIA-158V/F polymorphism on the efficacy of TNF inhibitors in RA (paper IV).

- Explore whether genetic variation in proteins of the inflammasome associates with RA susceptibility and/or severity (paper V).

Patients & Methods

Patients

The studies in this thesis are based on 3 different cohorts of RA patients. In Figure 4, a schematic illustration of time course and disease burden depicts which phases of RA that are represented by these patient populations. Thereafter, a brief description of each cohort will follow. All patients gave written informed consent to participation, and the study protocols were approved by the regional ethics boards in Lund (paper I), Linköping (paper II, III, V) and Stockholm (paper IV).

Figure 4. *Schematic illustration of the patient cohorts in relation to disease burden and time of diagnosis.*

The 'very early' arthritis cohort (paper I)

In order to estimate the annual incidence of inflammatory joint disease, a prospective Swedish population-based referral study was conducted by Dr. Maria Söderlin in Kronoberg County in southern Sweden [11]. Between May 1 1999 and May 1 2000, 151 patients with acute arthritis, *i.e.* at least one swollen joint, were rapidly referred to a rheumatologist. The 71 patients that had experienced symptom duration < 3 months were included in a 'very early' arthritis cohort. Two patients had osteoarthritis and were hence

excluded. After two years of follow-up, diagnosis was made in 69 patients by an experienced rheumatologist.

The 'TIRA' cohort (paper II, III, V)

In 1996, a multicentre inception cohort called 'TIRA' (Swedish acronym for "early interventions in rheumatoid arthritis") was initiated in cooperation with 10 rheumatology units in south-eastern Sweden [190]. One of its main purposes was, in light of the emerging awareness of the benefits of early aggressive therapy in RA, to find useful predictors of the disease course of RA, and ultimately to improve the management of early RA. In order to facilitate enrolment of early RA cases, patients were eligible for inclusion if they fulfilled either ≥4/7 of the 1987 ACR criteria [5], or had morning stiffness ≥60 minutes, symmetrical arthritis, and small joint arthritis (proximal interphalangeal/metacarpo-/metatarso-phalangeal joints/wrists). However, it turned out that the vast majority (≥95% of the patients in paper II, III and V) fulfilled the ACR classification requirements. Symptom duration, *i.e.* the patient's experience of ≥1 swollen joint, was 6-52 weeks. The exclusion criteria comprised psoriasis with negative RF, prior history of joint swelling, and serious liver or renal disease. No standardized treatment protocol was established, and hence DMARDs, non-steroidal anti-inflammatory drugs, corticosteroids, and analgesics were prescribed as judged appropriate by the physicians. The participants were followed up at standardized time-points and of the 320 patients included, 276 remained in the study after 3 years.

The anti-TNF cohort (paper IV)

Between 1999 and 2003, all RA patients fulfilling the 1987 ACR criteria and who were started on anti-TNF therapy with etanercept or infliximab at the rheumatology units of the Karolinska Hospital in Solna and Huddinge, Sweden, were eligible for inclusion. The 282 participants all had long disease duration, and had failed to response to conventional DMARD therapy. Clinical and laboratory measures of disease activity were obtained at the start of anti-TNF therapy and 3 months thereafter. Also, data on concurrent corticosteroid and DMARD therapy were available for 273 of the patients.

Referents

In paper I and II, 80 healthy blood donors (40 women and 40 men) served as reference population in the anti-CCP analyses. Unfortunately, demographic data were not available. In the genetic analyses in paper III and V, controls comprised individuals from a south-eastern Swedish reference material, where participants had been randomly recruited to match the distribution of age, gender, and residency of the population in the region. Upon acceptance to inclusion, a blood sample was taken and a questionnaire was answered. In paper III and V, the mean age and gender distribution of the control subjects did not differ significantly from the patients. Control subjects who reported 'rheumatic disease' in the questionnaire were omitted.

Serological analyses

ELISA, which was introduced by Engvall and Perlman in 1971 [191], is a versatile and relatively cheap method of antibody analysis. In this thesis, ELISA was the main approach to autoantibody analysis.

Anti-citrullinated protein antibody (ACPA) analysis

ACPA was consistently assessed by an anti-CCP2 ELISA kit (Immunoscan RA CCP2, Euro-Diagnostica, Arnhem, the Netherlands). In a strict sense, the anti-CCP2 test does not detect autoantibodies, since the antigen is synthetically constructed and cyclised to enhance citrulline residue exposure. Serum samples were stored at -72°C until use, and then thawed and diluted 1:50 in phosphate-buffered saline. All sera were tested according to the manufacturer's instructions and results given as mean values of duplicates. Values ≥25 units/ml were regarded as positive. The coefficient of variance (CV) for intra- and inter-assay variation was investigated by performing eight analyses each in one high-leveled (1360 units/ml) and one low-leveled (79 units/ml) anti-CCP positive serum sample. The intra- and inter-assay CVs of the high-leveled serum were 13.6% and 10.5%, respectively, and the corresponding figures for the low level serum were 6.6% and 7.8%.

Rheumatoid factor analysis

RFs were detected in two ways; by the latex particle agglutination technique, and isotype-specifically by ELISA. Agglutinating RF was measured at the patients' local hospitals, where the cut-off levels were determined by the 95th percentile of the reference population used at this particular hospital. IgM- and IgA-RFs were analysed by commercially available ELISAs (Autozyme RF, Cambridge Life Sciences, Cambridge, UK). Cut-off levels were set at ≥34 units/ml for IgM-RF and ≥15 units/ml for IgA-RF, according to the 95th percentile of 100 healthy blood donors (50 women, 50 men).

Genotyping

The polymerase chain reaction (PCR), invented in the 1980s, has revolutionized the research on genetic basis of disease [192]. In many cases, however, further characterization of the PCR-amplified sequence is required to detect specific genetic variations, *e.g.* mutations or polymorphisms. This is preferentially enabled by a rapid, accurate, and cheap method, of which there seem to be as many suggestions as there are manufacturers and scientists. Brief descriptions of the methods used in this thesis are given below. In all studies, deoxyribonucleic acid (DNA) was extracted from peripheral blood using standard techniques.

FcγRIIIA genotyping

Genetic investigations on FcγRIIIA are complicated by its sequence homology with FcγRIIIB. To enable specific amplification of FcγRIIIA, we used a forward primer previously described by Morgan *et al* [165], involving a discriminating nucleotide in its 3' end. Together with an intronic reverse primer, this corresponds to a 293 base-pair PCR-product, which then was subject to denaturing high performance liquid chromatography (DHLPC). This technique is based upon the formation of heteroduplexed DNA, *i.e.* the annealing of two DNA strands where nucleotides at the mutation or the polymorphic site are 'mismatched' (Figure 5), altering the helical structure of the molecule. PCR products were injected into an automated liquid chromatography system (Transgenomic inc., Dallas, TX), using a linear gradient of acetonitrile in triethylamine acetate (TEAA) buffer as suggested by the manufacturer. Heterozygous samples were clearly identified, while homozygous samples (158FF and 158VV) required annealing with a known 158FF sample, enabling 158VV to form heteroduplexes. Following another round of DHPLC 158VV then appeared heterozygous, while 158FF remained unchanged (Figure 5). The PCR products of 25 random samples were sequenced by a fluorescent-based automated DNA sequencing system (MegaBace™ 500, GE Healthcare, Bucks, UK), showing results concordant to DHPLC in every case. Also, the nucleotide in position 473, which differs between FcγRIIIA and FcγRIIIB, confirmed FcγRIIIA-specific amplification.

Figure 5. *Representative chromatogram appearance of the FcγRIIIA-158V/F polymorphism. The upper right box shows a homozygous sample, where the presence of 158FF or 158VV could not be dissolved. As shown in the lower right box, the first chromatogram peak represents heteroduplex PCR products, found in either heterozygous samples or when a 158VV sample has been annealed with 158FF. The second peak represents homoduplexes, 158FF and 158VV.*

FcγRIIB genotyping

PCR, primer extension and DHPLC were used to assess FcγRIIB-232 genotype [193]. The extension primer, designed to anneal with its 3'-end next to the polymorphic allele, was extended with one dideoxynucleotide triphosphate (ddNTP) regardless of genotype, hence creating equal fragment lengths. However, when TEAA is used as buffer, retention time is a function of both size and nucleotide composition [194], and in this case primer extension products of equal lengths yielded clearly separable retention times (Figure 6). A 493 base-pair fragment was amplified using the forward primer 5'-CTA-AGG-GGA-GCC-CTT-CCC-TCT-GT-3' and the reverse primer 5'-AAT-ACG-GGC-CTA-GAT-CTG-AAT-GTG-3', as described by Li et al [195]. PCR conditions were equal to the FcγRIIIA assay (described in paper III) except that annealing time was 45 seconds and the final extension step was 5 minutes. 5 µl of PCR product was treated with 0.5 units of

Shrimp Alkaline Phosphatase and 5 units of Exonuclease 1 (GE Healthcare) at 37ºC for 15 minutes to remove unincorporated primers and dNTPs, followed by enzyme inactivation at 80 ºC for 15 minutes. 7 µl of the purified PCR product was used in a 20µl primer extension reaction with 50mM of dideoxyadenine triphosphate (ddATP) and dideoxyguanine triphosphate (ddAGT), 12 pmol of primer (5'-ACA-ATG-GCC-GCT-ACA-GCA-3') and 0.5 units of Thermo Sequenase (USB Corp., Cleveland, OH). Amplification was carried out with an initial denaturation step at 94ºC for 2 minutes, followed by 50 cycles of 94ºC for 10 seconds, 43ºC for 10 seconds and 60ºC for 10 seconds. 10 µl of the samples were injected into a HT3 column (Transgenomic inc.) and eluted with a linear gradient of 0.1 M acetonitrile (20-40% over 6.4 minutes) at a flow rate of 1.5ml/minute. Representative elution profiles are given in Figure 6.

