

# Mechanisms of Disease: genetic susceptibility and environmental triggers in the development of rheumatoid arthritis

Lars Klareskog\*, Leonid Padyukov, Johnny Lorentzen and Lars Alfredsson

## SUMMARY

Rheumatoid arthritis (RA) is a complex disease in which environmental agents are thought to interact with genetic factors that influence susceptibility. This interaction triggers immunologic events that eventually result in the clinical signs of arthritis. Knowledge of the chain of etiological events that lead to the development of RA is incomplete. In this review, we describe the experimental approaches that are used to address the issue of gene–environment interactions in the etiology of RA, and discuss relevant examples of such interactions. We focus on how smoking, the best-known environmental risk factor for RA, interacts with HLA-DR shared epitope genes, the main genetic risk factors for RA, and result in a high risk of RA in individuals exposed to both of these risk factors. From these and other related findings, we can begin to define the distinct environmental risk factors (such as smoking) that in certain genetic contexts (for example, the presence of HLA-DR shared epitope alleles) can trigger immune reactions (such as autoantibodies to citrullinated peptides) many years before onset of RA, and consider how these immune reactions might contribute to clinical symptoms in a subset of affected patients. Increased knowledge about these and other events involved in the development of RA should enable the design of new tools for suppressing RA pathogenesis before the onset of disease.

**KEYWORDS** anticitrulline autoimmunity, environment, rheumatoid arthritis, shared epitope, smoking

## REVIEW CRITERIA

The review is based on data obtained from a structured but not fully systematic review of the scarce literature concerning gene–environment interactions in the etiology of rheumatoid arthritis. Only English-language papers were included.

*L Klareskog is Professor of Rheumatology at the Rheumatology Unit, L Padyukov and J Lorentzen are Senior Scientists in the Rheumatology Research Laboratory (linked to the Rheumatology Unit), Department of Medicine of the Karolinska Institutet, Karolinska University Hospital, and L Alfredsson is Professor of Epidemiology at the Institute of Environmental Medicine, Karolinska Institutet, and Stockholm Center for Public Health, Stockholm County Council, Stockholm, Sweden.*

## Correspondence

\*Rheumatology Unit, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, S-171 76 Stockholm, Sweden  
lars.klareskog@medks.ki.se

Received 18 July 2005 Accepted 8 June 2006

www.nature.com/clinicalpractice  
doi:10.1038/ncprheum0249

## INTRODUCTION

The etiology of rheumatoid arthritis (RA), like that of many other chronic inflammatory conditions, is largely unknown. Knowledge of the environmental and genetics factors that define susceptibility to disease, the specific immune reactions that drive disease development, and the measures that could be taken to prevent disease is limited. The lack of information in this area contrasts with the extensive progress that has been made in understanding disease pathogenesis at the level of cytokine regulation, which has provided the basis for the development of effective targeted therapies.<sup>1</sup>

In order to take additional steps towards preventing and treating RA, there is a need for additional knowledge of the basic etiologic factors involved in this disease. This review will describe some recent progress that has been made in elucidating the role of a few such factors, and will discuss concepts and methods that are needed to address the issues of genes, environment and adaptive immunity in the development of RA.

## GENES AND ENVIRONMENT SHOULD BE CONSIDERED IN THE SAME CONTEXT

A number of classic epidemiologic studies, many of them done in twins, have shown that both genetic and environmental factors contribute to the onset of RA.<sup>2</sup> Few studies (with one notable exception) have proceeded further, and studied how environmental and genetic factors might interact. The exception is a study by Silman and collaborators, in which the role of smoking was analyzed in 13 pairs of monozygotic twins who were discordant for RA as well as for smoking: the smoker was the twin with RA in 12 of these 13 pairs.<sup>3</sup> This elegant study illustrates that the effects of environmental agents should be studied in genetically well-defined contexts, and indicates that environmental influences can be dramatic if the genetic background is kept constant, as obviously occurs in individuals in real life. The design of this type of twin study,

**Box 1** Agents that cause joint inflammation in rodent models of arthritis when injected into the skin.<sup>a</sup>

**Adjuvants**

Microbial origin (e.g. glucans, lipopolysaccharide, CpG-containing DNA)  
Self origin (e.g. squalene)  
Environmental agents (e.g. mineral oils)

**Antigens**

Cartilage-derived antigens (e.g. collagen II, IX, X, XI, cartilage oligomeric matrix protein, proteoglycans)

<sup>a</sup>In all models, the arthritis-inducing capacity of the agent is limited to animals with certain genetic characteristics.

however, does not permit identification of the genes that interact with environmental stimuli, or elucidation of the mechanisms involved.

