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 co-morbidities 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.
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 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 2765195 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 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-citrullinated 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 developed 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 (APPIPRA 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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References
1 Turesson C, Jacobsson LT, Matteson EL. Cardiovascular co-morbidity in rheumatic diseases. Vasc Health Risk Manag 2008;4:605–14.
2 Finckh A, Choi HK, Wolfe F. Progression of radiographic joint damage in different eras: trends towards milder disease in rheumatoid arthritis are attributable to improved treatment. Ann Rheum Dis 2006;65:1192–7.
3 Crowson CS, Liao KP, Davis JM III et al. Rheumatoid arthritis and cardiovascular disease. Am Heart J 2013;166:622–8.
4 Myasoedova E, Davis JM III, Crowson CS, Gabriel SE. Epidemiology of rheumatoid arthritis: rheumatoid arthritis and mortality. Curr Rheumatol Rep 2010;12:379–85.
5 van Vollenhoven RF, Nagy G, Tak PP. Early start and stop of biologics: has the time come? BMC Med 2014;12:25.
6 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.
7 Nielen MM, van Schaardenburg D, Reesink HW et al. Increased levels of C-reactive protein in serum from blood donors before the onset of rheumatoid arthritis. Arthritis Rheum 2004;50:2423–7.
8 Jorgensen KT, Wilk A, Pedersen M et al. Cytokines, autoantibodies and viral antibodies in premorbid and postdiagnostic sera from patients with rheumatoid arthritis: case-control study nested in a cohort of Norwegian blood donors. Ann Rheum Dis 2008;67:860–6.
9 Deane KD, O’Donnell CI, Hueber W et al. The number of elevated cytokines and chemokines in preclinical seropositive rheumatoid arthritis predicts time to diagnosis in an age-dependent manner. Arthritis Rheum 2010;62:3161–72.
10 Kokkonen H, Soderstrom I, Rocklov J et al. Up-regulation of cytokines and chemokines precedes the onset of rheumatoid arthritis. Arthritis Rheum 2010;62:383–91.
11 Aho K, Hellowaara M, Maatela J, Tuomi T, Palosuo T. Rheumatoid factors antedating clinical rheumatoid arthritis. J Rheumatol 1991;18:1282–4.
12 de Hair MJ, Landewe RB, van de Sande MG et al. Smoking and overweight determine the likelihood of developing rheumatoid arthritis. Ann Rheum Dis 2013;72:1654–8.
13 Karlson EW, van Schaardenburg D, van der Helm-van Mil AH. Strategies to predict rheumatoid arthritis development in at-risk populations. Rheumatology 2016;55:6:15.
14 Stack RJ, van Tuyl LH, Sloots M et al. Symptom complexes in patients with seropositive arthralgia and in patients newly diagnosed with rheumatoid arthritis: a qualitative exploration of symptom development. Rheumatology 2014;53:1646–53.
15 Gerlag DM, Raza K, van Baarsen LG et al. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. Ann Rheum Dis 2012;71:638–41.
16 van de Sande MG, de Hair MJ, van der Leij C et al. Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. Ann Rheum Dis 2011;70:772–7.
17 Raza K, Gerlag DM. Preclinical inflammatory rheumatic diseases: an overview and relevant nomenclature. Rheum Dis Clin North Am 2014;40:569–80.
18 Stahl EA, Raychaudhuri S, Remmers EF et al. Genomewide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 2010;42:508–14.
19 Okada Y, Wu D, Trynka G et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 2014;506:371–86.
20 Catrina Al, Deane KD, Scher JU. Gene, environment, microbiome and mucosal immune tolerance in rheumatoid arthritis. Rheumatology 2016;55:391–402.
21 Tak PP, Smeets TJ, Daha MR et al. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. Arthritis Rheum 1997;40:217–25.
22 Tak PP. Is early rheumatoid arthritis the same disease process as late rheumatoid arthritis? Best Pract Res Clin Rheumatol 2001;15:17–26.
23 Kraan MC, Versendaal H, Jonker M et al. Asymptomatic synovitis precedes clinically manifest arthritis. Arthritis Rheum 1998;41:1481–8.
24 de Hair MJ, van de Sande MG, Ramwadhoebe TH et al. Features of the synovium of individuals at risk of developing rheumatoid arthritis: implications for understanding preclinical rheumatoid arthritis. Arthritis Rheumatol 2014;66:513–22.
25 Holmdahl R, Jonsson R, Larsson P, Klareskog L. Early appearance of activated CD4+ T lymphocytes and class II antigen-expressing cells in joints of DBA/1 mice immunized with type II collagen. Lab Invest 1988;58:53–60.
26 Nandakumar KS, Bajtner E, Hill L et al. Arthritogenic antibodies specific for a major type II collagen triple-helical epitope bind and destabilize cartilage independent of inflammation. Arthritis Rheum 2008;58:184–96.
27 Rodriguez-Palmero M, Pelegri C, Ferri MJ et al. Alterations of lymphocyte populations in lymph nodes but not in spleen during the latency period of adjuvant arthritis. Inflammation 1999;23:153–65.
28 de Hair MJ, Zijlstra IA, Bourmans MJ et al. Hunting for the pathogenesis of rheumatoid arthritis: core-needle biopsy of inguinal lymph nodes as a new research tool. Ann Rheum Dis 2012;71:1911–2.
29 van Baarsen LG, de Hair MJ, Ramwadhoebe TH et al. The cellular composition of lymph nodes in the earliest phase of inflammatory arthritis. Ann Rheum Dis 2013;72:1420–4.
30 Catrina AI, Ytterberg AJ, Reynisdottir G, Malmstrom V, Klareskog L. Lungs, joints and immunity against citrullinated proteins in rheumatoid arthritis. Nat Rev Rheumatol 2014;10:645–53.
31 Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of
seropositive rheumatoid arthritis. Arthritis Rheum 2004;50:3085–92.