Figure 6. *Representative elution profiles of the FcγRIIB-232I/T polymorphism. The first peak present in each chromatogram represents unincorporated primers and ddNTPs.*

CIAS1 and TUCAN genotyping

The CIAS1 and TUCAN SNPs were identified by a primer extension assay with a commercially available Megabace™ SnuPe genotyping kits (GE Healthcare). PCR products were purified as previously described and thermally cycled with SnuPe premix and a SNP-specific primer. Detection was performed on a MegaBACE™ 1000 DNA sequencing system (GE Healthcare).

Results & Discussion

Anti-CCP antibodies in relation to diagnosis and prognosis

In a cohort of incident early arthritis patients (n=69), the anti-CCP2 test was performed at baseline and related to diagnosis during a 2-year follow-up (Paper I). Of the 16 patients (23%) that were diagnosed with RA, 7 (44%) tested positive for anti-CCP2. No positive tests were found among the patients classified as having undifferentiated arthritis or 'other arthritides' (including psoriatic arthritis (PsoA), SLE, gluten enteropathy, sarcoid arthritis, Lyme arthritis, mixed connective tissue disease, ankylosing spondylitis, and polymyalgia rheumatica). Two of the 28 patients (7%) that were diagnosed with reactive arthritis (ReA) tested positive for anti-CCP2 (Figure 7). However, one of these patients, although diagnosed with a post-streptococcal ReA, actually fulfilled the ACR criteria for RA during the follow-up.

Figure 7. *Anti-CCP levels at inclusion according to diagnosis.*
● indicates RF+ and ○ RF-

If this patient instead was classified as having RA, the sensitivity turned out to be 47% and the specificity for RA was 98%. This was superior to the performance of agglutinating RF (35% and 94%, respectively). We found no correlation between the anti-CCP antibody level and laboratory or clinical variables such as ESR, CRP, or joint swelling. Since only 34 of the patients (49%) underwent x-ray examination at the 2 year follow-up, the predictive value of anti-CCP antibodies on radiographic progression could not be evaluated.

Further, we sought to determine the sensitivity of the anti-CCP2 test in early RA, its influence on disease course, and what happens with the levels longitudinally (paper II). Of the 242 early RA sera available inclusion in the 'TIRA' cohort, 156 (64%) tested positive for anti-CCP2. This was very similar to the results regarding agglutinating RF (155/242 =64%) and the co-occurrence with anti-CCP2 antibodies was highly significant ($P<0.001$). Serum samples from the 3-year follow-up were available for 121 patients, 96 of whom also had inclusion samples taken. Subjects lacking 3 year-samples did not differ with regard to disease severity, gender, or anti-CCP2 status at baseline compared to those with available sera. Although the mean anti-CCP2 level declined by 131 units/ml during 3 years from inclusion ($P=0.004$), 'seroconversion' was uncommon; only two patients changed from negative to positive, while three initially anti-CCP2 positive patients subsequently tested negative (Figure 8). None of the 80 healthy blood donors tested positive.

Figure 8. *Levels of anti-CCP antibodies in early RA patients (TIRA) at inclusion and after 3 years, and in healthy controls.*

The findings that anti-CCP status is stable over time has been reproduced by others [119, 196, 197], whereas the changes in levels have varied across study populations. A close relation between autoantibody level and disease activity and/or therapeutic response would strengthen the notion of a pathogenetic role. It seems that conventional DMARD therapy is associated with decreased anti-CCP antibody levels [119, 196], while

investigations on patients treated with TNF inhibitors have shown diverging results [197-200]. Likewise, whether or not changes in anti-CCP levels are associated with disease progression and/or therapeutic response remain to be clarified. RA therapy with rituximab, *i.e.* a B cell-depleting monoclonal antibody, would theoretically be an obvious long-term means to eliminate autoantibody production. However, although RF levels decline following rituximab therapy [201, 202], results regarding anti-CCP are conflicting. For instance, Cambridge *et al* reported that both anti-CCP and RF levels declined after rituximab therapy and that clinical relapses were closely related to rising levels of at least one of these autoantibodies [202]. Contrastingly, Toubi and colleagues reported that anti-CCP antibody levels did not decline upon rituximab therapy, whereas this was the case regarding RF, which also correlated to the clinical response [203]. Although the clinical implications of sequential variations of circulating autoantibody levels need further investigation, in the context of therapy-induced changes it seems that the RF and ACPA autoantibody families can be separated. As RF levels were not investigated passed inclusion in the TIRA cohort, such comparisons were not performed.

Rönnelid *et al* reported a significant drop in anti-CCP2 antibody levels, without correlation to disease progression, in patients treated with sulphasalazine but not in those receiving methotrexate [119]. However, when retrospectively investigating this in our cohort, we found that the mean anti-CCP2 antibody levels in patients receiving methotrexate at 3, 6 and 12 months (n=22) decreased by 195 units/ml (P=0.03). The same analysis for patients receiving sulphasalazine revealed a decrease of 134 units/ml, but due to the low number of subjects (n=7), this failed to reach statistical significance (P=0.2).

The disease course in relation to baseline anti-CCP2 antibody status was evaluated during three years of early RA (Paper II). Clinical and laboratory assessments revealed higher disease activity/severity in the anti-CCP2 positive patients compared to those testing negative in the anti-CCP2 assay. This was most pronounced regarding ESR, which was significantly higher in the anti-CCP2 positive patients at all time-points (Figure 9). Similar trends, but not as pronounced, were seen for RF. For instance, while the anti-CCP2 test at baseline predicted a significantly higher physician's assessment of disease activity

(PGA) score at all time points (except at the 3-year follow-up where $P=0.06$), IgM-RF did not predict higher PGA scores at any follow-up occasion. Most intriguingly, the more aggressive disease course seen in anti-CCP2 antibody-positive patients occurred despite more aggressive DMARD therapy throughout the study period (Figure 9).

Figure 9. *Disease activity measures and proportion of patients receiving DMARDs in relation to baseline anti-CCP status. Anti-CCP results were blinded to the physicians throughout the study period.*

Similar to the results presented in paper I, the levels of anti-CCP2 antibodies did not correlate to baseline disease activity measures, a finding which has been confirmed by others [119]. However, there are reports suggesting that the baseline level of anti-CCP antibodies correlate to future joint damage [204, 205].

Fcγ receptor polymorphisms in RA susceptibility and severity

The influence of FcγRIIB-232I/T and FcγRIIIA-158V/F on RA susceptibility and severity was evaluated in 181 early RA patients (paper III). The FcγRIIB genotype distribution was found to be very similar in patients and controls (Table 3), which is in line with other reports concluding that FcγRIIB-232I/T is not associated with an increased risk of RA [161, 162]. On the other hand, homozygousity for the high-affinity allele of FcγRIIIA (158VV) turned out to be significantly over-represented in RA patients (Table 3).

Table 3. *Allele and genotype distribution of the FcγRIIB and FcγRIIIA polymorphisms among patients and controls.*

	RA patients (%)	Controls (%)	Odds Ratio (95% CI)	*P*
Genotype frequencies:	*n* = 181	*n* = 362		
FcγRIIIA:				
158FF	70 (39)	168 (46)	1.0	
158VF	85 (47)	161 (45)	1.3 (0.9-1.9)	0.3
158VV	26 (14)	33 (9)	1.9 (1.01-3.5)	0.046
FcγRIIB:				
232II	137 (76)	269 (74)	1.0	
232IT	37 (20)	81 (22)	0.9 (0.6-1.4)	0.7
232TT	7 (4)	12 (3)	1.2 (0.4-3.2)	0.9
Allele frequencies:	*n* = 362	*n* = 724		
FcγRIIIA:				
158F	225 (62)	497 (69)	1.0	
158V	137 (38)	227 (31)	1.3 (1.02-1.8)	0.04
FcγRIIB:				
232I	311 (86)	619 (85)	1.0	
232T	51 (14)	105 (15)	0.9 (0.7-1.4)	0.9

After stratifying according to gender, it turned out that the increased risk of RA seen in the total cohort was predominantly harboured by its male subpopulation (Table 4). The increased risk for RA seen in the male population reached statistical significance in spite of the lower number of subjects as compared to the women. As previously discussed, results are conflicting regarding the impact of FcγRIIIA-158V/F on RA susceptibility, but this is the first report of a different influence in relation to gender.

Table 4. *FcγRIIIA genotype distribution in patients and controls after stratifying for gender.*

	RA patients (%)	Controls (%)	Odds Ratio (95% CI)	*P*
Fc γRIIIA genotype:				
Women:	*n* = 128	*n* = 228		
158FF	50 (39)	96 (42)	1.0	
158VF	61 (48)	109 (48)	1.1 (0.7-1.8)	0.9
158VV	17 (13)	23 (10)	1.4 (0.7-3.1)	0.4
Men:	*n* = 53	*n* = 134		
158FF	20 (38)	72 (54)	1.0	
158VF	24 (45)	52 (39)	1.7 (0.8-3.5)	0.2
158VV	9 (17)	10 (8)	3.2 (1.03-10.2)	0.04

No previous studies have reported gender-specific ORs regarding FcγR genetics, but their female-to-male ratios do not seem to explain the diverging results obtained regarding the FcγRIIIA-158V/F polymorphism and RA susceptibility [164-167]. Since the 158V allelic variant is stronger binding and more activating than 158F, the FcγRIIIA-158V/F polymorphism is likely to influence the pro-inflammatory response following IC encounter. Hence, 158VV individuals are hypothetically more prone to react with an aggressive and sustained inflammatory response upon IgG-IC encounter than those carrying 158FF. Although we did not find support for an increased risk among anti-CCP or RF positive patients, negative serum tests do not exclude local autoantibody production and occurrence of ICs in the joint. The mechanism by which men, but not women, carrying 158VV are at increased risk of developing RA is not obvious. Kramer and colleagues reported that incubation of human monocyte-derived macrophages with 17β-oestradiol caused a decrease in FcγRIIIA expression and FcγRIIIA-mediated release of TNF and IL-1β, and that this was reversed upon oestrogen removal [206]. The attenuation of the female dominance regarding RA incidence at higher ages is interesting in this context [207]. Although menopausal status was not investigated in this study, interestingly we noted that female patients aged 50-65 years (n=80), *i.e.* who were diagnosed with RA in proximity to probable menopause, displayed a genotype distribution (34% 158FF and 18% 158VV) very similar to that of male RA patients. Further, if these female patients were compared to female controls > 50 years of age, *i.e.* that theoretically had experienced the same 'menopausal risk', there was a higher OR for

158VV women (OR 2.5, 95%CI 0.8-7.5) than what was found in the total female population. However, since actual menopausal status and occurrence of hormonal replacement therapy were not evaluated, this finding does not allow any conclusions other than the need for further investigations on hormonal influences on FcγRIIIA function.

