General introduction: what precedes rheumatoid arthritis and can we prevent or delay disease onset by targeting in the preclinical phase?
Introduction

Rheumatoid arthritis (RA) is one of the most common chronic inflammatory joint diseases, affecting 0.5-1% of the adult population. The inflammation leads to joint destruction and is associated with significant disability and excess mortality.\textsuperscript{1,2} RA is considered to be a complex disease, with suspected interrelated contributions from genetic, infectious, environmental and hormonal factors. RA is frequently characterized by the presence of rheumatoid factor (IgM-RF) and more specifically by anti-citrullinated protein antibodies (ACPA) in both the preclinical and clinical phases of the disease. Although the full etiology still remains unclear,\textsuperscript{3} recent evidence points towards an important role for ACPA in disease susceptibility and severity, opening the option for early detection and prevention of RA related arthritis development.

Genetic risk factors

Evidence from twin studies demonstrates excess disease concordance between monozygotic (15\%) when compared to dizygotic twins (3.6\%).\textsuperscript{4} From such studies, genetic variation is thought to explain 50-60\% of the disease liability\textsuperscript{5} (for review on genetic factors implicated in RA; see\textsuperscript{6}).

\textbf{HLA-DRB1.} The major genetic risk factor (odds ratio [OR] ~ 6) is the shared epitope (SE) at the HLA-DRB1 locus.\textsuperscript{7,8} The SE hypothesis postulates that highly conserved amino acid sequences bordering the peptide binding groove of the HLA-DRB1 molecule are involved in the pathogenesis of RA, e.g. by enabling the presentation of arthritogenic peptides to T cells.\textsuperscript{7} In support of the latter concept it has been shown that the DRB1*0401 peptide binding groove allows for a high affinity interaction with citrullinated peptides, resulting in efficient antigen presentation.\textsuperscript{9} The frequency of SE alleles varies considerably between ethnic groups, however, the risk associated with the SE seems relatively constant.\textsuperscript{10} On the other hand, several protective HLA alleles such as DRB1*0103 have also been described, for instance in what is known as the ‘rheumatoid arthritis protection’ (RAP) hypothesis proposed by Zanelli.\textsuperscript{11}

\textbf{PTPN22.} The second largest genetic risk factor for RA, with an OR of ~ 1.8, is a single nucleotide polymorphism (SNP) in the protein tyrosine phosphatase non-receptor 22 (PTPN22) gene, found to be associated with multiple autoimmune diseases.\textsuperscript{12} These results have been replicated in several populations of European descent.\textsuperscript{13-19}

\textbf{STAT4.} Four SNPs in the third intron of the signal transducer and activator transcription gene STAT4\textsuperscript{20} are also associated with RA, the strongest being rs 7574865 (OR~1.4). This association was confirmed in a large replication cohort of North Americans, but the observed association and effect was much smaller in a Swedish cohort.\textsuperscript{20}

\textbf{6q23 and TRAF1/C5.} Recent whole genome association studies have confirmed the well-documented RA susceptibility genes HLA-DRB1 and PTPN22, and have resulted in two additional independent genetic risk factors for RA, namely a SNP in the intergenic region of 6q23 (OR ~1.2)\textsuperscript{21,22} and a SNP in the TRAF1/C5 region (OR~1.4).\textsuperscript{23} This association with RA was confirmed in a large study involving Dutch, Swedish and North-American validation samples.\textsuperscript{24}
Environmental and hormonal risk factors

**Smoking.** The most consistent environmental risk factor associated with RA is smoking.\(^2,25-28\) This association seems restricted to RA patients positive for ACPA.\(^{29-39}\) In these patients, a strong gene environment interaction between the RA associated HLA-DRB1 gene and smoking in the production of ACPA has been demonstrated. Therefore, a new hypothesis for the aetiology of RA has been proposed: smoking may trigger SE-restricted immune reactions to autoantigens modified by citrullination.\(^33\)

