REVIEW
Towards prevention of autoimmune diseases: The example of rheumatoid arthritis

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Prevention is the ultimate aim for clinicians and scientists concerned with severe diseases, like many immune-mediated conditions. Here, we describe recent progress in the understanding of etiology and molecular pathogenesis of rheumatoid arthritis (RA), which make this disease a potential prototype for prevention that may include both public health measures and targeted and personalized approaches that we call “personalized prevention.” Critical components of this knowledge are (i) better understanding of the dynamics of the RA-associated autoimmunity that may begin many years before onset of joint inflammation; (ii) insights into how this immunity may be triggered at mucosal surfaces after distinct environmental challenges; (iii) better understanding of which features of the pre-existing immunity may cause symptoms that precede joint inflammation and predict a high risk for imminent arthritis development; and (iv) how molecular events occurring before onset of inflammation might be targeted by existing or future therapies, ultimately by specific targeting of Major histocompatibility complex (MHC) class II restricted and RA-specific immunity. Our main conclusion is that studies and interventions in the phase of autoimmunity preceding RA offer new opportunities to prevent the disease and thereby also understand the molecular pathogenesis of its different variants.

Keywords: anticitrullinated protein antibodies · autoimmunity · prevention · rheumatoid arthritis · risk

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Search for causes and sources of autoimmunity, taking genes and environment into account

Most diseases occur due to a combination of environmental challenges in genetically susceptible hosts, with addition of risk—stochastic factors. For RA, studies on gene-environment interactions in the causation of disease have been performed in several different case controls studies and in a few longitudinal cohort studies. The major initial information came from our case-control study named Epidemiological Investigation of RA, where we described how one type of airway exposure, cigarette smoking, was associated with a very high risk for development of RA characterized by presence of antibodies to citrullinated proteins (ACPAs) in individuals carrying certain alleles of HLA-DRB1, often named “shared epitope (SE)” alleles [1]. The odds ratios for RA when combining heavy smoking with presence of two copies of the SE alleles as compared with nonsmokers without these HLA-DRB1 alleles were in the order of 40, thus, widely surpassing odds ratios provided by single genetic variants or single environmental exposures (OR 2.6 for heavy smoking alone, OR 11.5 for double SE alone) [2]. These data were rapidly confirmed in both longitudinal cohort studies, in particular the Nurses’ Health Study, and in other case control studies [3–5]. Interesting follow-up studies demonstrated that smoking was not the only airway exposures with increased risk for risk of seropositive RA when combined with presence of SE alleles; silica dust exposures, coal dust, as well as textile dust exposure yielded similar results [6–8]. Notably, other variants of RA with presence of rheumatoid factors (RFs) without ACPA showed smoking but not the HLA-DRB1 genes and gene-environment interactions to provide risk, whereas neither smoking nor the HLA-DRB1 risk alleles were risk factors for the variant of RA characterized by absence of ACPA as well as RF (the “seronegative” disease) [9].

These data provided a basis for the generation of a hypothesis on mucosal origins, particular the lung, for the immunity that defines the subset of RA where this gene-environment interaction was observed; the reasoning being that smoking and other air pollutants may cause local chronic exposure of neoantigens, such as citrullinated proteins [10, 11], as well as local activation of APCs in the lungs [1, 12]. Presentation of neoantigens from these APC to infiltrating T cells and generation of local GC-like structures in the lungs [13, 14] would according to this hypothesis enable activation of T- and B-cell immunity to the neoantigens and subsequent local production of autoantibodies in lungs as well as migration of antigen-specific T and B cells to LNs and further systemic immunity. This hypothesis has subsequently been supported by data showing the presence of autoantibodies to citrullinated proteins/peptides in sputum as well as bronchial alveolar fluids from individuals in early stages of RA as well as in autoantibody positive individuals before the onset of joint inflammation in RA [14, 15].

Subsequent to the proposal of this “mucosal hypothesis” for generation of pathogenic immunity in RA, studies have been performed also in other immune-mediated diseases. Thus, a big case-control study for MS, with the same design as the EIRA study, and called Epidemiological Studies in MS has shown gene-environment interaction between exposure to cigarette smoke and the MS-associated HLA-DRB1 allele (HLA-DRB1*15), with odds ratios of 6 for those exposed to smokers and harboring HLA-DRB1*15 genes, that is, being at risk as compared to nonsmokers without MS-associated HLA risk genes [16]. A similar hypothesis of a mucosal origin of immunity predisposing for MS has subsequently been formulated, so far however, without defining a specific antigen recognized by T and B cells in MS.