The disease course in relation to FcγRIIIA-158V/F genotype was found to differ between sexes. Mean values of the 3-year follow-up of HAQ and DAS28 were significantly higher in male patients carrying a 158V allele compared to those being 158F homozygous, while the opposite were seen among women. Also, the risk of baseline presence of radiographic joint damage was increased in 158VV patients (OR 6.1, 95%CI 1.4-28), but this finding must be interpreted cautiously, since data on symptom duration prior to inclusion are not available. Along with the report by Morgan and colleagues that 158VV is associated with the formation of rheumatoid nodules [166], this suggests that FcγRIIIA-158VV is an unfavourable prognostic marker in RA. However, given the evidence available today this issue remains to be settled.

We conclude that homozygousity of FcγRIIIA-158V confers increased risk for RA in men, and that future work on FcγRs in RA should be performed with a gender perspective.

Fcγ receptor type IIIA genotype and anti-TNF therapy

We investigated the influence of FcγRIIIA-158V/F on the therapeutic efficacy of etanercept and infliximab in 282 RA patients (paper IV). Both substances expose IgG1-Fc parts, which may interact with FcγRIIIA, hypothetically affecting the handling of the TNF-complexed drug. FcγRIIIA-expressing macrophages are abundant in rheumatoid synovium [208], and it has been shown that both etanercept and infliximab induce cell type-specific apoptosis of synovial macrophages to an extent that possibly associates with therapeutic response [209]. As the FcγRIIIA-158V/F polymorphism influences activation-induced apoptosis of NK cells [160], a role for FcγRIIIA in anti-TNF-induced apoptosis of synovial macrophages cannot be excluded. Also, opsonisation of cell surfaces by anti-TNF molecules would theoretically allow FcγRIIIA-mediated ligation of their Fc parts.

In our study we were unable to identify any significant differences in therapeutic response to infliximab or etanercept between patients with different FcγRIIIA genotypes (Figure 10). These findings contrast to those of Tutuncu *et al*, who reported that patients with RA or PsoA carrying the FcγRIIIA-158FF genotype responded most favourably to TNF inhibitors [210].

Figure 10. *Distribution of FcγRIIIA genotypes in relation to therapy response after 3 months as measured by the ACR response criteria.*

However, that study only comprised 35 subjects displaying considerable heterogeneity regarding genetic background, and the therapeutic response was not assessed by standard criteria. In line with our findings, a large study on adalimumab therapy outcome in RA

revealed no influence of neither FcγRIIIA-158V/F nor other polymorphisms in FcγRIIA and IIIB [211]. In Crohn's disease, an initial study reported that FcγRIIIA-158VV was beneficial regarding therapeutic response to infliximab, although this was subsequently contradicted [212]. Thus, FcγR polymorphisms do not seem useful to predict therapeutic response to TNF inhibitors. On the other hand, the role of FcγRIIIA for the therapeutic effect of rituximab in B cell malignancies is becoming increasingly evident. For instance, it has been shown that the FcγRIIIA-158VV genotype is associated with a superior therapeutic response in Waldenström's macroglobulinaemia [213] and non-Hodgkin's lymphomas [214-216]. Also, it has been shown that the valine variant of FcγRIIIA predicts a higher degree of rituximab-induced B cell depletion in SLE [217]. Of the putative mechanisms whereby rituximab depletes CD20+ cells, the relative importance of complement-dependent lysis, apoptosis, and ADCC by macrophages or NK cells remains unsettled. However, *in vitro* results on rituximab-induced ADCC by NK cells with different FcγRIIIA genotypes are in line with the clinical findings of therapeutic efficacy [218]. This issue needs to be addressed also in RA patients, since rituximab now is an established therapeutic option [219].

The inflammasome in early RA

Two recently described SNPs in genes encoding inflammasome components were studied regarding susceptibility and severity of RA (paper V). We found that, although being slightly more common among patients than in controls, the investigated SNPs of CIAS1 (Q705K) and TUCAN (C10X) did not individually confer increased risk of RA. However, when genotypes were combined and grouped according to the presence (+) or absence (-) of two wildtype alleles, the latter showed a significant association with RA. Thus, the carriage of at least one variant allele in both the CIAS1 and TUCAN SNPs was found to associate with an increased risk of RA, predominantly of the ACPA/SE positive phenotype (Figure 11). Although CIAS1 polymorphisms are closely related to MWS, NOMID, and FCAS [180], and TUCAN SNPs have been reported to influence susceptibility to Crohn's disease [220], there are no previous studies on RA patients regarding genetic variation in inflammasome proteins. Hence, this is the first report on this issue. Figure 11 shows the odds ratios in RA patients and subgroups, when compared to controls as CIAS1/TUCAN +/+ *vs* CIAS1/TUCAN -/-.

Figure 11. *Associations with* CIAS1/TUCAN -/- *in RA patients compared to controls (n=360). Odds ratios and 95% confidence intervals are shown.*

This intriguing and novel finding may, if confirmed, bring new insights to the role of innate immunity in RA aetiology and pathogenesis. In the context of autoinflammatory disorders, genetic variation in CIAS1 has been linked to deregulated IL-1β production, and also to clear-cut therapeutic response to IL-1 blocking therapy [181, 182]. Thus, functional studies of the CIAS1-Q705K and TUCAN-C10X polymorphisms are warranted.

It should be remembered that carriage of ≥1 variant allele in both the CIAS1 and TUCAN SNPs is relatively uncommon even among RA patients (11% in our study). Could these patients comprise a subgroup that would benefit from IL-1 blockade? A recent observational study reported that 14% of RA patients remained on IL-1 blocking therapy with sustained efficacy after 2 years [189]. This proportion is intriguingly alike that of CIAS1/TUCAN -/- patients found in the present study. We investigated the disease course and the prescription pattern of anti-rheumatic therapy in relation to the CIAS1 and TUCAN SNPs. At inclusion, there were no significant differences between CIAS1/TUCAN +/+ and CIAS1/TUCAN -/- patients regarding in ESR, CRP, PGA or DAS28. When followed longitudinally, CIAS1/TUCAN -/- patients showed signs of a more unfavourable disease course, and particularly so after 1 year. For instance, a significantly smaller proportion of CIAS1/TUCAN -/- patients were in remission after 3 years compared to CIAS1/TUCAN +/+ (13% *vs* 43%, *P*=0.035). Although there was a trend toward a higher prevalence of combination DMARD therapy among CIAS1/TUCAN -/- patients, no differences turned out to be significant. Anti-TNF therapy during the first 5 years was retrospectively assessed in 169 patients. No patients were prescribed TNF inhibitors in the first 2 years, but during the following 3 years this was instituted in 23 patients (14%). Seven out of these 23 patients (30%) carried CIAS1/TUCAN -/-, while only one had CIAS1/TUCAN +/+ (4%). Hence, CIAS1/TUCAN -/- patients had greatly increased probability of receiving anti-TNF therapy during 5 years of early RA. Further, presence of a variant allele in either CIAS1 or TUCAN was significantly associated with an increased likelihood of receiving TNF inhibitors (Figure 12).

Figure 12. *Risk of receiving anti-TNF therapy during 5 years from inclusion in relation to genotype. The relative risks shown are calculated as compared to their homozygous counterparts. Error bars indicate 95%CI.*

The pattern of which the disease course diverges according to CIAS1/TUCAN genotype is compatible with an impact on the responsiveness to conventional DMARD therapy. This notion is further strengthened by the fact that the frequency of anti-TNF therapy, which was instituted only after failure to respond to conventional DMARDs, was astonishingly associated with the CIAS1/TUCAN genotype. The clinical progression of RA varies considerably, and it is highly probable that differing cytokine patterns are, at least partly, connected to this heterogeneity [221]. Since the cryopyrin inflammasome is known to promote formation of bioactive IL-1β and IL-18, these cytokines deserve to be focused in this regard. For instance, given the suggested relation between IL-1 and joint destruction [222], it would be interesting to investigate whether CIAS1/TUCAN genotype influences radiological joint damage. Unfortunately, analysis of the efficacy of IL-1 blocking therapy in these patients was not possible, as none received such therapy during the study period.

Concluding remarks & Future perspectives

Not even the fields of rheumatology and immunology are resistant to whims and trends. B cells, autoantibodies and ICs were fashionable for several decades following the discovery of RF, but as the identities and functions of the T cell receptor and HLA molecules began to unravel, T cells inevitably went ahead during the 80s and 90s. However, humoral immunity has enjoyed a revival after the breakthrough regarding ACPA [95], the important findings in murine FcγR knock-out models of autoimmunity [145, 223], and the discovery of B-cell targeted therapy in RA and other autoimmune diseases previously regarded as merely T-cell induced conditions. New knowledge concerning the close interaction between humoral and cellular immune reactions makes the distinction between T- and B-cell immunity less meaningful today. Also, innate immunity is currently re-establishing its role in RA aetiopathogenesis, with the family of toll-like receptors as a rising star [224]. Its intracellular correlate, the inflammasome [176], is likely to join in shortly.

We conclude in this work that ACPA is not only a highly specific marker for RA, but also predicts a more aggressive disease. It seems not far-fetched that the widespread use of ACPA analysis in the 'real-world' has improved early RA management, although this is rather complicated to quantify. The critic could argue that ACPA analysis has not brought much new to rheumatology; the ACR classification criteria still remain the same as in 1987, bringing a growing number 'non-RA patients', but testing positive for ACPA, to the clinic. Further, no specific therapeutic guidelines according to anti-CCP status have been developed. However, anti-rheumatic therapy remains complex and decision-making in the management of arthritis patients must never remain solely on laboratory markers, but on the sound judgements of experienced clinicians. Patients presenting with a positive APCA test, but without the clinical RA diagnosis, need to be followed closely as they are highly at risk to develop RA, especially when risk genes are present [54, 112]. Concerning the classification of RA, it would be surprising if ACPA were not included in a new set of international arthritis classification criteria.

