

RA: from risk factors and pathogenesis to prevention

Towards prevention of autoantibody-positive rheumatoid arthritis: from lifestyle modification to preventive treatment

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Abstract

Recent advances in research into the earliest phases of RA have provided additional insights into the processes leading from the healthy to the diseased state. These insights have opened the way for the development of preventive strategies for RA, which represents a significant paradigm shift from treatment to prevention and will have major implications for patients as well as society. It would be a huge step forward if clinical signs and symptoms, disability, impaired quality of life and the need for chronic immunosuppressive treatment could be prevented. RA can be seen as a prototypic autoimmune disease, and discoveries about the preclinical diseased state for RA could potentially facilitate research into prevention of other immune-mediated inflammatory diseases such as type 1 diabetes, SLE and multiple sclerosis. This review focuses on the current knowledge of factors contributing to the development of RA and discusses the opportunities for intervention.

Key words: rheumatoid arthritis, prevention, treatment, rituximab.

Rheumatology key messages

- Preventive strategies in RA would represent a significant paradigm shift from treatment to prevention.
- Smoking and obesity are modifiable risk factors for RA and of interest for prevention.
- Treatment of individuals at risk of RA with rituximab is currently being tested as preventive therapy.

Rheumatoid arthritis

RA is a prototype immune-mediated inflammatory disease manifested in multiple joints, and it is associated with more aggressive articular disease, higher frequency of extra-articular manifestations and increased mortality when autoantibodies can be detected in the serum of patients. Despite major developments in antirheumatic treatment, the disease is still associated with long-term morbidity and early mortality, causing premature death due to cardiovascular disease, analogous to type I diabetes mellitus [1]. Although progression of radiographic joint damage has declined over the last

decades as a result of more effective use of DMARDs and the introduction of biologics, disease remission can still not be achieved in a significant proportion of patients [2], leading to disability, loss of quality of life, reduced ability to work and increased health care utilization by RA patients. In socio-economic terms, RA is the most common and most important of the inflammatory rheumatic diseases, with a prevalence of ~1% of the population worldwide, estimated to increase by ~22% between 2005 and 2025 due to the ageing population [3]. The relatively high prevalence, irreversible joint damage and widespread occurrence of comorbidities determine the huge societal impact of this disease. A therapeutic window of opportunity exists early in the course of the disease during which the introduction of aggressive antirheumatic therapy can result in a change in the course of disease, leading to protection against progressive joint destruction, prevention of disability and potential lowering of the risk of cardiovascular co-morbidity [4, 5]. Conceivably, there is a preventive window of opportunity during the preclinical stages of RA.

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The preclinical phase of RA

During recent years, research in the field of RA has focused on the earliest stages of disease, leading to the discovery that circulating autoantibodies and elevation of acute phase reactants, cytokines and chemokines can precede the clinical onset of the disease by many years [6–10]. With a median of 5 years before the onset of any signs of arthritis, elevated levels of autoantibodies such as IgM-RF and ACPA can be found in serum of subjects later diagnosed with RA [6, 11]. Subjects with autoantibodies and arthralgia have a 40–70% chance of developing RA within 4 years [12]. The detection of these and other RA-related autoantibodies against post-translationally modified proteins (such as those against carbamylated proteins) may help to identify individuals with systemic autoimmunity associated with RA without clinical evidence of arthritis, but who are at risk of developing RA [13]. Studies on the development of symptoms in these individuals are pivotal in research into the multifactorial aetiology of RA, and various groups have focused their research on this phase of the disease [14–16].

To facilitate communication between researchers in this field and for comparison between studies, new nomenclature on the various phases preceding the diagnosis of RA has been proposed by the Study Group for Risk Factors for RA established by the EULAR Standing Committee for Investigative Rheumatology [15, 17]. Clarity about terminology will help to describe the populations more precisely, which is critical for unravelling the factors that are important in the interaction between the susceptibility of the individual and environmental and lifestyle factors in the various phases. It will also help to determine the current gaps in our knowledge of the underlying pathophysiological processes and thereby help to focus the research agenda when studying the at-risk population.