32 Demoruelle MK, Weisman MH, Simonian PL et al. Brief report: airway abnormalities and rheumatoid arthritis-related autoantibodies in subjects without arthritis: early injury or initiating site of autoimmunity? Arthritis Rheum 2012;64:1756–61.

33 Willis VC, Demoruelle MK, Derber LA et al. Sputum autoantibodies in patients with established rheumatoid arthritis and subjects at risk of future clinically apparent disease. Arthritis Rheum 2013;65:2545–54.

34 Wegner N, Lundberg K, Kinloch A et al. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. Immunol Rev 2010;233:34–54.

35 Brusca SB, Abramson SB, Scher JU. Microbiome and mucosal inflammation as extra-articular triggers for rheumatoid arthritis and autoimmunity. Curr Opin Rheumatol 2014;26:101–7.

36 Mikuls TR, Thiele GM, Deane KD et al. Porphyromonas gingivalis and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. Arthritis Rheum 2012;64:3522–30.

37 Harvey GP, Fitzsimmons TR, Dhamarpatni AA et al. Expression of peptidylarginine deiminase-2 and -4, citrullinated proteins and anti-citrullinated protein antibodies in human gingiva. J Periodontal Res 2013;48:252–61.

38 Scher JU, Brez WA, Abramson SB. Periodontal disease and subgingival microbiota as contributors for rheumatoid arthritis pathogenesis: modifiable risk factors? Curr Opin Rheumatol 2014;26:424–9.

39 van der Woude D, Rantapaa-Dahlqvist S, Ioan-Facsinay A et al. Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. Ann Rheum Dis 2010;69:1554–61.

40 Sokolove J, Bronberg J, Deane KD et al. Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. PLoS One 2012;7:e35296.

41 Karlson EW, Mandl LA, Hankinson SE, Grodstein F. Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis? Results from the Nurses’ Health Study. Arthritis Rheum 2004;50:3458–67.

42 Brennan P, Silman A. Breast-feeding and the onset of rheumatoid arthritis. Arthritis Rheum 1994;37:808–13.

43 Hernandez AM, Liang MH, Willett WC et al. Reproductive factors, smoking, and the risk for rheumatoid arthritis. Epidemiology 1990;1:285–91.

44 Peschken CA, Robinson DB, Hitchon CA et al. Pregnancy and the risk of rheumatoid arthritis in a highly predisposed North American Native population. J Rheumatol 2012;39:2253–60.

45 Spector TD, Roman E, Silman AJ. The pill, parity, and rheumatoid arthritis. Arthritis Rheum 1990;33:782–9.

46 Carlens C, Jacobsson L, Brandt L et al. Perinatal characteristics, early life infections and later risk of rheumatoid arthritis and juvenile idiopathic arthritis. Ann Rheum Dis 2009;68:1159–64.