The aetiopathogenetic connection between ACPA and RA is, apart from the extreme specificity, supported by a number of observations. For instance, ACPA commonly occurs prior to symptom onset with higher prevalence and levels closer to diagnosis [103, 104]. As we and others have reported, baseline ACPA status usually is maintained during the disease course. ACPA development therefore seemingly correlates to disease initiation and its stable presence influences the disease course. This does not support the notion immunization against citrullinated proteins as a disease epiphenomenon, but may instead comprise a potential therapeutic target. Until now, murine models of arthritis have not been very informative on this issue, since ACPA formation is absent [116]. However, the development of an HLA-DR4-transgenic mouse model in which ACPA occurs, and which also mimics the gender bias of human RA, seems very promising [225].

The interactions between autoantibodies and FcγRs comprise an appealing target of pharmacotherapy in autoimmune diseases. More detailed insights into autoantibody-FcγR interplays are likely to follow close behind the interesting finding by Petkova *et al*, namely that the IgG fraction of human RA sera induced arthritis when transferred to mice lacking FcγRIIB [226]. Also, as antibody-based therapies are becoming increasingly popular, interactions between these substances and FcγRs need to be focused, since they in many instances are likely to modulate therapy response. In particular, IgG glycosylation seems to influence FcγR-mediated cellular responses [227-229].

Following its recent discovery, the interest in the inflammasome flourishes and studies are now expanding beyond the autoinflammatory syndromes. Still, the possible role of the inflammasome in RA remains to be elucidated. We found a compound polymorphism in proteins of the cryopyrin inflammasome to influence susceptibility and disease course in RA. If this finding is confirmed, a novel subtype of RA patients that is possibly more 'IL-1 dependent' may hypothetically have been identified in which IL-1 blocking therapy should be investigated.

Populärvetenskaplig sammanfattning

Kronisk ledgångsreumatism, reumatoid artrit (RA), är en sjukdom där kroppens eget immunförsvar orsakar inflammation i leder, vilket ofta leder till nedbrytning av brosk och lednära ben. Sjukdomens uppkomstmekanismer är till stora delar okända, men man vet att genetiska faktorer bidrar och att kvinnor drabbas ungefär dubbelt så ofta som män. De senaste 15 åren har forskning visat nyttan av tidig, aggressiv behandling av RA samtidigt som fler potenta läkemedel har utvecklats. Tack vare detta har behovet av tidigt ställd diagnos och prognostiska faktorer ökat. För dessa båda syften har blodprovet reumatoid faktor (RF) länge använts. Vi har undersökt vad ett nytt blodprov, antikroppar mot citrullinerade proteiner (anti-CCP), kan tillföra angående tidig diagnos och prognos vid RA. Två olika patientgrupper har undersökts och vi fann att förekomst av anti-CCP antikroppar både är en stark diagnostisk markör och förutsäger ett svårare sjukdomsförlopp. Detta gör att provet är av stort värde vid omhändertagandet av patienter med nydebuterad ledinflammation.

Genetiska analyser visade att en tidigare misstänkt riskgen, av betydelse för hur immunförsvarets celler reagerar på antikroppar, tycks vara förknippad med ökad risk att drabbas av RA. Dock sågs denna risk endast hos män och inte hos kvinnor, vilket kan antyda en inverkan av könshormoner. Fler studier behövs på detta område. Det har tidigare föreslagits att denna genvariant kan påverka behandlingsresultatet av de nya läkemedlen riktade mot en av immunförsvarets signalsubstanser, tumörnekrosfaktor alfa. Vi kunde dock inte, i den största studien hittills publicerad, finna någon sådan påverkan hos RA-patienter.

Cellernas produktion av en annan viktig signalsubstans i immunförsvaret, interleukin-1 (IL-1), styrs till del av en samling proteiner som kallas "inflammasom". Vissa genvarianter i dessa proteiner är starkt kopplade till olika återkommande febersjukdomar, men har inte tidigare undersökts vid RA. Vi fann att en viss kombination av genvarianter medförde en ökad risk att insjukna i RA och dessutom ett sämre sjukdomsförlopp. Om detta fynd upprepas bör det undersökas om patienter med denna genkombination kan behandlas framgångsrikt med läkemedel riktat mot IL-1.

Acknowledgements

This work was performed at the Department of Molecular and Clinical Medicine, Division of Rheumatology/Autoimmunity and Immune Regulation unit (AIR), Linköping University. I am sincerely grateful to everyone who contributed to the work underlying this thesis. In particular, I would like to thank the following persons:

My supervisor **Thomas Skogh**, for your tremendous ability to create a positive atmosphere. Any problems that come up, be scientific or be administrative, are short-lived in your hands. In the meantime, there's always room for jokes of any quality... I couldn't imagine a better boss!

Peter Söderkvist, co-supervisor, for introducing me to the field of genetics. Your enthusiasm and drive is impressive and inspiring.

Christopher Sjöwall, dear friend and colleague, for your support and guidance during these years. The fact that you will probably always be a couple of years ahead of me will indisputably be compensated by Giffarna in superettan 2007…

All **AIR colleagues**, for support and interesting discussions. Especially my roommate **Jonas Wetterö** for valuable help and fun tennis games. **Gunnel Almroth** for your excellent laboratory skills and for keeping all samples in order. **Helena Enocsson** for great efforts on the Fc receptors. **Petra Cassel**, my co-roommate, for answering all kinds of questions although 'you're not paid to do so'.

Annette Molbaek and **Åsa Schippert**, laboratory technicians at the genetics lab, for always being very friendly and helpful. It's always a pleasure to spend time in your lab.

All **TIRA-collaborators**, especially **Ylva Billing, Eva Hallert** and **Ingrid Thyberg** at the Linköping 'head-quarter'.

My co-authors, **Ahmad Ahmadi, Johan Bratt, Per Eriksson, Sofia Ernestam, Hannu Kautiainen, Marjatta Leirisalo-Repo, Jon Lampa, Leonid Padyukov, Maria Söderlin, Deepti Verma** and **Gun Wingren**, for fruitful collaboration.

Gunnar Strandberg, friend and former colleague, for great efforts during our work on anti-CCP.

The staff at the Rheumatology Clinic at the University Hospital in Linköping, especially **Ann Bengtsson**, head of the clinic, for creating such a research-friendly environment.

All the **patients** who participated in these studies

My family, for interest and encouragement.

The beloved **Hera**, for taking me out for walks and for being entertaining and supportive during the long hours in front of the laptop at home.

Figure 14. *Hera in a supportive mood.*

And finally, my princess **Lisa**; for your love and patience. Life is dreamlike with you by my side.

References

1. **Rothschild BM, Turner KR, DeLuca MA.** Symmetrical Erosive Peripheral Polyarthritis in the Late Archaic Period of Alabama. *Science* 1988;241:1498-1501.
2. **Buchanan WW.** Rheumatoid arthritis: Another new world disease? *Seminars in Arthritis and Rheumatism* 1994;23:289-294.
3. **Fontecchio G, Ventura L, Azzarone R, et al.** HLA-DRB genotyping of an Italian mummy from the 16th century with signs of rheumatoid arthritis. *Ann Rheum Dis* 2006;65:1676-1677.
4. **Landré-Beauvais AJ.** The first description of rheumatoid arthritis. Unabridged text of the doctoral dissertation presented in 1800. *Joint Bone Spine* 2001;68:130-143.
5. **Arnett FC, Edworthy SM, Bloch DA, et al.** The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
6. **Harrison BJ, Symmons DP, Barrett EM, Silman AJ.** The performance of the 1987 ARA classification criteria for rheumatoid arthritis in a population based cohort of patients with early inflammatory polyarthritis. American Rheumatism Association. *J Rheumatol* 1998;25:2324-30.
7. **Machold KP, Stamm TA, Eberl GJ, et al.** Very recent onset arthritis--clinical, laboratory, and radiological findings during the first year of disease. *J Rheumatol* 2002;29:2278-87.
8. **Symmons D, Turner G, Webb R, et al.** The prevalence of rheumatoid arthritis in the United Kingdom: new estimates for a new century. *Rheumatology (Oxford)* 2002;41:793-800.
9. **Gabriel SE, Crowson CS, O'Fallon WM.** The epidemiology of rheumatoid arthritis in Rochester, Minnesota, 1955-1985. *Arthritis Rheum* 1999;42:415-20.
10. **Kvien TK, Glennås A, Knudsrød OG, et al.** The prevalence and severity of rheumatoid arthritis in Oslo. Results from a county register and a population survey. *Scand J Rheumatol* 1997;26:412-8.
11. **Söderlin MK, Börjesson O, Kautiainen H, et al.** Annual incidence of inflammatory joint diseases in a population based study in southern Sweden. *Ann Rheum Dis* 2002;61:911-915.
12. **Uhlig T, Kvien TK, Glennås A, et al.** The incidence and severity of rheumatoid arthritis, results from a county register in Oslo, Norway. *J Rheumatol* 1998;25:1078-84.
13. **Riise T, Jacobsen BK, Gran JT.** Incidence and prevalence of rheumatoid arthritis in the county of Troms, northern Norway. *J Rheumatol* 2000;27:1386-9.
14. **Doran MF, Pond GR, Crowson CS, et al.** Trends in incidence and mortality in rheumatoid arthritis in Rochester, Minnesota, over a forty-year period. *Arthritis Rheum* 2002;46:625-31.
15. **Jacobsson LT, Hanson RL, Knowler WC, et al.** Decreasing incidence and prevalence of rheumatoid arthritis in Pima Indians over a twenty-five-year period. *Arthritis Rheum* 1994;37:1158-65.

16. **Shichikawa K, Inoue K, Hirota S***, et al.* Changes in the incidence and prevalence of rheumatoid arthritis in Kamitonda, Wakayama, Japan, 1965-1996. *Ann Rheum Dis* 1999;58:751-6.