**Infectious agents.** Indirect evidence has suggested that exposure to infectious agents may be the trigger for RA. The role of Epstein-Barr virus and human parvovirus B19 has been extensively studied and implicated in RA pathogenesis, but formal proof is lacking.\(^40-42\) In a study analysing preclinical RA samples, IgG antibodies against Epstein-Barr virus and parvovirus B19 did not differ significantly between case and control sera.\(^43\) Recently, infection with *Porphyromonas gingivalis* causing gingivitis has been implicated in priming autoimmunity in a subset of RA patients since the immunodominant peptide of citrullinated alpha-enolase (CEP-1), reactive with 37-62 % of rheumatoid patient sera and 2% of controls, shows 82% homology to bacterial enolase, present only in *P. gingivalis*.\(^44\)

**Nutritional factors.** Several dietary factors such as fatty acids, alcohol and coffee have been implicated in the protection or promotion of RA onset (reviewed by Pattison et. al.\(^45\)), although robust data are lacking and the mechanisms behind these associations are unclear.

**Hormonal factors.** The increased risk of RA in females points towards hormonal and pregnancy-related factors in disease incidence. Interestingly, only exogenous hormonal influences are implicated in disease risk, most clearly shown in the (temporary) protective effect of oral contraceptive use.\(^46,47\) Furthermore, the post-partum period, especially breastfeeding after the first pregnancy, is associated with an increased risk for RA.\(^48\) The latter may be attributed to the pro-inflammatory capacities of the hormone prolactin.\(^49\)

Linking genetic and environmental risk factors to ACPA production

There is a strong gene environment interaction between the RA associated HLA-DRB1 gene and smoking in the production of ACPA.\(^29-37,39,50\) Therefore, a new hypothesis for the aetiology of RA has been proposed: smoking may trigger SE-restricted immune reactions to auto-antigens modified by citrullination.\(^33\)

Recently, it was suggested that the increased risk for RA in SE positive undifferentiated arthritis is in fact not due to the SE, but to ACPA positivity.\(^51\) In the latter study, the presence of SE alleles was associated with significantly higher levels of anti-CCP antibodies, suggesting that the SE alleles act as classic immune response genes. Furthermore, novel statistical pathway analysis has indeed shown that the effect of the SE on RA and erosive phenotype is mediated by ACPA.\(^52,53\)
Pathophysiological role of ACPA

**Antibodies to citrullinated proteins.** One of the characteristics of RA is the presence of autoantibodies such as anti-citrullinated protein antibodies (ACPA) and IgM rheumatoid factor (IgM-RF). ACPA target citrullinated proteins and were first described in 1965 by Nienhuis and Mandema as the antiperinuclear factor. ACPA comprise a group of antibodies highly specific for RA, among those described are antibodies against cyclic citrullinated peptide (CCP), citrullinated fibrinogen, citrullinated alpha-enolase, citrullinated vimentin (MCV), and citrullinated forms of type I and II collagen. Several immunodominant citrullinated peptides have been recognized, pointing towards importance of flanking amino acids as antigenic determinants, such as serine and glycine. ACPA have repeatedly been found to be associated with the most severe and erosive forms of RA, are produced locally in the synovium where they probably interact with citrullinated proteins and are present in preclinical disease.

Citrullination of proteins is a normal post-translational modification in which deimination of the amino-acid arginine forms the non-standard amino acid citrulline, a calcium dependent process catalyzed by the enzyme peptidyl arginine deiminase (PAD). There are at least 5 isoforms of PAD, two of which (PAD2 and 4) have been found in monocytes and macrophages of the inflamed synovium. Furthermore, polymorphisms in the PAD4 gene are associated with susceptibility for RA in the Japanese population, although this finding has not been replicated in a UK population. Citrullination is thought to have a physiological role in aging, gene expression, regulation, apoptosis, trauma and inflammation, although the precise function is still unknown. Citrullinated proteins have been detected at sites of inflammation such as the inflamed joints of RA patients, but also in non-RA inflamed joints and other tissues. These data suggest that the antibody response against, but not the presence of citrullinated proteins, is specific for RA. Somehow, tolerance for citrullinated proteins is broken and therefore, a pathophysiological role for ACPA in RA has been suggested.