A third example of the same principle of mucosal origins of potentially pathogenic immunity has been formulated for idiopathic inflammatory myopathies a.k.a. myositis, where a gene-environment interaction between cigarette smoking and HLA-DRB1*03 has been observed in the subset of this disease characterized by the presence of antibodies against the Jo-1 autoantigen [17]. Notably, the myositis subset characterized by anti-Jo 1 and other anti-tRNA synthetase antibodies is typically associated with severe pulmonary symptoms characterized by inflammation and subsequent pulmonary fibrosis [18]. Further analyses of the potential origins of this immunity in lung have shown exposure of otherwise hidden epitopes of the His tRNA synthetase in cells exposed to TLR-driven activation similar to what is seen in patients exposed to cigarette smoke [19].

The more generalized hypothesis coming from these studies suggest that exposure to noxious agents, like cigarette smoke, silica, air pollutants etcetera may contribute to the exposures of a number of different neoepitopes in the lung compartment. The ability to mount immune responses against such neoepitopes is by this hypothesis dependent in part of different MHC class II genes that enable immune activation against different neoepitopes, where this immunity may exist many years before onset of the actual inflammatory disease but subsequently contribute to inflammation in joints, nerves, or muscle in some but not all of those with the actual autoimmunity. For a unifying hypothesis concerning the role of MHC class II molecules and environment in the triggering of disease-specific immunity, see Fig. 1.

However, it remains unclear how much of the autoimmunity in these diseases that results from immune activation at mucosal surfaces such as the lung or elsewhere. In RA, which is the condition with most data on the origins of disease-specific autoimmunity, there is evidence that immunity may also be triggered in the gum mucosa, specifically in the context of local inflammation such as in periodontitis. Here, there is also evidence that periodontitis-associated bacteria, in particular Porphyromonas gingivalis harbor mechanisms that enable this bacterium to present PTM, mainly citrullinated, antigens to local T and B cells and that autoimmunity cross-reactive with the bacterial antigens may be triggered in a “molecular mimicry” scenario [20, 21].
When and where can potentially pathogenic immunity be recognized and targeted to accomplish prevention?

An inherent question in diseases named “autoimmune” is whether and which aspects of the specific observed autoimmunity may be causatively involved in disease development or rather representing a subsequent or bystander phenomenon. As will be argued later in this review, this predicament will likely not be resolved before we have methods to specifically eliminate or modify candidate immune reactions and determine effects of such interventions on disease onset and progression.

We argue that RA offers an interesting study case for investigations of these questions, as (i) immunity to post-translationally modified (PTM) citrullinated proteins/peptides as well as RFs is present many years before onset of joint inflammation [22–25]; (ii) this immunity can be triggered in defined ways at mucosal sites [14, 26, 27]; and (iii) as the autoimmunity, so far investigated mainly for B cells and antibodies, is very dynamic with a number of changes in B cells and antibody specificity and characteristics when autoimmunity is translated into an autoimmune disease, here seropositive RA [24, 28]. Understanding these processes in detail may provide information on what immunity we need specifically to eliminate or modify in order to prevent development of arthritis. In the following sections, we will focus on specificity and functionality of B and T cells that have been described in different phases of RA development.

B-cell development and autoantibody characteristics

After an initial triggering which may occur at mucosal surfaces, autoreactive B cells from patients with RA have undergone multiple rounds of antigen- and T-cell-driven maturation with extensive somatic hypermutations in the immunoglobulin variable genes [29–31]. Interestingly, these extensive mutations may be driven from recognition of different PTM antigens as the reactivity patterns of antibodies captured at different stages towards the development of arthritis display an increased reactivity against several different PTM epitopes [29, 32–34]. Of interest is also that RFs cloned from single B cells display much fewer somatic mutations than ACPAs from the same patient [42]. The observation that serum antibodies as well as human monoclonal autoantibodies cloned from individual B cells and plasma cells from RA patients, show reactivity with several different, often linear epitopes of citrullinated, sometimes homocitrullinated and sometimes also acetylated antigens [29, 30, 34] has led to the redesignation of these antibodies as AMPA in certain contexts. Notably, there is a clear hierarchy as well as cross-reactivity between antibodies against citrullinated, carbamylated, and acetylated self-antigens.
with citrullinated antigens being the most widely recognized antigens, with few antibodies recognizing carbamylated or acetylated antigen but not the citrullinated ones [30]. Based on these and other data indicating a primary role of ACPA in RA pathogenesis, these antibodies will be the main ones discussed in the rest of this review, with the precaution that there may still be targets of antibodies to carbamylated or acetylated (or possibly otherwise modified antigen) that have not yet been recognized.