17. **Uhlig T, Kvien TK.** Is rheumatoid arthritis disappearing? *Ann Rheum Dis* 2005;64:7-10.

18. **Olsson ÅR, Skogh T, Wingren G.** Aetiological factors of importance for the development of rheumatoid arthritis. *Scand J Rheumatol* 2004;33:300-6.

19. **Silman AJ, Newman J, MacGregor AJ.** Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins. *Arthritis Rheum* 1996;39:732-5.

20. **Stolt P, Bengtsson C, Nordmark B***, 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;62:835-41.

21. **Stolt P, Källberg H, Lundberg I***, et al.* Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Ann Rheum Dis* 2005;64:582-6.

22. **Sverdrup B, Källberg H, Bengtsson C***, 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:R1296-303.

23. **Lundberg I, Alfredsson L, Plato N***, et al.* Occupation, occupational exposure to chemicals and rheumatological disease. A register based cohort study. *Scand J Rheumatol* 1994;23:305-10.

24. **Pedersen M, Jacobsen S, Klarlund M, Frisch M.** Socioeconomic status and risk of rheumatoid arthritis: a Danish case-control study. *J Rheumatol* 2006;33:1069-74.

25. **Wilson C, Rashid T, Tiwana H***, et al.* Cytotoxicity responses to peptide antigens in rheumatoid arthritis and ankylosing spondylitis. *J Rheumatol* 2003;30:972-8.

26. **Peterlana D, Puccetti A, Beri R***, et al.* The presence of parvovirus B19 VP and NS1 genes in the synovium is not correlated with rheumatoid arthritis. *J Rheumatol* 2003;30:1907-10.

27. **van der Heijden IM, Wilbrink B, Tchetverikov I***, et al.* Presence of bacterial DNA and bacterial peptidoglycans in joints of patients with rheumatoid arthritis and other arthritides. *Arthritis Rheum* 2000;43:593-8.

28. **Osler M, Clausen J, Ibsen KK, Jensen G.** Maternal smoking during childhood and increased risk of smoking in young adulthood. *Int J Epidemiol* 1995;24:710-4.

29. **MacGregor AJ, Snieder H, Schork NJ, Spector TD.** Twins. Novel uses to study complex traits and genetic diseases. *Trends Genet* 2000;16:131-4.

30. **Lawrence JS.** Heberden Oration, 1969. Rheumatoid arthritis--nature or nurture? *Ann Rheum Dis* 1970;29:357-79.

31. **Silman AJ, MacGregor AJ, Thomson W***, et al.* Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br J Rheumatol* 1993;32:903-7.

32. **Aho K, Koskenvuo M, Tuominen J, Kaprio J.** Occurrence of rheumatoid arthritis in a nationwide series of twins. *J Rheumatol* 1986;13:899-902.

33. **del Junco D, Luthra HS, Annegers JF***, et al.* The familial aggregation of rheumatoid arthritis and its relationship to the HLA-DR4 association. *Am J Epidemiol* 1984;119:813-29.

34. **MacGregor AJ, Snieder H, Rigby AS, et al.** Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum* 2000;43:30-7.

35. **Grant SF, Thorleifsson G, Frigge ML, et al.** The inheritance of rheumatoid arthritis in Iceland. *Arthritis Rheum* 2001;44:2247-54.

36. **Ghosh S, Collins FS.** The geneticist's approach to complex disease. *Annu Rev Med* 1996;47:333-53.

37. **van der Helm-van Mil AH, Wesoly JZ, Huizinga TW.** Understanding the genetic contribution to rheumatoid arthritis. *Curr Opin Rheumatol* 2005;17:299-304.

38. **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;30:1205-13.

39. **du Montcel ST, Michou L, Petit-Teixeira E, et al.** New classification of HLA-DRB1 alleles supports the shared epitope hypothesis of rheumatoid arthritis susceptibility. *Arthritis Rheum* 2005;52:1063-8.

40. **Cvetkovic JT, Wållberg-Jonsson S, Stegmayr B, et al.** Susceptibility for and clinical manifestations of rheumatoid arthritis are associated with polymorphisms of the TNF-alpha, IL-1beta, and IL-1Ra genes. *J Rheumatol* 2002;29:212-9.

41. **Pawlik A, Florczak M, Ostanek L, et al.** TNF-alpha -308 promoter polymorphism in patients with rheumatoid arthritis. *Scand J Rheumatol* 2005;34:22-6.

42. **Hajeer AH, Dababneh A, Makki RF, et al.** Different gene loci within the HLA-DR and TNF regions are independently associated with susceptibility and severity in Spanish rheumatoid arthritis patients. *Tissue Antigens* 2000;55:319-25.

43. **Wilson AG, de Vries N, van de Putte LB, Duff GW.** A tumour necrosis factor alpha polymorphism is not associated with rheumatoid arthritis. *Ann Rheum Dis* 1995;54:601-3.

44. **Brinkman BM, Huizinga TW, Kurban SS, et al.** Tumour necrosis factor alpha gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? *Br J Rheumatol* 1997;36:516-21.

45. **Swanberg M, Lidman O, Padyukov L, et al.** MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. *Nat Genet* 2005;37:486-94.

46. **Iikuni N, Ikari K, Momohara S, et al.** MHC2TA is associated with rheumatoid arthritis in Japanese patients. *Ann Rheum Dis* 2006; Epub Nov 7. DOI:10.1136/ard.2006.063347.

47. **Seidl C, Donner H, Fischer B, et al.** CTLA4 codon 17 dimorphism in patients with rheumatoid arthritis. *Tissue Antigens* 1998;51:62-6.

48. **Lei C, Dongqing Z, Yeqing S, et al.** Association of the CTLA-4 gene with rheumatoid arthritis in Chinese Han population. *Eur J Hum Genet* 2005;13:823-8.

49. **Eyre S, Bowes J, Spreckley K, et al.** Investigation of the MHC2TA gene, associated with rheumatoid arthritis in a Swedish population, in a UK rheumatoid arthritis cohort. *Arthritis Rheum* 2006;54:3417-22.

50. **Barton A, Myerscough A, John S, et al.** A single nucleotide polymorphism in exon 1 of cytotoxic T-lymphocyte-associated-4 (CTLA-4) is not associated with rheumatoid arthritis. *Rheumatology (Oxford)* 2000;39:63-6.

51. **Milicic A, Brown MA, Wordsworth BP.** Polymorphism in codon 17 of the CTLA-4 gene (+49 A/G) is not associated with susceptibility to rheumatoid arthritis in British Caucasians. *Tissue Antigens* 2001;58:50-4.

52. **Begovich AB, Carlton VE, Honigberg LA*, 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;75:330-7.

53. **Hinks A, Barton A, John S*, et al.* Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. *Arthritis Rheum* 2005;52:1694-9.

54. **Johansson M, Ärlestig L, Hallmans G, Rantapää-Dahlqvist S.** PTPN22 polymorphism and anti-cyclic citrullinated peptide antibodies in combination strongly predicts future onset of rheumatoid arthritis and has a specificity of 100% for the disease. *Arthritis Res Ther* 2006;8:R19.

55. **Harrison P, Pointon JJ, Farrar C*, et al.* Effects of PTPN22 C1858T polymorphism on susceptibility and clinical characteristics of British Caucasian rheumatoid arthritis patients. *Rheumatology (Oxford)* 2006;45:1009-11.

56. **Simkins HM, Merriman ME, Highton J*, et al.* Association of the PTPN22 locus with rheumatoid arthritis in a New Zealand Caucasian cohort. *Arthritis Rheum* 2005;52:2222-5.

57. **Plenge RM, Padyukov L, Remmers EF*, 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;77:1044-60.

58. **Orozco G, Sanchez E, Gonzalez-Gay MA*, et al.* Association of a functional single-nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum* 2005;52:219-24.

59. **Steer S, Abkevich V, Gutin A*, et al.* Genomic DNA pooling for whole-genome association scans in complex disease: empirical demonstration of efficacy in rheumatoid arthritis. *Genes Immun* 2006;8:57-68.

60. **Bottini N, Musumeci L, Alonso A*, et al.* A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 2004;36:337-8.

61. **Kyogoku C, Langefeld CD, Ortmann WA*, et al.* Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 2004;75:504-7.

62. **Velaga MR, Wilson V, Jennings CE*, et al.* The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *J Clin Endocrinol Metab* 2004;89:5862-5.

63. **Konttinen YT, Ainola M, Valleala H*, et al.* Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. *Ann Rheum Dis* 1999;58:691-7.

64. **Skogh T, Kastbom A, Sjöwall C.** The B cells are back! *Drug Discovery Today: Disease Mechanisms* 2005;2:351-357.

65. **Schröder AE, Greiner A, Seyfert C, Berek C.** Differentiation of B cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. *Proc Natl Acad Sci U S A* 1996;93:221-5.

66. **Andreakos ET, Foxwell BM, Brennan FM*, et al.* Cytokines and anti-cytokine biologicals in autoimmunity: present and future. *Cytokine Growth Factor Rev* 2002;13:299-313.

67. **Carswell EA, Old LJ, Kassel RL*, et al.* An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* 1975;72:3666-70.

68. **Bertolini DR, Nedwin GE, Bringman TS*, et al.* Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. *Nature* 1986;319:516-8.

69. **Cavender D, Saegusa Y, Ziff M.** Stimulation of endothelial cell binding of lymphocytes by tumor necrosis factor. *J Immunol* 1987;139:1855-60.

70. **Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA.** Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc Natl Acad Sci U S A* 1985;82:8667-71.

71. **Reginato AM, Sanz-Rodriguez C, Diaz A*, et al.* Transcriptional modulation of cartilage-specific collagen gene expression by interferon gamma and tumour necrosis factor alpha in cultured human chondrocytes. *Biochem J* 1993;294:761-9.

72. **Saklatvala J.** Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature* 1986;322:547-9.

73. **Elliott MJ, Maini RN, Feldmann M*, et al.* Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum* 1993;36:1681-90.

74. **Dinarello CA.** Biologic basis for interleukin-1 in disease. *Blood* 1996;87:2095-147.