So far, most other studies have investigated environmental and genetic risk factors for RA separately. In addition, few of the environmental factors and genetic polymorphisms implicated in the pathogenesis of RA to date have been confirmed in multiple studies. The most solid evidence for environmental influences exists for smoking, which is discussed in detail below, followed by that for silica exposure, which has been reviewed elsewhere.<sup>4</sup> The literature is sparse or contradictory concerning the influence of occupational agents such as solvents<sup>5</sup> or dietary factors such as coffee or meat,<sup>6–10</sup> despite reports published in 2004 that related the consumption of red meat to the risk for RA.<sup>11,12</sup> The strongest evidence for the influence of genetic polymorphisms on risk of RA relates to MHC class II antigens and, in particular, to various HLA-DR alleles; of these the structure defined by the shared epitope (SE)<sup>13</sup> is the most important. The second major polymorphism occurs in the *PTPN22* gene, which encodes protein tyrosine phosphatase, nonreceptor type 22, a tyrosine phosphatase of importance in T cell signaling: several groups have confirmed a relationship between one variant allele and susceptibility to RA.<sup>14,15</sup> Several other polymorphisms have been described, but extended replication studies are required before definite conclusions on their significance can be reached.<sup>15</sup> Most notable in the context of the present review, there are no reports on relationships between these genetic polymorphisms and environmental influences, apart from the studies on smoking and silica that are described below.

**Lessons from animal models**

In order to proceed with studies that investigate how specific genes, environmental factors and immune reactions interact in RA, we can learn from experiments in animal models of arthritis. In contrast to previous human etiology studies, it has been mandatory to study the genetics of experimentally induced disease in models where the trigger is well defined, in inbred and genetically well-defined strains of animals. In proceeding to human studies, we should learn from the general methodology of these studies, and also can gain information on candidate triggers, genes and pathogenetic immune reactions.

Two general types of triggers are used to induce the development of arthritis in (predominantly rodent) animal models. The classical approach is to extract autoantigens from the target organ, here cartilage, and inject these together with an adjuvant into the skin of recipient animals. A series of such autoantigens, of which collagen II is the most commonly used, have been defined (Box 1), and immunization with these antigens results in disease with many similarities to human RA.<sup>16,17</sup> Interestingly, post-translational modification of collagen II by means of citrullination renders this autoantigen more prone to induce experimental arthritis;<sup>18</sup> however, the immunology behind this reaction is not identical to that seen in human RA patients. The second type of stimuli are known as adjuvants. The first arthritis adjuvant to be described was composed of a mixture of mycobacterial antigens, which induced disease in the recipient animal and, therefore, suggested that adaptive immunity to bacterial products might be involved in disease pathogenesis.<sup>19,20</sup> Later, however, it was shown that a series of simple adjuvant molecules, such as glucans from yeast,<sup>21</sup> unmethylated DNA from bacteria<sup>22</sup> and chemicals such as mineral oils,<sup>23,24</sup> are able to stimulate the innate immune system and cause arthritis without being recognized by the adaptive immune system.

Animal systems provide much better opportunities than the outbred human population for defining candidate genes that predispose to the development of arthritis. A large series of genetic loci have now been defined that influence arthritis phenotypes; interestingly, distinct genetic regions influence different aspects of the disease, from general susceptibility to time of onset, degree of chronicity and degree of erosiveness, among others.<sup>25</sup> Notably,

these genetic polymorphisms have primarily been defined in situations where one specified agent is used to trigger arthritis. Some, but not all, of these genetic polymorphisms have subsequently been shown to influence susceptibility to arthritis triggered by other agents.<sup>26</sup> As summarized in Figure 1, genes and triggers should be studied in the same context in order to provide a further basis for the understanding of the molecular pathogenesis of the different variants of disease that are seen in different gene or trigger combinations.

We are now in a situation where knowledge of many candidate genes that have first been identified in rodent arthritis models can be used to define candidate genes in human arthritis, via identification of homologous genes in rodent and humans respectively. This method has already identified several genes of interest with respect to the development of human RA.<sup>27,28</sup> We believe that this comparative study approach should be expanded, not only to demonstrate the importance of gene–environment interactions in arthritis, but also to provide hypotheses on the entire chain of events, from gene–environment interactions to pathogenetic pathways involved in different subsets of RA patients.

### DIVISION OF RHEUMATOID ARTHRITIS INTO CLINICALLY RELEVANT SUBGROUPS

RA is defined by a set of disease criteria that were formulated on the basis of clinical experience,<sup>29</sup> rather than on an understanding of a common etiology and molecular pathogenesis.<sup>30</sup> When trying to elucidate the etiology of RA, it is important that the disease should be divided into subtypes, in which etiological factors and molecular pathways might be more uniform. Without *a priori* knowledge of which subdivisions are appropriate, we should collect and consider a multitude of phenotypic data from our patients, and be prepared to investigate a number of such subdivisions.

At present, two partially overlapping subdivisions seem to be most compelling, namely division by the presence of rheumatoid factor (RF) and antibodies that can bind cyclic citrullinated proteins (anti-CCP), respectively. Individuals who are positive for either or both of these biomarkers have a more severe disease course, compared with those who lack both of them;<sup>31</sup> and of the two, anti-CCP antibodies