A further interesting feature of the variable gene segments of ACPA present prior to the development of joint inflammation is the emergence of sequences in the variable (Fab) segments that enable N-linked glycosylation [35]. The result is that virtually all ACPAs identified in late stages toward disease development [36] and in established RA [37] display such Fab glycosylation which may be used as an additional predictor for development of arthritis. The presence of all these features show how knowledge is accumulating concerning the dynamics of T-cell driven evolution of ACPAs which may contribute to disease development thereby providing more precise information on when and how to interfere with these processes in order to prevent development of RA.

**Autoreactive CD4+ T cells**

The dynamic features of T-cell development prior to the development of RA are far less known and investigated as compared with the B cells and antibodies. What has been proposed, is that an IL-17-dependent mechanism is involved in the T-cell driven events that change IgG Fc glycosylation patterns toward asialylation [38]. This observation contrasts with the apparent lack of a major contribution from IL-17 in the early phases of joint inflammation in RA, thereby emphasizing that different cytokine-driven processes may be involved in different phases of the development from autoimmunity to autoimmune arthritis.

The knowledge we have on specificity and functions of T cells with relevant TCR specificities in RA relates to established RA with some emphasis on early RA [39–44]. Here, HLA-DRB1-peptide tetramer assays have demonstrated the presence of a moderately increased number of T cells specific for certain citrullinated peptides and where such T cells from RA patients display an activated and memory phenotype as compared to similarly labelled cells from HLA-DRB1 matched healthy controls [40]. However, we still lack substantial knowledge about the presence and evolution of antigen-specific T cells during the phases of autoimmunity that precede the development of seropositive RA. Even though one may extrapolate from information gained from recent onset RA patients, there is an urgent need to characterize specificity, phenotype, and function of antigen-specific T cells in phases of RA preceding arthritis. Thus, whereas interventions targeting antigen-specific T cells may not yet be an option for prevention and therapy, broader interference with T-cell activation, for example, using CTLA-4-Ig is an attractive and currently actively investigated option for prevention of RA (see also Table 2).

**Risk phases and evolution of early disease symptoms**

In RA, there is a suggested terminology for defining specific risk subgroups during the different phases of autoimmune disease development. Individuals can pass through a phase of autoimmunity (such as ACPA-positivity), a phase of symptoms without clinical arthritis, and a phase of unclassified arthritis leading to development of RA [45]. N.B. the order of the phases is not strict; individual can skip some of the phases or pass through them at once, or even go through the phases but never develop RA. There are also reports that individuals with specific genetic predisposition and autoimmunity, specifically those with low concentration of specific IgG, can leave this phase and spontaneously revert to seronegative [46, 47]. Another characteristic of the phase of autoimmunity is the lack of sign of inflammation in the target organ, synovial tissue, and in the peripheral blood, however, there are studies reporting a trend, of gradual increase of acute phase reactants and cytokines closer to disease onset [48–50].

There is further expert opinion of a specific symptom complex with a high risk for future development of RA designated “clinical suspect arthralgia” [51]. Qualitative studies and interviews among ACPA-positive risk individuals have further identified 13 different symptoms linked to the risk phase and associated with high risk for RA development such as, joint pain, stiffness, and fatigue, with substantial impact on daily life [52, 53]. Overall “risk RA” individuals are currently mainly identified due to these symptoms that make them seek health care and where a positive test for ACPA and/or RF may lead to referral to rheumatologist. In some centers, further risk prediction and efforts toward prevention of RA, also introducing genetic risks and risk scores are ongoing, so far mainly in research contexts. See Table 1 for presentation of Risk RA cohorts [47, 54–60].

**Options to target other cells and/or molecular processes before onset of inflammatory arthritis**

The joint inflammation in RA is preceded not only by changes in B and T cells but also by alterations in a number of other cell types, sometimes driven by the adaptive immunity, in particular the antibodies described above, but often without us knowing which molecular mechanisms drive the observed cellular changes. Alterations that are known include:

**Osteoclasts**: Osteoclasts participate in generating a general and joint specific bone loss that has been observed before onset of joint inflammation in ACPA-positive individuals [61] and ACPA has been shown to induce osteoclast activation and bone loss in vitro and in vivo [62–64]. Additional experiments have suggested that ACPA may cause this osteoclast activation by means of a PAD- and IL-8-dependent activation process [65]. These observations provide a basis for targeting
Table 1. Risk-RA prospective cohorts, studies of individuals without clinical arthritis and future risk for arthritis and rheumatoid arthritis