75. **Rooney M, Symons JA, Duff GW.** Interleukin 1 beta in synovial fluid is related to local disease activity in rheumatoid arthritis. *Rheumatol Int* 1990;10:217-9.

76. **van den Berg WB.** Uncoupling of inflammatory and destructive mechanisms in arthritis. *Semin Arthritis Rheum* 2001;30:7-16.

77. **Waaler E.** On the occurence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. *Acta Pathol Microbiol Scand* 1940;17:172-188.

78. **Lacroix-Desmazes S, Kaveri SV, Mouthon L*, et al.* Self-reactive antibodies (natural autoantibodies) in healthy individuals. *J Immunol Methods* 1998;216:117-37.

79. **Poletaev A, Osipenko L.** General network of natural autoantibodies as immunological homunculus (Immunculus). *Autoimmun Rev* 2003;2:264-71.

80. **Combe B, Eliaou JF, Daures JP*, et al.* Prognostic factors in rheumatoid arthritis. Comparative study of two subsets of patients according to severity of articular damage. *Br J Rheumatol* 1995;34:529-34.

81. **De Rycke L, Peene I, Hoffman IE*, et al.* Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. *Ann Rheum Dis* 2004;63:1587-93.

82. **Schellekens GA, Visser H, de Jong BA*, et al.* The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000;43:155-63.

83. **Palosuo T, Tilvis R, Strandberg T, Aho K.** Filaggrin related antibodies among the aged. *Ann Rheum Dis* 2003;62:261-3.

84. **van der Heijde DM, van Riel PL, van Leeuwen MA*, et al.* Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis. A prospective follow-up study of 147 patients. *Br J Rheumatol* 1992;31:519-25.

85. **Guillemin F, Gerard N, van Leeuwen M*, et al.* Prognostic factors for joint destruction in rheumatoid arthritis: a prospective longitudinal study of 318 patients. *J Rheumatol* 2003;30:2585-9.

86. **Sihvonen S, Korpela M, Mustila A, Mustonen J.** The predictive value of rheumatoid factor isotypes, anti-cyclic citrullinated peptide antibodies, and antineutrophil cytoplasmic antibodies for mortality in patients with rheumatoid arthritis. *J Rheumatol* 2005;32:2089-94.

87. **Machold KP, Stamm TA, Nell VP*, et al.* Very recent onset rheumatoid arthritis: clinical and serological patient characteristics associated with radiographic progression over the first years of disease. *Rheumatology (Oxford)* 2007;46:342-9.

88. **Wolfe F, Mitchell DM, Sibley JT*, et al.* The mortality of rheumatoid arthritis. *Arthritis Rheum* 1994;37:481-94.

89. **Jonsson T, Thorsteinsson J, Kolbeinsson A*, et al.* Population study of the importance of rheumatoid factor isotypes in adults. *Ann Rheum Dis* 1992;51:863-8.

90. **Päi S, Päi L, Birkenfeldt R.** Correlation of serum IgA rheumatoid factor levels with disease severity in rheumatoid arthritis. *Scand J Rheumatol* 1998;27:252-6.

91. **Visser H, Gelinck LB, Kampfraath AH*, et al.* Diagnostic and prognostic characteristics of the enzyme linked immunosorbent rheumatoid factor assays in rheumatoid arthritis. *Ann Rheum Dis* 1996;55:157-61.

92. **Nienhuis RL, Mandema E.** A New Serum Factor in Patients with Rheumatoid Arthritis; the Antiperinuclear Factor. *Ann Rheum Dis* 1964;23:302-5.

93. **Young BJ, Mallya RK, Leslie RD*, et al.* Anti-keratin antibodies in rheumatoid arthritis. *Br Med J* 1979;2:97-9.

94. **Simon M, Girbal E, Sebbag M*, et al.* The cytokeratin filament-aggregating protein filaggrin is the target of the so-called "antikeratin antibodies," autoantibodies specific for rheumatoid arthritis. *J Clin Invest* 1993;92:1387-93.

95. **Schellekens GA, de Jong BA, van den Hoogen FH*, et al.* Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1998;101:273-81.

96. **Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ.** PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. *Bioessays* 2003;25:1106-18.

97. **Vossenaar ER, van Venrooij WJ.** Citrullinated proteins: sparks that may ignite the fire in rheumatoid arthritis. *Arthritis Res Ther* 2004;6:107-11.

98. **Masson-Bessière C, Sebbag M, Girbal-Neuhauser E*, et al.* The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. *J Immunol* 2001;166:4177-84.

99. **van Gaalen FA, Visser H, Huizinga TW.** A comparison of the diagnostic accuracy and prognostic value of the first and second anti-cyclic citrullinated peptides (CCP1 and CCP2) autoantibody tests for rheumatoid arthritis. *Ann Rheum Dis* 2005;64:1510-2.

100. **Zendman AJ, van Venrooij WJ, Pruijn GJ.** Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. *Rheumatology (Oxford)* 2006;45:20-5.

101. **van Gaalen FA, Linn-Rasker SP, van Venrooij WJ***, et al.* Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004;50:709-15.

102. **Vittecoq O, Incaurgarat B, Jouen-Beades F***, et al.* Autoantibodies recognizing citrullinated rat filaggrin in an ELISA using citrullinated and non-citrullinated recombinant proteins as antigens are highly diagnostic for rheumatoid arthritis. *Clin Exp Immunol* 2004;135:173-80.

103. **Rantapää-Dahlqvist S, de Jong BA, Berglin E***, et al.* Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;48:2741-9.

104. **Nielen MM, van Schaardenburg D, Reesink HW***, et al.* Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380-6.

105. **Suzuki A, Yamada R, Chang X***, et al.* Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003;34:395-402.

106. **Barton A, Bowes J, Eyre S***, et al.* A functional haplotype of the PADI4 gene associated with rheumatoid arthritis in a Japanese population is not associated in a United Kingdom population. *Arthritis Rheum* 2004;50:1117-21.

107. **Caponi L, Petit-Teixeira E, Sebbag M***, et al.* A family based study shows no association between rheumatoid arthritis and the PADI4 gene in a white French population. *Ann Rheum Dis* 2005;64:587-93.

108. **Klareskog L, Stolt P, Lundberg K***, 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;54:38-46.

109. **van Gaalen FA, van Aken J, Huizinga TW***, et al.* Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. *Arthritis Rheum* 2004;50:2113-21.

110. **Forslind K, Ahlmén M, Eberhardt K***, et al.* Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). *Ann Rheum Dis* 2004;63:1090-5.

111. **Hill JA, Southwood S, Sette A***, et al.* Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J Immunol* 2003;171:538-41.

112. **Berglin E, Padyukov L, Sundin U***, et al.* A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. *Arthritis Res Ther* 2004;6:R303-8.

113. **Vossenaar ER, Smeets TJ, Kraan MC***, et al.* The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. *Arthritis Rheum* 2004;50:3485-94.

114. **Makrygiannakis D, af Klint E, Lundberg IE***, et al.* Citrullination is an inflammation-dependent process. *Ann Rheum Dis* 2006;65:1219-22.

115. **Vossenaar ER, Nijenhuis S, Helsen MM*, et al.* Citrullination of synovial proteins in murine models of rheumatoid arthritis. *Arthritis Rheum* 2003;48:2489-500.

116. **Vossenaar ER, van Boekel MA, van Venrooij WJ*, et al.* Absence of citrulline-specific autoantibodies in animal models of autoimmunity. *Arthritis Rheum* 2004;50:2370-2.

117. **Dubucquoi S, Solau-Gervais E, Lefranc D*, et al.* Evaluation of anti-citrullinated filaggrin antibodies as hallmarks for the diagnosis of rheumatic diseases. *Ann Rheum Dis* 2004;63:415-9.

118. **Pinheiro GC, Scheinberg MA, Aparecida da Silva M, Maciel S.** Anti-cyclic citrullinated peptide antibodies in advanced rheumatoid arthritis. *Ann Intern Med* 2003;139:234-5.

119. **Rönnelid J, Wick MC, Lampa J*, et al.* Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. *Ann Rheum Dis* 2005;64:1744-9.

120. **Lindqvist E, Eberhardt K, Bendtzen K*, et al.* Prognostic laboratory markers of joint damage in rheumatoid arthritis. *Ann Rheum Dis* 2005;64:196-201.

121. **Turesson C, Jacobsson LT, Sturfelt G*, et al.* Rheumatoid factor and antibodies to cyclic citrullinated peptides are associated with severe extra-articular manifestations in rheumatoid arthritis. *Ann Rheum Dis* 2007;66:59-64.

122. **Rantapää-Dahlqvist S.** Diagnostic and prognostic significance of autoantibodies in early rheumatoid arthritis. *Scand J Rheumatol* 2005;34:83-96.

123. **Scott DL, Smith C, Kingsley G.** Joint damage and disability in rheumatoid arthritis: an updated systematic review. *Clin Exp Rheumatol* 2003;21:S20-7.

124. **Jacobsson LT, Turesson C, Nilsson JÅ*, et al.* Treatment with TNF-blockers and mortality risk in patients with rheumatoid arthritis. *Ann Rheum Dis* 2006.

125. **Jacobsson LT, Turesson C, Gülfe A*, et al.* Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis. *J Rheumatol* 2005;32:1213-8.

126. **Prevoo ML, van 't Hof MA, Kuper HH*, et al.* Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44-8.

127. **Fransen J, Creemers MC, Van Riel PL.** Remission in rheumatoid arthritis: agreement of the disease activity score (DAS28) with the ARA preliminary remission criteria. *Rheumatology (Oxford)* 2004;43:1252-5.

128. **Fries JF, Spitz P, Kraines RG, Holman HR.** Measurement of patient outcome in arthritis. *Arthritis Rheum* 1980;23:137-45.

129. **Ekdahl C, Eberhardt K, Andersson SI, Svensson B.** Assessing disability in patients with rheumatoid arthritis. Use of a Swedish version of the Stanford Health Assessment Questionnaire. *Scand J Rheumatol* 1988;17:263-71.

130. **van Gestel AM, Prevoo ML, van 't Hof MA*, et al.* Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis Rheum* 1996;39:34-40.

131. **Felson DT, Anderson JJ, Boers M*, et al.* American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum* 1995;38:727-35.