47 Pattison DJ, Harrison RA, Symmons DP. The role of diet in susceptibility to rheumatoid arthritis: a systematic review. J Rheumatol 2004;31:1310–9.

48 Pedersen M, Stripp C, Klarlund M et al. Diet and risk of rheumatoid arthritis in a prospective cohort. J Rheumatol 2005;32:1249–52.

49 Sugiyama D, Nishimura K, Tamaki K et al. Impact of smoking as a risk factor for developing rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis 2010;69:70–81.

50 Di GD, Discacciati A, Orsini N, Wolk A. Cigarette smoking and risk of rheumatoid arthritis: a dose–response meta-analysis. Arthritis Res Ther 2014;16:R61.

51 Crowson CS, Matteson EL, Davis JM III, Gabriel SE. Contribution of obesity to the rise in incidence of rheumatoid arthritis. Arthritis Care Res 2013;65:71–7.

52 Hazes JM, Dijkmans BA, Vandenbroucke JP, de Vries RR, Cats A. Lifestyle and the risk of rheumatoid arthritis: cigarette smoking and alcohol consumption. Ann Rheum Dis 1990;49:980–2.

53 Heliovaara M, Aho K, Aromaa A, Knekt P, Reunanen A. Smoking and risk of rheumatoid arthritis. J Rheumatol 1993;20:1830–5.

54 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.

55 Karlson EW, Lee IM, Cook NR et al. A retrospective cohort study of cigarette smoking and risk of rheumatoid arthritis in female health professionals. Arthritis Rheum 1999;42:910–7.

56 Bhatia SS, Majka DS, Koteljohn JM et al. Rheumatoid factor seropositivity is inversely associated with oral contraceptive use in women without rheumatoid arthritis. Ann Rheum Dis 2007;66:267–9.

57 Wesley A, Bengtsson C, Elkan AC et al. Association between body mass index and anti-citrullinated protein antibody seropositivity is inversely associated with oral contraceptive use in women without rheumatoid arthritis. Ann Rheum Dis 2007;66:267–9.

58 Lu B, Hiraki LT, Sparks JA et al. Autoantibody antibody response meta-analysis. Arthritis Rheum 2014;73:1914–22.

59 Lu B, Hiraki LT, Sparks JA et al. Being overweight or obese and risk of developing rheumatoid arthritis among women: a prospective cohort study. Ann Rheum Dis 2014;73:1914–22.

60 Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y. Obesity in autoimmune diseases: not a passive bystander. Autoimmun Rev 2014;13:981–1000.

61 Gremese E, Tolusso B, Gigante MR, Ferraccioli G. Obesity as a risk and severity factor in rheumatic diseases (autoimmune chronic inflammatory diseases). Front Immunol 2014;5:576.
Danielle M. Gerlag et al.

63 Harbig LS. Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. Lipids 2003;38:323-41.

64 Merlino LA, Curtis J, Mikuls TR et al. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women’s Health Study. Arthritis Rheum 2004;50:72-7.

65 Nielsen MM, van SD, Lems WF et al. Vitamin D deficiency does not increase the risk of rheumatoid arthritis: comment on the article by Merlino et al. Arthritis Rheum 2006;54:3719-20.

66 Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 2008;8:349-61.

67 Lee YH, Bae SC, Song GG. Omega-3 polyunsaturated fatty acids and the treatment of rheumatoid arthritis: a meta-analysis. Arch Med Res 2012;43:356-62.

68 James M, Proudman S, Cleland L. Fish oil and rheumatoid arthritis: past, present and future. Proc Nutr Soc 2010;69:316-23.

69 Di GD, Wallin A, Bortoli M, Askling J, Wolk A. Long-term intake of dietary long-chain n-3 polyunsaturated fatty acids and risk of rheumatoid arthritis: a prospective cohort study of women. Ann Rheum Dis 2014;73:1949-53.

70 Rosell M, Wesley AM, Rydin K, Klareskog L, Alfredsson L. Dietary fish and fish oil and the risk of rheumatoid arthritis. Epidemiology 2009;20:896-901.

71 Shapiro JA, Koeppel TD, Voigt LF et al. Diet and rheumatoid arthritis in women: a possible protective effect of fish consumption. Epidemiology 1996;7:256-63.

72 Pedersen M, Stripp C, Klarlund M et al. Diet and risk of rheumatoid arthritis in a prospective cohort. J Rheumatol 2005;32:1249-52.