**In vitro and animal data.** Recent in vitro and animal data have reinforced the hypothesis of the pathophysiological importance of ACPA. First, mice have been shown to develop IgM and IgG ACPA in a collagen-induced arthritis (CIA) model. These autoantibodies enhanced arthritis when co-administered with a submaximal dose of anti-collagen antibodies and bound targets within the inflamed synovium of mice with CIA. However, despite the presence of citrullinated proteins in the inflamed synovia of rodent arthritis models, the finding of murine ACPA antibodies has not been replicated. Second, passive transfer of human RA autoantibodies can induce inflammation and histological lesions consistent with arthritis in Fcγ-Receptor IIB deficient mice. Third, citrullinated fibrinogen, but not the uncitrullinated form, induces arthritis in HLA-DRB1*0401 transgenic mice, as was recently shown by Hill et. al. Immunological analysis of these mice through T cell epitope scanning and antibody microarray analysis identified a unique profile of citrulline-specific reactivity. Fourth, ACPA complexed with fibrinogen are capable of selective and dose dependent induction of TNFα in monocyte-derived macrophages. TNFα production could be prevented by selective blockade of the Fcγ-RIIa. Addition of citrullinated fibrinogen derived peptides also abolished formation of macrophage activating immune complexes, thereby preventing TNFα production. Recently, Zhao et.
al.95 have shown the presence of circulating immunocomplexes (CICs) containing citrullinated fibrinogen in ~50% of ACPA positive RA patients. These CICs colocalize with the complement component C3 in the rheumatoid synovium, suggesting that they contribute to synovitis in (a subset of) RA patients and complete ‘the rheumatoid arthritis cycle’, as proposed by Vossenaar and van Venrooij, in which ACPA are the major driving force in the perpetuation of rheumatoid inflammation.96:97

Detection of individuals at risk

Autoantibodies in the preclinical phase. A wealth of data is available on genetic and environmental risk factors, and the role of autoantibodies, especially ACPA, in the development of RA. Furthermore, autoantibodies have been detected in preclinical serum samples of persons who later developed RA. Several groups have described the autoantibody profile in preclinical RA in high risk98-100 and healthy populations,43;71;72;75;101-103 opening the possibility of detecting those at risk for the development of RA.

Nielen et al. reported the presence of IgM-RF and/or ACPA autoantibodies in 49% of patients who would later develop RA at a median of 5 years before the onset of symptoms, providing a possible window of opportunity for treating those at risk of developing rheumatoid arthritis.72 From these retrospective data calculations have been made estimating the 5-year risk of developing RA in the presence of IgM-RF or ACPA in individuals without arthritis. The risk rises to 69 % in ACPA positive individuals in a high-risk population (two first-degree relatives with RA).72 The contribution of acute phase reactants in the preclinical phase has also been studied, and results were not uniform.104-108

Autoantibodies before the first symptoms were also found in other autoimmune diseases, such as systemic lupus erythematosus109;110 and insulin-dependent diabetes mellitus.111 In diabetes, intervention strategies aiming at prevention of disease onset in selected populations have already been studied,112-115 with the result that disease onset was not prevented. However, as the authors stated it should not be concluded that it is impossible to delay or prevent type 1 diabetes; rather, it may require testing of more potent interventions or combinations of therapies, guided by better understanding of the immunopathogenesis of the disease, to demonstrate attenuation or amelioration of the destructive immune process leading to type 1 diabetes.