Genetics and environment

Genetic risks for RA have been acknowledged for a number of years and genome-wide association study (meta-) analyses have identified various RA-associated genes, such as *HLA-DRB1*, *PADI4*, *PTPN22*, *TNFAIP3*, *STAT4* and *CCR6* [18, 19]. The contribution of these individual risk loci to the development of RA appears to be variable. Concordance rates among monozygotic and dizygotic twins are relatively low for both ACPA-positive healthy individuals and ACPA-positive RA patients, indicating that only a limited number of determinants for these two phenotypes have been identified [20]. The focus has therefore shifted towards the regulation of the genes identified, because effects of environmental factors and epigenetic regulation may influence the risk of developing of RA in a susceptible population. What the exact role (as well as the interplay between the various environmental and epigenetic factors identified to date on the specific genetic make-up) is in the various phases of the disease still needs to be elucidated, and this is a fast-moving research field.

Changes in the synovium during the earliest stages of RA: how and why does inflammation in the joints begin?

While the presence of the RA-specific serum autoantibodies is an indication that the risk of subsequent development of RA is increased, not all autoantibody-positive subjects develop clinically manifest disease. The factors leading to arthritis in autoantibody-positive individuals at risk of RA are currently poorly understood. Histological studies in patients with early active RA have shown that all features of chronic synovial inflammation can be found within weeks to months after the first clinical evidence of arthritis [21, 22]. These data indicate that so-called early RA in fact represents chronic synovitis, and this has led to the hypothesis that the development of clinical signs and symptoms may be preceded by asymptomatic synovial inflammation [23]. To explore this hypothesis, investigation of the synovial tissue by MRI and immunohistochemical analyses before the onset of clinical evidence of arthritis has been carried out in a prospective study in autoantibody-positive subjects who were at risk of developing RA. Results have shown that neither the presence of inflammatory cells nor the number of blood vessels in the synovial tissue are associated with the development of arthritis [16, 24]. Consistent with these findings, MRI showed no indication of synovial inflammation during the weeks and months prior to clinical onset of the disease, although a subtle synovial T-cell infiltration in subjects who subsequently developed arthritis compared with those who did not develop arthritis was detected [24]. The notion that subclinical synovial inflammation usually does not coincide with the appearance of serum autoantibodies during preclinical RA is supported by data in animal models of RA, although these models typically lack the presence of antibodies directed against citrullinated proteins [25, 26]. Based on these findings in experimental studies of RA and in our studies in humans, systemic autoimmunity appears to precede the development of synovitis, suggesting that a second hit (due to, for instance, a trauma or a viral infection) is required, leading to citrullination in the synovial tissue and followed by subsequent epitope spreading [16]. These factors remain speculative, however, and are currently the focus of investigation in various cohorts of individuals at risk. Conceivably, the best opportunity for preventive intervention would be before the synovial tissue gets involved.

Changes at sites other than the joints during the earliest stages of RA

The observation that ACPAs during the preclinical phases of RA are not necessarily directed against joint-specific antigens suggests that other tissues may be early sites of RA-related autoimmunity. Based on the development of arthritis in animal models, where changes in lymph nodes precede those in the joints [27], it has been hypothesized that changes might be evident in the lymph nodes of individuals at risk of developing RA [28].

Therefore, lymph node biopsy samples were obtained from individuals who were ACPA- and/or IgM-RF-positive, and we (D.M.G. and P.-P.T. and colleagues) were able to demonstrate increased immune cell activation compared with samples from lymph nodes from healthy volunteers [29]; this demonstrated that abnormalities can be found at sites other than the synovium in apparently healthy individuals. These data provide a rationale for further work on the functional capacities of these immune cells and their interaction with stromal cells residing in the same lymph nodes, which could lead to the identification of new targets for preventive intervention.

Other sites where the immune system may be activated during preclinical RA include mucosal surfaces, such as the lung, the periodontium and the gut, where cells of the innate as well as the adaptive immune system of the host interact with the external environment [30]. Studies focusing on these sites have yielded some exciting data on the potential role of a local immune response in response to, for instance, cigarette smoke and the microbiome in RA pathogenesis. Smoking may, for example, result in citrullination of proteins in the lung, with subsequent processing and presentation of citrullinated antigens in the genetically susceptible individual, resulting in breach of tolerance and systemic autoimmunity. There is, indeed, a strong association between smoking and the presence of ACPAs in the serum of RA patients with distinct major histocompatibility complex class II alleles (the so-called shared epitope) [12, 31]. The hypothesis that tobacco smoke (and other environmental exposures to the lungs, such as silica) can lead to a mucosal immune response giving rise to ACPA production has been supported by studies using high-resolution imaging techniques of the lungs, as well as by analysis of immune cells and autoantibodies in sputum and bronchial alveolar lavage from subjects at risk of developing RA [30, 32, 33].