132. **Möttönen T, Hannonen P, Korpela M*, et al.* Delay to institution of therapy and induction of remission using single-drug or combination-disease-modifying antirheumatic drug therapy in early rheumatoid arthritis. *Arthritis Rheum* 2002;46:894-8.

133. **Nell VP, Machold KP, Eberl G*, et al.* Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. *Rheumatology (Oxford)* 2004;43:906-14.

134. **Lipsky PE, van der Heijde DM, St Clair EW*, et al.* Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med* 2000;343:1594-602.

135. **Klareskog L, van der Heijde D, de Jager JP*, et al.* Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. *Lancet* 2004;363:675-81.

136. **Weinblatt ME, Keystone EC, Furst DE*, et al.* Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum* 2003;48:35-45.

137. **Pincus T, Yazici Y, Sokka T*, et al.* Methotrexate as the "anchor drug" for the treatment of early rheumatoid arthritis. *Clin Exp Rheumatol* 2003;21:S179-85.

138. **O'Dell JR.** Therapeutic strategies for rheumatoid arthritis. *N Engl J Med* 2004;350:2591-602.

139. **Furst DE, Breedveld FC, Kalden JR*, et al.* Updated consensus statement on biological agents, specifically tumour necrosis factor alpha (TNFalpha) blocking agents and interleukin-1 receptor antagonist (IL-1ra), for the treatment of rheumatic diseases, 2005. *Ann Rheum Dis* 2005;64 Suppl 4:iv2-14.

140. **Svensson B, Boonen A, Albertsson K*, et al.* Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. *Arthritis Rheum* 2005;52:3360-70.

141. **Kirwan J, Bijlsma J, Boers M, Shea B.** Effects of glucocorticoids on radiological progression in rheumatoid arthritis. *Cochrane Database Syst Rev* 2007;1:CD006356.

142. **Askling J, Fored CM, Brandt L*, et al.* Risk and case characteristics of tuberculosis in rheumatoid arthritis associated with tumor necrosis factor antagonists in Sweden. *Arthritis Rheum* 2005;52:1986-92.

143. **De Bandt M, Sibilia J, Le Loet X*, et al.* Systemic lupus erythematosus induced by anti-tumour necrosis factor alpha therapy: a French national survey. *Arthritis Res Ther* 2005;7:R545-51.

144. **Jonsdottir T, Forslid J, van Vollenhoven A*, et al.* Treatment with tumour necrosis factor alpha antagonists in patients with rheumatoid arthritis induces anticardiolipin antibodies. *Ann Rheum Dis* 2004;63:1075-8.

145. **Kleinau S.** The impact of Fc receptors on the development of autoimmune diseases. *Curr Pharm Des* 2003;9:1861-70.

146. **Story CM, Mikulska JE, Simister NE.** A major histocompatibility complex class I-like Fc receptor cloned from human placenta: possible role in transfer of immunoglobulin G from mother to fetus. *J Exp Med* 1994;180:2377-81.

147. **Nimmerjahn F, Ravetch JV.** Fcgamma receptors: old friends and new family members. *Immunity* 2006;24:19-28.

148. **Kindmark CO.** Stimulating effect of C-reactive protein on phagocytosis of various species of pathogenic bacteria. *Clin Exp Immunol* 1971;8:941-8.

149. **Bharadwaj D, Stein MP, Volzer M*, et al.* The major receptor for C-reactive protein on leukocytes is fcgamma receptor II. *J Exp Med* 1999;190:585-90.

150. **Van den Herik-Oudijk IE, Capel PJ, van der Bruggen T, Van de Winkel JG.** Identification of signaling motifs within human Fc gamma RIIa and Fc gamma RIIb isoforms. *Blood* 1995;85:2202-11.

151. **Muta T, Kurosaki T, Misulovin Z*, et al.* A 13-amino-acid motif in the cytoplasmic domain of Fc gamma RIIB modulates B-cell receptor signalling. *Nature* 1994;369:340.

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

153. **Diaz de Stahl T, Andren M, Martinsson P*, et al.* Expression of FcgammaRIII is required for development of collagen-induced arthritis. *Eur J Immunol* 2002;32:2915-22.

154. **Yuasa T, Kubo S, Yoshino T*, et al.* Deletion of fcgamma receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. *J Exp Med* 1999;189:187-94.

155. **Corr M, Crain B.** The role of FcgammaR signaling in the K/B x N serum transfer model of arthritis. *J Immunol* 2002;169:6604-9.

156. **Bruin M, Bierings M, Uiterwaal C*, et al.* Platelet count, previous infection and FCGR2B genotype predict development of chronic disease in newly diagnosed idiopathic thrombocytopenia in childhood: results of a prospective study. *Br J Haematol* 2004;127:561-7.

157. **Carcao MD, Blanchette VS, Wakefield CD*, et al.* Fcgamma receptor IIa and IIIa polymorphisms in childhood immune thrombocytopenic purpura. *Br J Haematol* 2003;120:135-41.

158. **Edberg JC, Langefeld CD, Wu J*, et al.* Genetic linkage and association of Fcgamma receptor IIIA (CD16A) on chromosome 1q23 with human systemic lupus erythematosus. *Arthritis Rheum* 2002;46:2132-40.

159. **Kyogoku C, Dijstelbloem HM, Tsuchiya N*, et al.* Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. *Arthritis Rheum* 2002;46:1242-54.

160. **Wu J, Edberg JC, Redecha PB*, et al.* A novel polymorphism of FcgammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J Clin Invest* 1997;100:1059-70.

161. **Kyogoku C, Tsuchiya N, Matsuta K, Tokunaga K.** Studies on the association of Fc gamma receptor IIA, IIB, IIIA and IIIB polymorphisms with rheumatoid arthritis in the Japanese: evidence for a genetic interaction between HLA-DRB1 and FCGR3A. *Genes Immun* 2002;3:488-93.

162. **Radstake TR, Franke B, Wenink MH*, et al.* The functional variant of the inhibitory Fcgamma receptor IIb (CD32B) is associated with the rate of radiologic

joint damage and dendritic cell function in rheumatoid arthritis. *Arthritis Rheum* 2006;54:3828-37.

163. **Wijngaarden S, van de Winkel JG, Jacobs KM*, et al.*** A shift in the balance of inhibitory and activating Fcgamma receptors on monocytes toward the inhibitory Fcgamma receptor IIb is associated with prevention of monocyte activation in rheumatoid arthritis. *Arthritis Rheum* 2004;50:3878-87.

164. **Nieto A, Caliz R, Pascual M*, et al.*** Involvement of Fcgamma receptor IIIA genotypes in susceptibility to rheumatoid arthritis. *Arthritis Rheum* 2000;43:735-9.

165. **Morgan AW, Griffiths B, Ponchel F*, et al.*** Fcgamma receptor type IIIA is associated with rheumatoid arthritis in two distinct ethnic groups. *Arthritis Rheum* 2000;43:2328-34.

166. **Morgan AW, Keyte VH, Babbage SJ*, et al.*** FcgammaRIIIA-158V and rheumatoid arthritis: a confirmation study. *Rheumatology (Oxford)* 2003;42:528-33.

167. **Milicic A, Misra R, Agrawal S*, et al.*** The F158V polymorphism in FcgammaRIIIA shows disparate associations with rheumatoid arthritis in two genetically distinct populations. *Ann Rheum Dis* 2002;61:1021-3.

168. **Brun JG, Madland TM, Vedeler CA.** Immunoglobulin G fc-receptor (FcgammaR) IIA, IIIA, and IIIB polymorphisms related to disease severity in rheumatoid arthritis. *J Rheumatol* 2002;29:1135-40.

169. **Radstake TR, Petit E, Pierlot C*, et al.*** Role of Fcgamma receptors IIA, IIIA, and IIIB in susceptibility to rheumatoid arthritis. *J Rheumatol* 2003;30:926-33.

170. **Matsumoto I, Zhang H, Muraki Y*, et al.*** A functional variant of Fcgamma receptor IIIA is associated with rheumatoid arthritis in individuals who are positive for anti-glucose-6-phosphate isomerase antibodies. *Arthritis Res Ther* 2005;7:R1183-8.

171. **Wijngaarden S, van Roon JA, van de Winkel JG*, et al.*** Down-regulation of activating Fcgamma receptors on monocytes of patients with rheumatoid arthritis upon methotrexate treatment. *Rheumatology (Oxford)* 2005;44:729-34.

172. **Bhatia A, Blades S, Cambridge G, Edwards JC.** Differential distribution of Fc gamma RIIIa in normal human tissues and co-localization with DAF and fibrillin-1: implications for immunological microenvironments. *Immunology* 1998;94:56-63.

173. **Wijngaarden S, van Roon JA, Bijlsma JW*, et al.*** Fcgamma receptor expression levels on monocytes are elevated in rheumatoid arthritis patients with high erythrocyte sedimentation rate who do not use anti-rheumatic drugs. *Rheumatology (Oxford)* 2003;42:681-8.

174. **Abrahams VM, Cambridge G, Lydyard PM, Edwards JC.** Induction of tumor necrosis factor alpha production by adhered human monocytes: a key role for Fcgamma receptor type IIIa in rheumatoid arthritis. *Arthritis Rheum* 2000;43:608-16.

175. **Martinon F, Burns K, Tschopp J.** The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* 2002;10:417-26.

176. **Martinon F, Tschopp J.** NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* 2005;26:447-54.

177. **Agostini L, Martinon F, Burns K*, et al.*** NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* 2004;20:319-25.

178. **Kummer JA, Broekhuizen R, Everett H*, et al.* Inflammasome Components NALP 1 and 3 Show Distinct but Separate Expression Profiles in Human Tissues, Suggesting a Site-specific Role in the Inflammatory Response. *J Histochem Cytochem* 2006; Epub Dec 12. DOI: 10.1369/jhc.6A7101.2006.

179. **Rosengren S, Hoffman HM, Bugbee W, Boyle DL.** Expression and regulation of cryopyrin and related proteins in rheumatoid arthritis synovium. *Ann Rheum Dis* 2005;64:708-14.