Towards intervention strategies in individuals at risk

Targeting autoantibodies. Presence and levels of ACPA (and IgM-RF) have been suggested to influence the risk of development of RA in synovitis of recent onset, as well as disease severity and radiographic progression.116-121 Therefore, the effect of treatment strategies such as dexamethasone and anti-TNF treatment on autoantibody levels in RA patients has been extensively studied, and data are controversial122-134 (for review see135). Corticosteroid treatment reduces IgM-RF levels in RA patients,122-124 the effects on ACPA (subclasses) have not previously been studied. Most authors report a significant decrease of ACPA after TNF-treatment,125;130-132 but in those responding to treatment only.125;130;131 On the other hand, unaltered or temporarily decreased ACPA levels in patients treated with anti-TNF agents have also been reported.126;128;129;134 IgM-RF, an antibody which targets the Fc fragment of IgG, is observed in about 75 % of RA patients, but it is also frequently observed in
other inflammatory diseases. All studies reporting on IgM-RF levels after anti-TNF treatment have shown a reduction of these levels, in some restricted to those responding to treatment. IgM-RF and ACPA levels also decreased in response to diverse non-biological treatments. In this study, the reduction in IgM-RF was associated with effective treatment, whereas the decrease in ACPA was associated with shorter disease duration.

Taken together, the properties of IgM-RF and ACPA and the apparent differential response of these autoantibodies during antirheumatic treatment suggests that IgM-RF acts as a marker for inflammation in RA and other (non)-rheumatic diseases, while ACPA, being more disease specific, is less susceptible to aggressive antirheumatic treatment. These data also suggest that antibodies may provide a useful target or marker for effective intervention strategies in the preclinical phase.

**Window of opportunity.** In recent years, great progress has been made in targeting the ‘window of opportunity’ in RA; i.e. a timeframe in which there is a greater than usual response to therapy resulting in long term sustained benefits. Despite these efforts, a large proportion of patients with early, active, RA already have radiographic joint erosions at first presentation. Therefore, targeting even before the onset of arthritis in patients at risk for the development of RA seems a conceivable next step in the prevention of the disease burden associated with RA.

**Suggested reading:**

- van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, De Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arthritis Rheum* 2006;54:1117-21.
Thesis outline

The aim of this thesis was to investigate whether arthritis development can be predicted in persons at risk for developing rheumatoid arthritis (RA) and whether disease onset can be prevented or delayed in the preclinical phase. Furthermore, to gain more insight in the properties of anti-citrullinated protein antibodies (ACPA) and IgM rheumatoid factor (IgM-RF), the influence of antirheumatic treatment on the presence and levels of these autoantibodies in the clinical and preclinical phase of RA was studied.

Therefore, a prospective cohort and intervention study of arthralgia patients positive for ACPA and/or IgM-RF was initiated in 2004 aiming at 1) identifying those arthralgia patients most at risk for the development of arthritis and 2) postponing or suspending arthritis development in arthralgia patients by applying glucocorticoid treatment. Additional cohorts used in the present thesis comprised of blood donors who later developed RA, patients with early onset arthritis, and established RA patients starting intensive antirheumatic treatment using TNF-alpha blockade.

In chapter 2, the effect of anti-TNF treatment in RA patients on IgM-RF and ACPA levels are reported. These observations are extended in chapters 3 and 4. In chapter 3, the change in IgM-RF and ACPA is correlated to clinical response and acute phase reactants, whereas in chapter 4 the effect of anti-TNF treatment on ACPA IgG subclass levels is reported.

Chapter 5 shows the relationship between age at seroconversion of ACPA and IgM-RF in the preclinical phase and the period until onset of RA related symptoms.

The effects of ACPA and IgM-RF presence and levels on the progression to arthritis in arthralgia patients are presented in chapter 6 and 7. A cross-sectional comparison between shared epitope status and ACPA levels in arthralgia and arthritis patients is reported in chapter 6, whereas the role of ACPA presence and levels on arthritis development is prospectively studied in chapter 7. Gene-expression profiling in arthralgia patients used to detect novel biomarkers for the development of RA is reported in chapter 8. The results of a placebo-controlled trial of dexamethasone in the primary prevention of rheumatoid arthritis in arthralgia patients as well as the effects on ACPA and IgM-RF levels are reported in chapter 9.

Finally, a summary of the results, general discussion and recommendations for future research are presented in chapter 10.
Reference list


51. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, De Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arthritis Rheum* 2006;54:1117-21.


General introduction