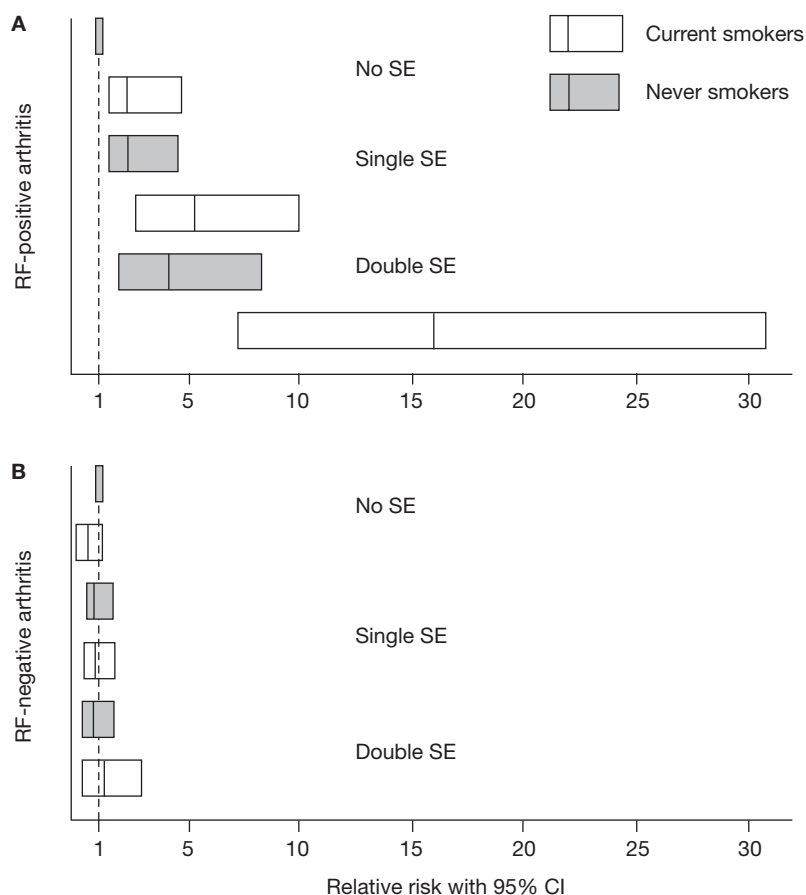
MDP	0.8	ND	ND	5.0	6.8	ND	11.8
Pristane	0	0.2	0.5	7.4	11.0	ND	9.7
β-Glucan	ND	ND	2.5	ND	5.5	ND	7.9
Avridine	ND	0	0	6.3	9.0	ND	11.0
Squalene	ND	ND	0	0	0.3	5.4	9.8
IFA	0	0	0	0	0	3.2	6.1
Olive oil	ND	ND	ND	ND	ND	ND	0
	F344	E3	PVG.1AV1	LEW	LEW.1AV1	DA.1H	DA
	Inbred rat strain						

**Figure 1** Gene–environment interactions seem to have a threshold effect that determines arthritis susceptibility, as interpreted from experimental data for multiple combinations of adjuvants in inbred rat strains. Published, numerical data from these experiments were transformed to a 0–16 scoring scale,<sup>21,50</sup> with the median value presented in cases of several reports. Adjuvants and rat strains are ranked along the axes in order of increasing disease association. Shaded areas depict combinations that result in disease, or are suspected to do so. This figure, originally by Lorentzen, illustrates the relative nature of concepts such as arthritogenicity and disease susceptibility or resistance. It also illustrates influences from MHC genes (rat strains DA and DA.1H) and non-MHC genes (rat strains DA, PVG.1AV1 and LEW.1AV1). β-Glucan is derived from yeast, muramyl dipeptide from mycobacteria and avridine is a synthetic lipoidal amine (avridine and muramyl dipeptide are co-injected with incomplete Freund's adjuvant in the respective models). Abbreviations: IFA, incomplete Freund's adjuvant; MDP, muramyl dipeptide; ND, not determined.

seem to have the best prognostic value.<sup>32,33</sup> The anti-CCP-positive phenotype is also more stable over time than the RF-positive phenotype.<sup>34</sup> We expect more subdivisions to be developed soon when additional biomarkers, as well as additional clinically relevant features, are identified that associate with different genetic polymorphisms and environmental triggers.

### IMMUNE REACTIONS ASSOCIATED WITH RHEUMATOID ARTHRITIS

A number of different immune reactions have been demonstrated to be more frequent in patients with RA than in healthy individuals or patients with other inflammatory diseases. In considering which of these potentially pathogenic reactions might be important, it seems rational to ask three initial questions: which of the reactions can induce arthritis in animal



**Figure 2** Gene–environment interactions between HLA-DR shared epitope genes and smoking, in **(A)** rheumatoid-factor-positive and **(B)** rheumatoid-factor-negative individuals. Relative risks of developing rheumatoid arthritis (with 95% CI limits) are shown for each genotype. The figure was created from original data from Padyukov *et al.*<sup>41</sup> Abbreviations: RF, rheumatoid factor; SE, shared epitope.

models; which of the reactions occur before rather than after onset of RA; and which of the reactions are reasonably common in RA, and occur in a clinically meaningful subset of RA patients? So far, antibodies to collagen II<sup>35</sup> and glucose-6-phosphate isomerase<sup>36</sup> fulfill the first criterion, but neither of the other two. RF fulfils the last two criteria but not the first. Autoantibodies to citrullinated proteins come close to fulfilling all three criteria: citrullination of cartilage antigen collagen type II makes the modified antigen more arthritogenic in rodents;<sup>18</sup> anti-CCP antibodies can be present up to 10 years before onset of RA<sup>37,38</sup> and few patients become anti-CCP-positive after disease onset;<sup>34</sup> and anti-CCP antibodies are common in RA and define a clinically meaningful subset of disease<sup>39</sup> (see above).