There has also been considerable interest in the possible role of periodontitis in the pathogenesis of RA. Epidemiological studies have suggested an association between RA and periodontitis, and antibodies against bacteria involved in periodontitis, such as *Porphyromonas gingivalis*, have been detected in patients with RA [34, 35]. Interestingly, this bacterium expresses endogenous citrullinated peptides and its own bacterial peptidylarginine deiminase, which may citrullinate other peptides. Moreover, immunity to *P. gingivalis* is associated with the presence of RA-related autoantibodies in individuals at risk for RA [36], suggesting that infection might play a role in the early loss of tolerance to self-antigens in RA pathogenesis. RA synovitis shares common features with gingivitis, including infiltration by inflammatory cells, increased expression of proinflammatory cytokines and peptidylarginine deiminases, citrullination of proteins and formation of ACPAs [37].

Variation in the microbiome present in the gut has also been linked to autoimmune diseases, including RA. Although various species of bacteria have been suggested to be involved in the pathogenesis, most of the support for a potential role for the gut microbiome

comes from animal data [35]. A more thorough discussion of the potential role of the microbiome in the development of RA can be found in recent publications, which conclude that more research on individuals at risk of developing RA needs to be performed in a prospective manner to understand the importance of the associations found [20, 38].

Although more work needs to be done to understand the possible role of changes in extra-articular tissues such as lymph nodes, lung, gingiva and gut, understanding the initial autoimmune response resulting in ACPA formation and the subsequent process of synovial inflammation and epitope spreading [39, 40] could lead to the development of novel preventive strategies, such as induction of tolerance during the preclinical stage, and perhaps cessation of smoking, treatment of periodontitis and targeting of the microbiome.

Lifestyle risk factors

Many risk factors associated with an increased risk of RA or inflammatory arthritis have been reported in the literature, ranging from infections and vaccinations to hormonal and reproductive risk factors, such as breastfeeding [41, 42] and the timing, number and outcomes of pregnancies [43–46], as well as lifestyle-related factors, such as diet [47, 48], smoking [12, 49, 50] and obesity [12, 51]. In addition, silica exposure and periodontitis have been associated with an increased risk of RA.

The risk factor that has the most unequivocal strong association with RA is cigarette smoking, which has been extensively studied and repeatedly shown in a variety of cohorts to increase the risk of ACPA-positive RA [12, 31, 49, 50, 52–55]. Smoking has a dose-dependent effect on the risk of ACPA-positive RA in both males and females, with odds ratios reported of up to 3.02 for developing RA [49], and was associated with RF positivity in women at risk for RA [56], suggesting that smoking acts early in the development of the immune dysregulation that occurs in RA.

The results of cross-sectional studies on the association between obesity and RA have been variable [51, 57–59]. Of note, disease activity may be a confounding factor in cross-sectional studies because lack of mobility due to impaired function may promote obesity, whereas active inflammation could lead to loss of body weight. Furthermore, alcohol consumption resulting in obesity and having some protective effect against RA (see below) could be a confounding factor. Therefore, prospective studies may be more informative. Two of the authors (P.-P.T. and D.M.G.) and their colleagues prospectively followed 55 ACPA- and/or IgM-RF-positive individuals who had never had any evidence of arthritis [12]. Fifteen of these (27%) developed arthritis during follow-up after a median duration of 13 months. After a median of 27 months follow-up, the overall arthritis risk was increased from 28 to 60% in individuals with a smoking history combined with overweight, defined as having a BMI of 25 or higher [12]. In contrast, the risk of developing arthritis in never smokers with normal weight was only 2%. The identification of obesity as a risk factor

for the development of RA was subsequently supported in a much larger prospective study of subjects who were not selected based on autoantibody positivity: during 2 765 195 person-years of follow-up, 1181 incident cases of RA were identified [59]. There was a clear trend toward increased risk of all RA among overweight and obese women. The association between obesity, autoimmunity and inflammation might perhaps be explained by pro-inflammatory pathways that are activated in adipose tissue, leading to the production of pro-inflammatory adipocytokines [60, 61] and a more pro-inflammatory state. It should also be noted that the synovium is in proximity to articular adipose tissue, which can produce (adipo-) cytokines that may stimulate, for instance, fibroblast-like synoviocytes in the synovial tissue, promoting synovial inflammation [62]. Together, these studies suggest that lifestyle modification aimed at smoking cessation and weight loss could have important consequences for arthritis prevention in RA-prone individuals. Clearly, this hypothesis needs to be tested in a prospective interventional study.