180. **McDermott MF, Aksentijevich I.** The autoinflammatory syndromes. *Curr Opin Allergy Clin Immunol* 2002;2:511-6.

181. **Dowds TA, Masumoto J, Zhu L*, et al.* Cryopyrin-induced interleukin 1beta secretion in monocytic cells: enhanced activity of disease-associated mutants and requirement for ASC. *J Biol Chem* 2004;279:21924-8.

182. **Hawkins PN, Lachmann HJ, McDermott MF.** Interleukin-1-receptor antagonist in the Muckle-Wells syndrome. *N Engl J Med* 2003;348:2583-4.

183. **Goldbach-Mansky R, Dailey NJ, Canna SW*, et al.* Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. *N Engl J Med* 2006;355:581-92.

184. **Hoffman HM, Rosengren S, Boyle DL*, et al.* Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. *Lancet* 2004;364:1779-85.

185. **Kanneganti TD, Body-Malapel M, Amer A*, et al.* Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J Biol Chem* 2006;281:36560-8.

186. **Martinon F, Petrilli V, Mayor A*, et al.* Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 2006;440:237-41.

187. **Martinon F, Agostini L, Meylan E, Tschopp J.** Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr Biol* 2004;14:1929-34.

188. **Gartlehner G, Hansen RA, Jonas BL*, et al.* The comparative efficacy and safety of biologics for the treatment of rheumatoid arthritis: a systematic review and metaanalysis. *J Rheumatol* 2006;33:2398-408.

189. **den Broeder AA, de Jong E, Franssen MJ*, et al.* Observational study on efficacy, safety, and drug survival of anakinra in rheumatoid arthritis patients in clinical practice. *Ann Rheum Dis* 2006;65:760-2.

190. **Hallert E, Thyberg I, Hass U*, et al.* Comparison between women and men with recent onset rheumatoid arthritis of disease activity and functional ability over two years (the TIRA project). *Ann Rheum Dis* 2003;62:667-70.

191. **Engvall E, Perlman P.** Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 1971;8:871-4.

192. **Saiki RK, Scharf S, Faloona F*, et al.* Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985;230:1350-4.

193. **Hoogendoorn B, Owen MJ, Oefner PJ*, et al.* Genotyping single nucleotide polymorphisms by primer extension and high performance liquid chromatography. *Hum Genet* 1999;104:89-93.

194. **Huber CG, Oefner PJ, Bonn GK.** High-resolution liquid chromatography of oligonucleotides on nonporous alkylated styrene-divinylbenzene copolymers. *Anal Biochem* 1993;212:351-8.

195. **Li X, Wu J, Carter RH***, et al.* A novel polymorphism in the Fcgamma receptor IIB (CD32B) transmembrane region alters receptor signaling. *Arthritis Rheum* 2003;48:3242-52.

196. **Mikuls TR, O'Dell JR, Stoner JA***, et al.* Association of rheumatoid arthritis treatment response and disease duration with declines in serum levels of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibody. *Arthritis Rheum* 2004;50:3776-82.

197. **Bobbio-Pallavicini F, Caporali R, Alpini C***, et al.* High IgA Rheumatoid factor levels are associated with poor clinical response to TNFalpha inhibitors in rheumatoid arthritis. *Ann Rheum Dis* 2006; Epub Nov 1. DOI:10.1136/ard.2006.060608.

198. **De Rycke L, Verhelst X, Kruithof E***, et al.* Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. *Ann Rheum Dis* 2005;64:299-302.

199. **Atzeni F, Sarzi-Puttini P, Dell' Acqua D***, et al.* Adalimumab clinical efficacy is associated with rheumatoid factor and anti-cyclic citrullinated peptide antibody titer reduction: a one-year prospective study. *Arthritis Res Ther* 2006;8:R3.

200. **Braun-Moscovici Y, Markovits D, Zinder O***, et al.* Anti-cyclic citrullinated protein antibodies as a predictor of response to anti-tumor necrosis factor-alpha therapy in patients with rheumatoid arthritis. *J Rheumatol* 2006;33:497-500.

201. **Cohen SB, Emery P, Greenwald MW***, et al.* Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum* 2006;54:2793-806.

202. **Cambridge G, Leandro MJ, Edwards JC***, et al.* Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. *Arthritis Rheum* 2003;48:2146-54.

203. **Toubi E, Kessel A, Slobodin G***, et al.* Macrophage function changes following rituximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2006; Epub Dec 5. DOI:10.1136/ard.2006.062505.

204. **Berglin E, Johansson T, Sundin U***, et al.* Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset. *Ann Rheum Dis* 2006;65:453-8.

205. **Bongi SM, Manetti R, Melchiorre D***, et al.* Anti-cyclic citrullinated peptide antibodies are highly associated with severe bone lesions in rheumatoid arthritis anti-CCP and bone damage in RA. *Autoimmunity* 2004;37:495-501.

206. **Kramer PR, Kramer SF, Guan G.** 17beta-estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. *Arthritis Rheum* 2004;50:1967-1975.

207. **Masi AT.** Incidence of rheumatoid arthritis: do the observed age-sex interaction patterns support a role of androgenic-anabolic steroid deficiency in its pathogenesis? *Br J Rheumatol* 1994;33:697-9.

208. **Edwards JC, Blades S, Cambridge G.** Restricted expression of Fc gammaRIII (CD16) in synovium and dermis: implications for tissue targeting in rheumatoid arthritis (RA). *Clin Exp Immunol* 1997;108:401-6.

209. **Catrina AI, Trollmo C, af Klint E*, et al.* Evidence that anti-tumor necrosis factor therapy with both etanercept and infliximab induces apoptosis in macrophages, but not lymphocytes, in rheumatoid arthritis joints: extended report. *Arthritis Rheum* 2005;52:61-72.

210. **Tutuncu Z, Kavanaugh A, Zvaifler N*, et al.* Fcgamma receptor type IIIA polymorphisms influence treatment outcomes in patients with inflammatory arthritis treated with tumor necrosis factor alpha-blocking agents. *Arthritis Rheum* 2005;52:2693-6.

211. **Miceli-Richard C, Ohresser M, Comets E*, et al.* Analysis of Fc gamma-receptor IIA, IIIA and IIIB gene polymorphisms as predictive factors of response to adalimumab in rheumatoid arthritis patients treated in the ReAct study. *Arthritis Rheum* 2006;54 (Suppl):S128-S129.

212. **Louis EJ, Watier HE, Schreiber S*, et al.* Polymorphism in IgG Fc receptor gene FCGR3A and response to infliximab in Crohn's disease: a subanalysis of the ACCENT I study. *Pharmacogenet Genomics* 2006;16:911-4.

213. **Treon SP, Hansen M, Branagan AR*, et al.* Polymorphisms in FcgammaRIIIA (CD16) receptor expression are associated with clinical response to rituximab in Waldenstrom's macroglobulinemia. *J Clin Oncol* 2005;23:474-81.

214. **Weng WK, Levy R.** Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol* 2003;21:3940-7.

215. **Cartron G, Dacheux L, Salles G*, et al.* Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood* 2002;99:754-8.

216. **Kim DH, Jung HD, Kim JG*, et al.* FCGR3A gene polymorphisms may correlate with response to frontline R-CHOP therapy for diffuse large B-cell lymphoma. *Blood* 2006;108:2720-5.

217. **Anolik JH, Campbell D, Felgar RE*, et al.* The relationship of FcgammaRIIIa genotype to degree of B cell depletion by rituximab in the treatment of systemic lupus erythematosus. *Arthritis Rheum* 2003;48:455-9.

218. **Dall'Ozzo S, Tartas S, Paintaud G*, et al.* Rituximab-dependent cytotoxicity by natural killer cells: influence of FCGR3A polymorphism on the concentration-effect relationship. *Cancer Res* 2004;64:4664-9.

219. **Smolen JS, Keystone EC, Emery P*, et al.* Consensus statement on the use of rituximab in patients with rheumatoid arthritis. *Ann Rheum Dis* 2007;66:143-50.

220. **McGovern DP, Butler H, Ahmad T*, et al.* TUCAN (CARD8) genetic variants and inflammatory bowel disease. *Gastroenterology* 2006;131:1190-6.

221. **Ulfgren AK, Gröndal L, Lindblad S*, et al.* Interindividual and intra-articular variation of proinflammatory cytokines in patients with rheumatoid arthritis: potential implications for treatment. *Ann Rheum Dis* 2000;59:439-47.

222. **Smeets TJ, Barg EC, Kraan MC*, et al.* Analysis of the cell infiltrate and expression of proinflammatory cytokines and matrix metalloproteinases in arthroscopic synovial biopsies: comparison with synovial samples from patients with end stage, destructive rheumatoid arthritis. *Ann Rheum Dis* 2003;62:635-8.

223. **Nabbe KC, Blom AB, Holthuysen AE*, et al.* Coordinate expression of activating Fc gamma receptors I and III and inhibiting Fc gamma receptor type II in the determination of joint inflammation and cartilage destruction during immune complex-mediated arthritis. *Arthritis Rheum* 2003;48:255-65.
224. **Brentano F, Kyburz D, Schorr O*, et al.* The role of Toll-like receptor signalling in the pathogenesis of arthritis. *Cell Immunol* 2005;233:90-6.
225. **Taneja V, Behrens M, Mangalam A*, et al.* New humanized HLA-DR4-transgenic mice that mimic the sex bias of rheumatoid arthritis. *Arthritis Rheum* 2007;56:69-78.
226. **Petkova SB, Konstantinov KN, Sproule TJ*, et al.* Human antibodies induce arthritis in mice deficient in the low-affinity inhibitory IgG receptor Fc gamma RIIB. *J Exp Med* 2006;203:275-80.
227. **Shields RL, Lai J, Keck R*, et al.* Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. *J Biol Chem* 2002;277:26733-40.
228. **Niwa R, Sakurada M, Kobayashi Y*, et al.* Enhanced natural killer cell binding and activation by low-fucose IgG1 antibody results in potent antibody-dependent cellular cytotoxicity induction at lower antigen density. *Clin Cancer Res* 2005;11:2327-36.
229. **Kaneko Y, Nimmerjahn F, Ravetch JV.** Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 2006;313:670-3.