**GENES, TRIGGERS, AUTOIMMUNE REACTIONS AND DISEASE SUBTYPES**

We can conclude from the previous discussions that studies on the etiology of RA will need appropriate subdivision of the RA syndrome, as well as definition of potential environmental triggers that act in defined genetic contexts and (in some cases) are associated with autoimmune reactions. Few such studies have been done so far, but we will illustrate the use and feasibility of this approach by describing some studies performed by our own as well as other research groups.

**Smoking and HLA-DR genes interact as risk factors in rheumatoid arthritis**

An population-based case–control study, the Epidemiological Investigation of Risk Factors in Rheumatoid Arthritis (EIRA) study, performed by our group, was initiated in Sweden in 1996. Data collection from patients with recent-onset RA and well-matched controls is ongoing, so that genetic factors, environmental triggers, immune reactions and phenotypic features of the disease can be studied in concert (the study design has been described elsewhere<sup>40</sup>). So far, information from over 2,000 cases and 2,000 controls has been collected, and we have begun investigating the feasibility of the general concepts. In the first analysis, we subdivided RA by the most classical phenotype (i.e. RF-positive versus RF-negative disease) to investigate the most classical gene polymorphisms of importance for RA (i.e. HLA-DR SE alleles<sup>13</sup>) and the best-known environmental agent (i.e. smoking).

The results, which are summarized in Figure 2, were striking—we found a profound gene–environment interaction between smoking and HLA-DR SE genes as risk factors for RA.<sup>41</sup> In individuals who are HLA-DR SE-negative, smoking is a relatively modest risk factor. This finding contrasts with the situation in individuals who carry one or two sets of the SE genes, in whom smoking dramatically increases the risk for RA. Equally striking was the finding that the genetic risk factor (HLA-DR SE) as well as the environmental risk factor (smoking) exerted their effects almost exclusively in one subtype of RA, the RF-positive variant, and not in the other, the RF-negative variant.

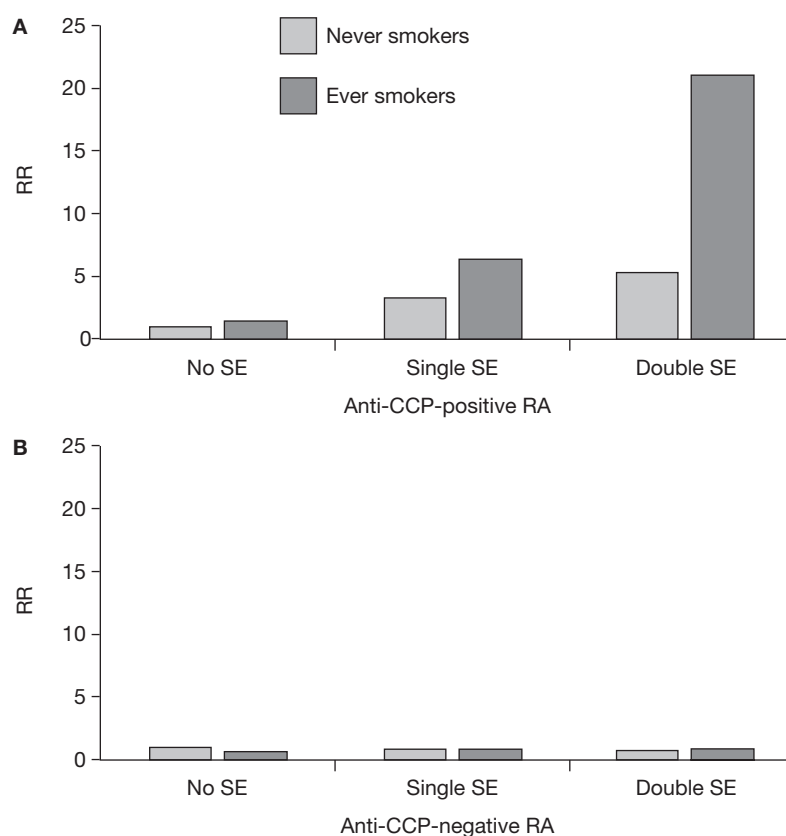
These findings illustrate several principal points regarding etiologic studies in RA. First, the influence of genes on the susceptibility to RA might be highly dependent on which environmental factors are present. Second,

environmental factors should be studied in defined genetic contexts. Third, RA is a heterogeneous disease with respect to both genetic and environmental influences, and should be appropriately subdivided into subtypes in order to permit appropriate recognition of genetic as well as environmental risk factors. Finally, concepts relating to the pathogenesis of RA need to be modified to take into account the finding that the principal environmental and genetic risk factors act in different ways in two major subtypes of RA. This implies that molecular pathways involved in the adaptive immune response act differently in the two forms of RA.

The results from this initial study of gene–environment interactions in RA pose several new questions: what is the biological explanation for the finding that HLA-DR SE genes are risk factors only for RF-positive disease; why does smoking interact so strongly with the HLA-DR SE genes; and why does this happen only in RF-positive RA?