Dietary intake of vitamin D and omega-3 fatty acids could be of interest because of their potential anti-inflammatory properties [63]. Conflicting results have been reported on the effects of vitamin D intake and exposure to sunlight [64, 65]. The omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid are of particular interest, as they serve as substrates for lipid mediators actively involved in the resolution of inflammation [66]. A recent meta-analysis demonstrated that omega-3 fatty acid supplements reduced the consumption of non-steroidal anti-inflammatory drugs in RA patients [67], suggesting omega-3 fatty acid supplements might have utility in RA [68]. In addition, some epidemiologic studies suggest a protective association between consumption of fish high in omega-3 fatty acids and risk of RA [69–71] (that is not observed for fish consumption in general) [72, 73], implicating omega-3 fatty acids as the likely component of interest. Of note, although the studies suggesting a beneficial effect of fish consumption on established RA take various lifestyle factors into account, the influence of obesity and exercise as potential confounding factors were not specifically mentioned. In a cohort at risk for RA, individuals who were ACPA positive were significantly less likely than autoantibody-negative individuals to be taking omega-3 fatty acid supplements and had significantly lower levels of eicosapentaenoic acid and docosahexaenoic acid in the erythrocyte membranes than autoantibody-negative individuals [74], suggesting that low levels of omega-3 fatty acids may play a role in the development of RA-related autoimmunity.

Other lifestyle factors that may influence RA development include alcohol consumption. It has been shown that there is an inverse relationship between alcohol consumption and the risk of developing seropositive RA, as found in both retrospective studies of established RA patients as well as in a prospective study on individuals with arthralgias and positive RA-related serology [75–77]. Also, the exposure to silica has been associated with a moderate

increase in the risk of developing ACPA-positive RA, specifically when combined with smoking [78, 79], as has periodontitis [80], as discussed above.

Primary prevention of RA by modification of risk factors

Primary prevention of RA, defined as interventions targeted at preventing the development of RA-related systemic autoimmunity, is very well-suited to modification of lifestyle factors such as smoking and overweight that will generally result in improved health, but could potentially also contribute to the prevention of RA and related comorbidities in the susceptible population. Indeed, a Finnish study on the effects of an intensive prevention programme aiming at dietary changes and smoking cessation showed long-term prevention of cardiovascular diseases as well as a decline in the incidence of RA [81]. Therefore, campaigns aimed at reduction of the risks of these inherently modifiable lifestyle factors, for instance by education of individuals at risk of developing RA, could provide valuable strategies that need further consideration. Recently, a randomized controlled clinical trial evaluating RA risk education in first-degree relatives of RA patients without RA has been designed to compare willingness to change behaviours when confronted with personalized RA risk information compared with general information on RA [82].

It is currently unclear whether smoking cessation and/or weight reduction may be effective in reducing the risk of RA in every phase preceding the diagnosis [83]. Apart from lowering cardiovascular risks, both factors do have an effect on response to treatment, various disease outcome measures and cardiovascular risk when patients are diagnosed with RA [77, 84], which justifies efforts aimed at cessation of smoking and weight reduction in obese subjects.

Influencing mucosal immune regulation in the oral cavity and the gut are potentially interesting preventive strategies once we have a better understanding of the interaction between the microbiome and the host. Although more evidence is needed for the role of periodontitis in the development of RA, treatment of periodontitis and education on periodontal care are justified from a general health perspective and can be implemented relatively easily. It is at present too early to suggest any interventions aimed at changing the microbiome of the oral cavity or the gut as a way to influence the risk of RA development [38].