| Location                  | Number individuals | Inclusion criteria                                                                 | Arthritis progression, Number (prevalence) | References |
|---------------------------|--------------------|-----------------------------------------------------------------------------------|--------------------------------------------|------------|
| Amsterdam, Netherlands    | 374                | ACPA(+) or IgM-RF(+) at two timepoints and arthralgia.                             | 131 (35%)                                  | [54, 96]   |
| Leiden, Netherlands       | 241                | Rheumatologist clinical suspicious arthralgia in small joints, duration <1 year   | 45 (19%) RA***ACPA(-), 14 (11%)***ACPA(+), 16 (67%) | [55, 56]   |
| Leeds, UK                 | 419                | ACPA(+) and incident MSK symptom                                                  | 123 (31%)                                  | Di Matteo et al. (2020) |
| Manitoba, Canada          | 374                | FDR RA                                                                            | 18 (5%)***ACPA(-), 8 (2%)***ACPA(+), 10 (24%) | [47]       |
| Colorado, USA             | 242                | FDR RA and/or arthralgia and/or screening                                         | 18 (7%)***ACPA(-), 0 (0%)***ACPA(+), 18 (21%) | [58]       |
| Linköping, Sweden         | 82                 | ACPA(+) and MSK pain                                                              | 39 (48%)                                   | [60]       |
| Stockholm, Sweden         | 268                | ACPA(+) and MSK symptom and lack of ultrasound arthritis                           | 75 (28%)                                   | [59]       |

of PADs and IL-8 to interfere with the osteoclast-dependent part of arthritis development. The same potential may exist for pain preceding RA as polyclonal ACPA purified from serum of seropositive RA patients have been suggested to initiate an IL-8- and PAD-dependent pain like behavior when injected into mice [66].

Synovial fibroblasts are major players in the joint pathology in RA and during recent years a number of different subsets of these cells have been described in the fully inflamed RA joints, some originating from the thin lining of healthy joint synovium and being associated with tissue damage, others originating from the sublining layer and associated mainly with immune effector functions [67]. Also, synovial fibroblasts, experimentally derived from biopsies of inflamed synovia can be activated by proinflammatory cytokines and chemokines (including IL-8) [68]. Certain ACPAs can further promote activated synovial fibroblasts into an enhanced migratory phenotype [68]. Based on the new data on synovial fibroblasts and their diverse functions, several options for targeting these synovial fibroblasts are currently being considered.

Neutrophils: ACPA may typically interfere with NETs which display both acetylated and citrullinated molecules including histones as a typical feature of NETosis. This activation may also be enhanced by IL-8 suggesting a possible role of products from ACPA-activated osteoclasts and fibroblasts in local neutrophil activation. However, also many other mechanisms, including direct effects of ACPA may exist locally in the joints that contribute to the production of NETosis-dependent inflammatory mediators and other proinflammatory features of activated neutrophils [69, 70].

Peptidylarginine deiminase (PAD) enzymes: These enzymes are expressed in different tissues and during different phases of health and disease [71] and are present also in the inflamed RA synovial tissue [72, 73]. There are five PAD isotype enzymes, where PAD2 is the mostly widely expressed and PAD4 is expressed by immune cells and granulocytes [74]. PADS are essential for the citrullination process, where citrullinated-proteins are formed during an enzymatic process catalyzed by the enzyme in which an arginine residue is converted to citrulline. It has been shown that PAD-dependent citrullination of certain peptides allows a better binding to certain allelic forms of HLA-DR molecules on APCs, enabling activation of T cells against these neopeptides [41, 75–77]. The effect of smoking in RA pathogenesis is suggested to be mediated through induction of post-translational modifications of proteins in lungs by inducing PAD expression and citrullination of proteins and peptides that may enable a specific T-cells response and subsequent B-cell differentiation and production of ACPA [10, 14, 78, 79]. Interestingly, there are also reports on B-cell responses targeting PAD and creating anti-PAD reactivity in RA patients [80]. All these data have suggested that specific PAD inhibition may constitute a way towards inhibition of anticitrulline immunity, even before onset of joint inflammation.

How can we translate knowledge about etiology and molecular pathogenesis of the early- and risk-arthritis phases of RA to prevent development of the disease?

Public health measures: The epidemiologic studies of risk factors for RA have revealed a number of modifiable environmental and life-style factors that may be addressed in public health as well as in more targeted contexts [1-4, 6, 7, 81]. The most obvious
modifiable risk factor is cigarette smoking, where a historic study in our own EIRA cohort showed that more than 20% of all RA cases, more than 30% of all seropositive RA cases, and more than 50% of all cases occurring in individuals carrying HLA-DRB1 SE genes would not have happened if nobody had smoked (estimation by so called population attribution) [2]. This was in a population where around 50% were ever smokers. This number has decreased dramatically in many countries in recent years, but smoking is still a common habit in many countries in the world, not least in Asia.