#### Anticitrulline autoimmunity and the link between smoking and HLA-DR shared epitope genes

To further understand the molecular basis behind this gene–environment interaction, which occurs in a distinct subset of RA, we turned our attention to the recently published observation<sup>42</sup> that citrullination of certain peptides makes these peptides more prone to bind to HLA-DR-SE-containing MHC class II molecules and, thus, that an immune response to citrullinated proteins might occur preferentially in individuals carrying the HLA-DR genotypes. Together with the knowledge that anticitrulline autoimmunity in RA is closely linked to the presence of RF, this finding suggested that the previous demonstration of a relationship between HLA-DR SE and RF-positive RA could be secondary to a relationship between HLA-DR SE and anti-CCP-positive RA. We reanalyzed data from our case–control study, for interactions between HLA-DR SE and smoking in anti-CCP-positive and anti-CCP-negative RA, respectively. We also analyzed data from individuals who were discordant for RF and anti-CCP antibodies, in order to determine whether the relationship with HLA-DR SE was primarily related to RF or to the anti-CCP antibodies. The results of this study are summarized in Figure 3.<sup>43</sup> As shown, an even more dramatic gene–environment interaction

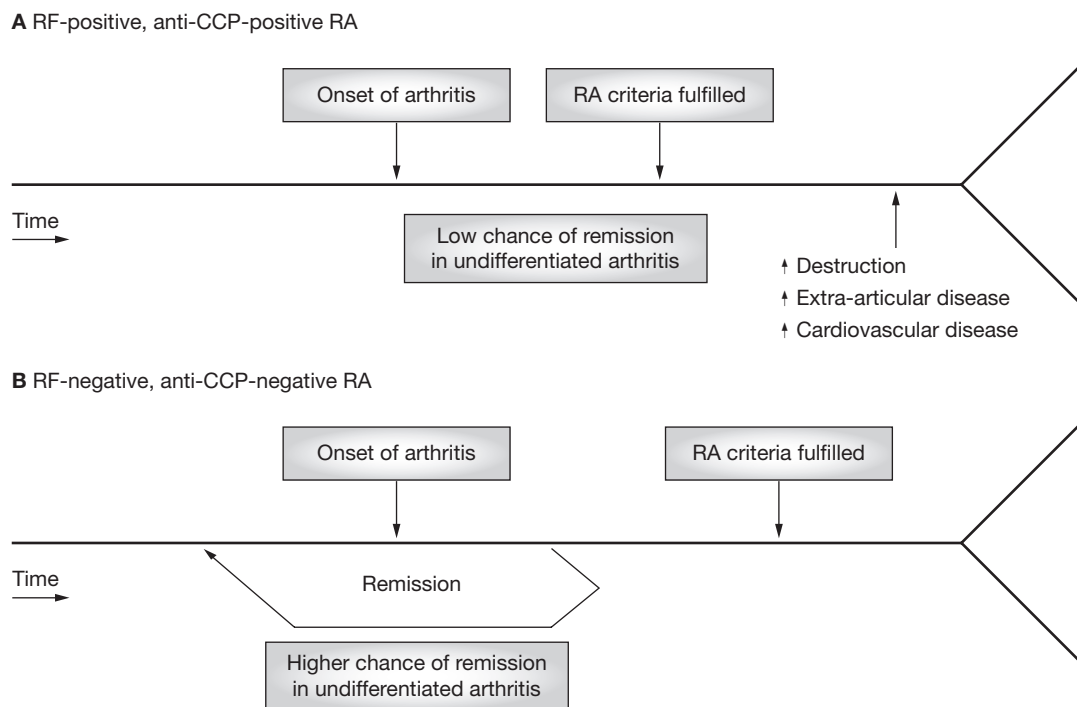


**Figure 3** Gene–environment interactions between HLA-DR shared epitope genotype and smoking in (A) anti-CCP-positive and (B) anti-CCP-negative individuals. Relative risks of developing RA are shown. The figure was created from original data by Klareskog *et al.*<sup>43</sup> Abbreviations: CCP, cyclic citrullinated peptide; RA, rheumatoid arthritis; RR, relative risk; SE, shared epitope.

exists between smoking and HLA-DR SE in anti-CCP-positive RA, than in RF-positive RA. No effects of either smoking or HLA-DR SE on the development of anti-CCP-negative RA were seen. In addition, HLA-DR SE is linked primarily with the presence of anti-CCP antibodies, rather than the presence of RF. This gene–environment interaction between HLA-DR SE and smoking, and its restriction to anti-CCP-positive RA, has also recently been confirmed in another independent study.<sup>44</sup>

#### A new etiologic hypothesis: smoking might induce arthritogenic anticitrulline autoimmunity

The epidemiologic studies summarized above, together with previously available data on autoimmunity to citrullinated peptides, suggest that anti-CCP antibodies could be of pathogenetic significance in RA: they can be detected years before onset of disease, citrullinated