Potential secondary preventive treatments

Secondary prevention is defined as interventions targeted at preventing the development of RA in individuals with pre-existing systemic autoimmunity. Of note, some of the interventions described above, such as cessation of smoking and weight loss might also be studied in the context of secondary prevention of RA. At present, no

pharmacological interventions are known that would prevent the onset of clinical signs and symptoms associated with arthritis in subjects with systemic autoimmunity. Preventive interventions aimed at mechanisms that are operative in the preclinical stage of the disease, such as antigen presentation and autoantibody production could in theory be effective.

B cells play an important role in the pathogenesis of RA, based on the marked expression of B cells and plasma cells in the inflamed synovium, the formation of germinal centre-like structures at the site of inflammation in a significant proportion of patients, and the known association of RA with a variety of autoantibodies, including ACPA, IgM-RF, anti-carbamylated protein antibodies and anti-collagen type II antibodies. As circulating autoantibodies may be found years before the clinical onset of the disease, targeting B cells during the preclinical stage of RA might provide a preventive approach in autoantibody-positive subjects at risk of developing RA. There are several mechanisms by which B cells may participate in the earliest phases of the disease process. B cells are efficient antigen-presenting cells; they may activate T cells in the context of co-stimulatory signals; they produce a variety of cytokines; and B-lineage cells produce immunoglobulins, including IgM-RF and ACPA. Rituximab, a depleting anti-CD20 antibody, has been approved for the treatment of established RA patients. This medicine is generally well tolerated and is effective, especially in autoantibody-positive RA patients in late stage [85, 86] as well as in earlier phases of the disease [87]. To test whether rituximab treatment could be used as a preventive approach, we (D.M.G. and P.-P.T.) designed a randomized, double-blind, placebo-controlled clinical trial in individuals at a high risk of developing RA, defined by the presence of both serum autoantibodies as well as elevated CRP levels (the PRAIRI study: NTR 1969). In this currently ongoing clinical trial study, subjects receive either a single infusion of 1000 mg of rituximab or placebo, after premedication with 100 mg methylprednisolone, and they are subsequently followed for the development of arthritis. The aim of this proof-of-principle study is to accomplish a decrease in development of arthritis of at least 75% in a 4-year period; we believe such a large effect could justify the introduction of a single infusion with rituximab for the prevention of RA in this patient population in terms of safety and costs. The intervention and planned research will also lead to a deeper understanding of the pathogenetic processes in this phase of the disease. The results of this clinical trial are as yet not available, but as the first clinical trial with a biologic in this population (i.e. before the onset of arthritis), it may represent a paradigm shift in our approach towards RA, from active treatment of late-stage RA to the implementation of the recent treat-to-target principle in early disease, with the possible next step being intervention with a biologic during preclinical RA to prevent symptomatic disease.

In addition to rituximab, abatacept, a fusion protein of cytotoxic T lymphocyte-associated antigen-4 and immunoglobulin G1 that selectively modulates the CD80/

CD86-CD28 costimulatory signal required for full T-cell activation [88], is currently considered to be a potential treatment that could be tested in a comparable population (APPIRA study; ISCTRN No. 46017566). Abatacept has been approved for treatment of early- as well as late-stage RA patients [89, 90]. The hypothesis would be that interfering with the interaction between T cells and antigen-presenting cells could stop disease progression towards established RA.

Other potential ways of intervening in the preclinical phase of the disease that have been suggested include induction of tolerance by vaccination with dendritic cells, promoting bystander immunity by inducing autoantigen-specific Tregs [91], or desensitization using various antigens, which might be more effective in the phases preceding the diagnosis compared with in fully established disease. None of these interesting potential approaches have been studied yet.

Conclusion

As we start to better understand the processes underlying the aetiopathogenesis of RA, options for preventive treatment, with the ultimate goal of preventing the development of clinically manifest disease, appear on the horizon [92]. Studies of modifiable risk factors like smoking and obesity, and a clinical trial with rituximab in preclinical RA, which could be a game changer in terms of testing biologic therapy before the clinical onset of the disease, are currently underway. Developing preventive strategies in RA would represent a significant paradigm shift from treatment to prevention and will have major implications for patients as well as society. It would be a huge step forward if clinical signs and symptoms, disability, impaired quality of life and the need for chronic immunosuppressive treatment could be prevented. Of note, RA can be seen as a prototypic autoimmune disease, and discoveries about the preclinical diseased state in RA could potentially facilitate research into prevention of other immune-mediated diseases.

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