73 Benito-Garcia E, Feskianch D, Hu FB, Mandal LA, Karlson EW. Protein, iron, and meat consumption and risk for rheumatoid arthritis: a prospective cohort study. Arthritis Res Ther 2007;9:R16.

74 Gan R, Young K, Zerbe G et al. Lower omega-3 fatty acids are associated with the presence of anti-cyclic citrullinated peptide autoantibodies in a population at risk for future rheumatoid arthritis: a nested case-control study. Rheumatology 2016;55:367-76.

75 Maxwell JR, Gowers IR, Moore DJ, Wilson AG. Alcohol consumption is inversely associated with risk and severity of rheumatoid arthritis. Rheumatology 2010;49:2140-6.

76 van de Stadt LA, Witte BI, Bos WH, van Schaardenburg D. A prediction rule for the development of arthritis in seropositive arthralgia patients. Ann Rheum Dis 2013;72:1920-6.

77 Lu B, Solomon DH, Costenbader KH, Karlson EW. Alcohol consumption and risk of incident rheumatoid arthritis in women: a prospective study. Arthritis Rheumatol 2014;66:1998-2005.

78 Stolt P, Yahya A, Bengtsson C et al. Silica exposure among male current smokers is associated with a high risk of developing ACPA-positive rheumatoid arthritis. Ann Rheum Dis 2010;69:1072-6.

79 Yahya A, Bengtsson C, Larsson P et al. Silica exposure is associated with an increased risk of developing ACPA-positive rheumatoid arthritis in an Asian population: evidence from the Malaysian MyEIRA case-control study. Mod Rheumatol 2014;24:271-4.

80 de Pablo D, Dietrich T, McAlindon TE. Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. J Rheumatol 2008;35:70-6.

81 Kääpiäinen-Seppänen O, Kautiainen H. Declining trend in the incidence of rheumatoid factor-positive rheumatoid arthritis in Finland 1980-2000. J Rheumatol 2006;33:2132-8.

82 Sparks JA, Iversen MD, Miller KR et al. Personalized Risk Estimator for Rheumatoid Arthritis (PRE-RA) Family Study: rationale and design for a randomized controlled trial evaluating rheumatoid arthritis risk education to first-degree relatives. Contemp Clin Trials 2014;39:145-57.

83 Andersson ML, Bergman S, Soderlin MK. The effect of stopping smoking on disease activity in rheumatoid arthritis (RA). Data from BARFOT, a multicenter study of early RA. Open Rheumatol J 2012;6:303-9.

84 Soderlin MK, Petersson IF, Geborek P. The effect of smoking on response and drug survival in rheumatoid arthritis patients treated with their first anti-TNF drug. Scand J Rheumatol 2012;41:1-9.

85 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;54:793-806.

86 Mease PJ, Revicki DA, Szechinski J et al. Improved health-related quality of life for patients with active rheumatoid arthritis receiving rituximab: results of the Dose-Ranging Assessment: International Clinical Evaluation of Rituximab in Rheumatoid Arthritis (DANCER) Trial. J Rheumatol 2008;35:20-30.

87 Tak PP, Rigby W, Rubbert-Roth A et al. Sustained inhibition of progressive joint damage with rituximab plus methotrexate in early active rheumatoid arthritis: 2-year results from the randomised controlled trial IMAGE. Ann Rheum Dis 2012;71:351-7.

88 Moreland LW, Alten R, Van den Bosch F et al. Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4ig and LEA299 eighty-five days after the first infusion. Arthritis Rheum 2002;46:1470-9.

89 Westhovens R, Robles M, Ximenes AC et al. Clinical efficacy and safety of abatacept in methotrexate-naive patients with early rheumatoid arthritis and poor prognostic factors. Ann Rheum Dis 2009;68:1870-7.

90 Genovese MC, Schiff M, Luggen M et al. Efficacy and safety of the selective co-stimulation modulator abatacept following 2 years of treatment in patients with rheumatoid arthritis and an inadequate response to anti-tumour necrosis factor therapy. Ann Rheum Dis 2008;67:547-54.

91 Pascual DW, Yang X, Holderness K et al. Regulatory T-cell vaccination independent of auto-antigen. Exp Mol Med 2014;46:e82.

92 Tak PP. Are we ready to change the pace of arthritis treatment? Treating pre-arthritis and very early arthritis. Acta Reumatol Port 2011;36:8-9.