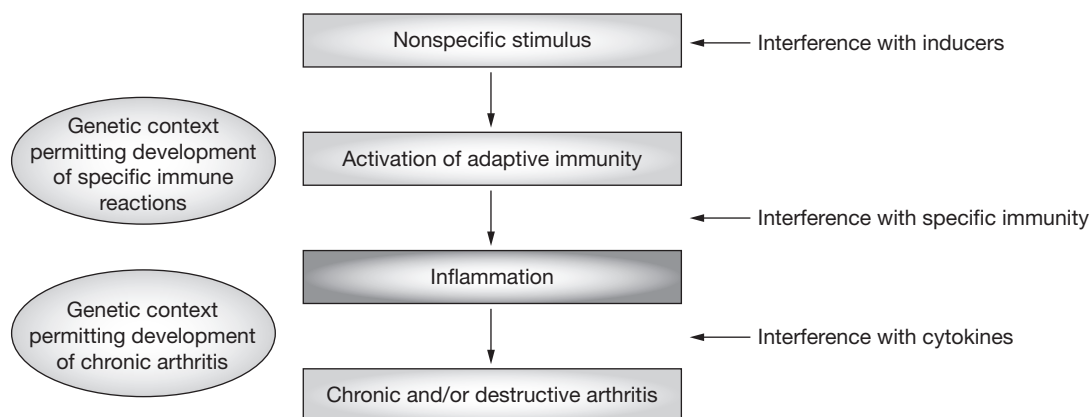
**Figure 4** Longitudinal course of anti-CCP-positive and anti-CCP-negative rheumatoid arthritis. The figure illustrates how the disease course is more severe for anti-CCP-positive rheumatoid arthritis (**A**) than for anti-CCP-negative rheumatoid arthritis (**B**). Erosions, as well as other comorbidities, such as cardiovascular disease and lymphomas, might develop in the course of either subtype of disease. Abbreviations: CCP, cyclic citrullinated peptide; RA, rheumatoid arthritis; RF, rheumatoid factor.

peptides can induce arthritis in rodents, and now it has been shown that the main genes that influence susceptibility and the major environmental trigger for disease associate only with anti-CCP-positive RA. We still need to know how smoking interacts with HLA-DR SE, and why this interaction only occurs for anti-CCP-positive RA. One possible explanation is that smoking works as an environmental trigger for development of anticitrulline autoimmunity. This possibility was tested experimentally by investigating the presence of citrullinated peptides at the site where the smoke primarily encounters the body (i.e. in the lungs). Bronchoalveolar lavage studies in smokers and nonsmokers, respectively, showed that a high percentage of cells in the aspirated fluid from smokers contained citrullinated proteins, whereas this was not the case in nonsmokers.<sup>43</sup>

Taken together, these data have enabled us to suggest a hypothetical etiology for anti-CCP-positive RA, in which an environmental agent (smoking) induces citrullination of lung proteins. Adjuvants in the smoke also stimulate

the innate immune system and, specifically, help to induce immunity to citrullinated proteins, which occurs preferentially in individuals carrying the HLA-DR SE genes. This autoimmunity might be present years before disease onset, and could ultimately contribute to the pathogenesis of arthritis, as suggested previously from studies in rodents (Figure 4).

Although compelling, this scenario leaves many questions unanswered. We do not know how autoantibodies to citrullinated antigens develop, or why most HLA-DR SE-positive individuals exposed to smoke actually do not develop anti-CCP antibodies. We also do not know which citrullinated peptides are recognized by immune cells in the lung or in the joints, or both, and moreover we do not know how autoimmunity to ubiquitously present citrullinated antigens might be involved in the development of joint-specific inflammation. These studies on environmental risk factors, genetic risk factors, autoimmunity and subtypes of RA, have nevertheless produced an etiologic hypothesis that can now be tested by immunologists, molecular biologists and



**Figure 5** Possible options for intervention in different stages of rheumatoid arthritis pathogenesis. Current treatments for rheumatoid arthritis focus mainly on the innate immune system, for example blocking cytokine signaling with anti-tumor-necrosis-factor agents. This figure illustrates two attractive future alternatives: interference with specific reactions of the adaptive immune system, such as autoimmunity to citrullinated proteins, and interference with environmental risk factors, for example antismoking programs.

epidemiologists respectively. It would be of particular interest to follow individuals who are at a high risk of RA in prospective longitudinal studies aimed at understanding which immune reactions, triggered by which environmental agents, enhance or counteract the development of clinical signs of arthritis.

#### Other pathways to disease development

The example discussed above is just one possible pathway for one variant of RA; there are several other possibilities. For example, the pathway involving the detoxifying enzyme glutathione S transferase M1 deserves a mention, as the *GSTM1*-null genotype has been shown to interact with smoking and be associated with a more-severe course of RA than other genotypes.<sup>45</sup> Also, as described above, a number of potential exogenous stimuli in addition to smoking have been described in epidemiologic studies as being linked to subtypes of disease or to certain immune processes, but few links between these stimuli and specific genes have been shown. The most compelling association is that of silica exposure—another example of an environmental exposure that occurs mainly via the airways—which is associated with an increased risk for RA,<sup>4,46,47</sup> even after adjustment for the effects of smoking.<sup>48</sup> Another example of an exogenous stimulus with a relationship to RA and to genotype, has however, recently been elucidated in the EIRA study. Exposure to

mineral oils is a well-known trigger of arthritis in some strains of rats,<sup>23,24</sup> and was reported (in 2005) to be associated with an increased risk of RA.<sup>49</sup> The increased risk, similarly to smoking, was confined to the anti-CCP-positive subtype of disease, but was, in contrast to smoking, not significantly related to the presence or absence of HLA-DR SE genes.<sup>49</sup> It is tempting to speculate that similar mechanisms to those responsible for oil (and adjuvant)-induced arthritis in rats, could also be active in humans exposed to mineral oils.

#### CONCLUDING REMARKS

The heterogeneous, chronic and complex nature of RA has hampered efforts to decipher the critical etiologic factors that would permit us to suppress the disease before damage occurs. The main message of this review is that the use of modern genetic and biomarker technology might soon permit us to divide RA patients into appropriate subgroups, to identify environmental and genetic etiologic factors, and possibly to link these findings to specific disease-associated or even disease-inducing immune reactions (Figure 5). These results would in turn mean that it should be possible to intervene early in the disease, possibly before clinical signs and symptoms develop. Intervention could be based not only on currently available therapies, but also on approaches that interfere with specific immune responses and environmental triggers.

## KEY POINTS

- Rheumatoid arthritis (RA) is a heterogeneous disease, and the genetic and environmental risk factors for the major disease subtypes (rheumatoid-factor-positive, rheumatoid-factor-negative, positive for antibodies that bind cyclic citrullinated proteins [anti-CCP], or anti-CCP-negative) might be different
- There is a major gene–environment interaction between HLA-DR shared epitope genes as a central genetic risk factor, and smoking as a major environmental risk factor, in the etiology of anti-CCP-positive (but not anti-CCP-negative) RA
- A possible mechanism behind the interaction between smoking, HLA-DR shared epitope genes and anticitrulline autoimmunity is discussed
- Several other environmental agents, such as silica dust and mineral oil, have been identified as risk factors for RA
- The dramatically increased risk of RA that has been identified in individuals carrying certain genes should lead to counseling against smoking in patients who have relatives with RA

## References

- 1 Feldmann M *et al.* (2004) The transfer of a laboratory based hypothesis to a clinically useful therapy: the development of anti-TNF therapy of rheumatoid arthritis. *Best Pract Res Clin Rheumatol* **18**: 59–80
- 2 MacGregor AJ *et al.* (2000) Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum* **43**: 30–37
- 3 Silman AJ *et al.* (1996) Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins. *Arthritis Rheum* **39**: 732–735
- 4 Cooper GS *et al.* (2002) Occupational exposures and autoimmune diseases. *Int Immunopharmacol* **2**: 303–313
- 5 Olsson AR *et al.* (2004) Occupations and exposures in the work environment as determinants for rheumatoid arthritis. *Occup Environ Med* **61**: 233–238
- 6 Heliovaara M *et al.* (2000) Coffee consumption, rheumatoid factor, and the risk of rheumatoid arthritis. *Ann Rheum Dis* **59**: 631–635
- 7 Pattison DJ *et al.* (2004) The role of diet in susceptibility to rheumatoid arthritis: a systematic review. *J Rheumatol* **31**: 1310–1319
- 8 Aho K and Heliovaara M (2004) Risk factors for rheumatoid arthritis. *Ann Med* **36**: 242–251
- 9 Grant WB (2000) The role of meat in the expression or rheumatoid arthritis. *Br J Nutr* **84**: 589–595
- 10 Symmons D and Harrison B (2000) Early inflammatory polyarthritis: results from the Norfolk Arthritis Register with a review of the literature. I. Risk factors for the development of inflammatory polyarthritis and rheumatoid arthritis. *Rheumatology* **39**: 835–843
- 11 Pattison DJ *et al.* (2004) Dietary risk factors for the development of inflammatory polyarthritis: evidence for a role of high level of red meat consumption. *Arthritis Rheum* **50**: 3804–3812
- 12 Choi HK (2004) Diet and rheumatoid arthritis: red meat and beyond. *Arthritis Rheum* **50**: 3745–3747
- 13 Gregersen PK *et al.* (1987) The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* **30**: 1205–1213
- 14 Begovich AB *et al.* (2004) A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (*PTPN22*) is associated with rheumatoid arthritis. *Am J Hum Genet* **75**: 330–337
- 15 Plenge *et al.* (2005) Replication of putative candidate gene associations with rheumatoid arthritis in over 4,000 samples from North America and Sweden: association of susceptibility with *PTPN22*, *CTLA4* and *PADI4*. *Am J Hum Genet* **77**: 1044–1060
- 16 Brand DD *et al.* (2003) Immunopathogenesis of collagen arthritis. *Springer Semin Immunopathol* **25**: 3–18
- 17 Klareskog L and McDevitt H (1999) Rheumatoid arthritis and its animal models: the role of TNF-alpha and the possible absence of specific immune reactions. *Curr Opin Immunol* **11**: 657–662
- 18 Lundberg K *et al.* (2005) Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity. *Arthritis Res Ther* **7**: R458–R467
- 19 Waksman BH *et al.* (1960) Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. II. Evidence that the disease is a disseminated immunologic response to exogenous antigen. *J Immunol* **85**: 403–417
- 20 Van Eden W and Waksman BH (2003) Immune regulation in adjuvant-induced arthritis: possible implications for innovative therapeutic strategies in arthritis. *Arthritis Rheum* **48**: 1788–1796
- 21 Lorentzen JC (1999) Identification of arthritogenic adjuvants of self and foreign origin. *Scand J Immunol* **49**: 45–50
- 22 Svelander L *et al.* (2004) Oligodeoxynucleotides containing CpG motifs can induce T cell-dependent arthritis in rats. *Arthritis Rheum* **50**: 297–304
- 23 Kleinau S *et al.* (1991) Adjuvant oils induce arthritis in the DA rat. I. Characterization of the disease and evidence for an immunological involvement. *J Autoimmun* **4**: 871–880
- 24 Holmdahl R *et al.* (2001) Arthritis induced in rats with nonimmunogenic adjuvants as models for rheumatoid arthritis. *Immunol Rev* **184**: 184–202
- 25 Holmdahl R *et al.* (2003) Dissection of the genetic complexity of arthritis using animal models. *J Autoimmun* **21**: 99–103
- 26 Remmers EF *et al.* (2002) Modulation of multiple experimental arthritis models by collagen-induced arthritis quantitative trait loci isolated in congenic rat lines: different effects of non-major histocompatibility complex quantitative trait loci in males and females. *Arthritis Rheum* **46**: 2225–2234
- 27 Barton A *et al.* (2001) High resolution linkage and association mapping identifies a novel rheumatoid arthritis susceptibility locus homologous to one linked to two rat models of inflammatory arthritis. *Hum Mol Genet* **10**: 1901–1906
- 28 Swanberg M *et al.* (2005) *MHC2TA* is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. *Nat Genet* **37**: 486–494
- 29 Arnett FC *et al.* (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* **31**: 315–324
- 30 Huizinga TWJ *et al.* (2002) Criteria for early arthritis. From Bayes' Law revisited to new thoughts on pathogenesis. *Arthritis Rheum* **46**: 1155–1159

- 31 van Gaalen FA *et al.* (2004) Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* **50**: 709–715
- 32 Forslind K *et al.* (2004) Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). *Ann Rheum Dis* **63**: 1090–1095
- 33 Kastbom A *et al.* (2004) Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project). *Ann Rheum Dis* **63**: 1085–1089
- 34 Ronnelid J *et al.* (2005) Longitudinal analysis of anti-citrullinated protein/peptide antibodies (anti-CP) during 5 year follow-up in early rheumatoid arthritis: anti-CP status is a stable phenotype that predicts worse disease activity and greater radiological progression. *Ann Rheum Dis* **64**: 1744–1749
- 35 Nandakumar KS *et al.* (2003) Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes. *Am J Pathol* **163**: 1827–1837
- 36 Maccioni M *et al.* (2002) Arthritogenic monoclonal antibodies from K/B×N mice. *J Exp Med* **195**: 1071–1077
- 37 Rantapaa-Dahlqvist S *et al.* (2003) Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* **48**: 2741–2749
- 38 Nielen MM *et al.* (2004) Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* **50**: 380–386
- 39 Vossenaar ER and WJ van Venrooij (2004) Citrullinated proteins: sparks that may ignite the fire in rheumatoid arthritis. *Arthritis Res Ther* **6**: 107–111
- 40 Stolt P *et al.* (2003) Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann Rheum Dis* **62**: 835–841
- 41 Padyukov L *et al.* (2004) A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum* **50**: 3085–3092
- 42 Hill JA *et al.* (2003) 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* **171**: 538–541
- 43 Klareskog L *et al.* (2006) Smoking may trigger HLA-DR (SE)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum* **54**: 38–46
- 44 Linn-Rasker SP *et al.* (2005) Smoking is a risk factor for anti-CCP antibodies only in RA patients that carry HLA-DRB1 shared epitope alleles. *Ann Rheum Dis*, **65**: 366–371
- 45 Matthey DL *et al.* (2002) Relationship among the HLA-DRB1 shared epitope, smoking, and rheumatoid factor production in rheumatoid arthritis. *Arthritis Rheum* **47**: 403–407
- 46 Klockars M *et al.* (1987) Silica exposure and rheumatoid arthritis: a follow up study of granite workers 1940–1981. *Br Med J (Clin Res Ed)* **294**: 997–1000
- 47 Olsson AR *et al.* (2004) Occupations and exposures in the work environment as determinants for rheumatoid arthritis. *Occup Environ Med* **61**: 233–238
- 48 Stolt P *et al.* (2005) Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Ann Rheum Dis* **64**: 582–586
- 49 Sverdrup B *et al.* (2005) Association between occupational exposure to mineral oil and rheumatoid arthritis. Results from the Swedish EIRA case-control study. *Arthritis Res Ther* **7**: R1296–R1303
- 50 Bäckdahl L (2005) Genetic dissection of experimental arthritis in the DA rat [thesis]. Stockholm: Karolinska Institutet ISBN 91-7140-227-6 [<http://diss.kib.ki.se/2005/91-7140-227-6/thesis.pdf>] (accessed 22 June 2006)

### Acknowledgments

We thank members of our research team for valuable discussions, and members of the Epidemiological Investigation of Risk Factors in Rheumatoid Arthritis (EIRA) study group for contributing data on patients in the EIRA study. Funding for the EIRA study was provided by the Swedish Research Council, the Swedish Council for Working Life and Social Research, the Swedish Rheumatism Association, King Gustaf V's 80-year Foundation, the Torsten och Ragnar Söderberg Foundation and the insurance company American Fidelity Assurance.

### Competing interests

The authors declared they have no competing interests.