Further, also protection against exposure to several occupational noxious airborne substances, including silica dust, coal dust, other inorganic dusts, solvents, textile dust, and most probably asbestos should be measures preventing RA in a public health context (for a recent review, see [82]). One example, again from population attributions studies have indicated that coal dust is responsible for one-third of all cases of RA in coalminers [8].

Presence of different pulmonary inflammatory conditions, including chronic obstructive pulmonary disease, bacterial pneumonia, and interstitial lung disease, have been associated with an increased risk for both seropositive and seronegative RA [83, 84]. We still do not know to which extent successful treatment of these diseases also reduce risk for RA, but an interesting possibility is that use of local anti-inflammatory drugs may affect not only the inflammatory lung disease but also the risk for RA. Finally, it is possible that also treatment of periodontitis by proper tooth hygiene and other measures may diminish risk for subsequent RA.

Notably, individuals with genetic predisposition for RA, in particular those carrying HLA-DR SE genes, typical commonly occurring in European populations (30-50% frequency), should be particularly encouraged to change eventual smoking habits and also try to avoid airborne noxious occupational exposures. Notably, most of us do not know our precise HLA-DR allele type, but addressing those with a family history of RA may be particularly relevant; as the relative risk for RA increases if having several first-degree relatives FDRs [84, 85]. Clinicians may also encourage their patients with seropositive RA to advice their children and other first-degree relatives to modify lifestyle and/or other environmental exposures to reduce risk for RA.

**Personalized prevention aiming at influencing triggering and perpetuation of potentially pathogenic immunity**

Whereas the long period of autoimmunity, and sometimes also symptoms, such as arthralgia and fatigue, often precede the development of RA, these subjects are in most cases not identified as risk for RA individuals and not referred to health care. We and others have recently developed an e-health tool for identifying at risk individuals using a screening protocol involving both an electronic questionnaire for symptoms and a multiplex serology chip for determination of autoantibody patterns [86]. Such tools may enable patients with symptoms of arthralgia or other musculoskeletal symptoms to be included in prevention programs that are so far only run in a few centers in the world in research settings but may become routine if effective means for prevention are developed. Other e-health tools that are currently developed as “personal digital companions” will enable patients to modify lifestyles and environment that are associated with increases risk for RA. Major tasks for centers involved in these efforts are to improve risk prediction and establish cohorts where new preventive strategies can be tested. Prediction is so far mainly dependent on a combination of clinical symptoms and serology (titers and fine specificities of ACPA and combination with RF). It remains to be determined if as yet experimental procedures (such as analysis of Fab glycosylation,Fc glycan asialylation, and possibly analysis of phenotypes and numbers of antigen-specific B and T cells) might enhance prediction [87].

As indicated above, we now have access to a rather large number of interventions that may be tested for prevention of RA in individuals at sufficiently high risk for imminent RA. The choice of interventions to be investigated will obviously depend on the balance between the possible effect towards disease prevention and the potential risk associated with the treatment. Notably, several of the so far described mechanisms in the autoimmune phase preceding joint inflammation in RA are different from mechanisms targeted by drugs that are effective in established arthritis. Some may be similar, such as drugs targeting the adaptive immunity, that is, anti-CD20 (rituximab) or CTLA4Ig (abatacept), but others may be different.

As for the broader public health measures, elimination or modification of environmental and lifestyle risk factors is the most straightforward and least risky intervention. A few recent studies indicate that such targeted behavioral interventions may have effect [88-90] on behavior, for a recent review of this area, see Ref. [91].

Also, pharmacological interferences aimed at modifying the pre-existing adaptive immunity are increasingly investigated. The first of these using targeted therapies, including biologics, used rituximab in a population of individuals at risk for RA. Here, the onset of RA was delayed significantly (up to 1 year from one injection of anti-CD20 treatment, rituximab, at a timepoint were 25% of all the subjects had develop arthritis but the effect on delaying arthritis was attenuated over time) [92]. This indicates that repeated use of rituximab may indeed function as a prevention, but no such data exist as yet. Notably, the patients included in the trial were ACPA and RF positive and they also had a subclinical inflammation as seen from a rise in CRP levels. A few prevention studies are also ongoing using abatacept aimed at reducing T-cell activity as well as subsequent autoantibody production ([93] and ClinicalTrials.gov identifier: NCT02778906). First results from these studies are expected during 2021. Finally, studies are also ongoing with other antirheumatic drugs, see Table 2 [88-90, 92-96]), also awaiting results during coming years.
| Study name; location | Inclusion criteria | Intervention | Primary endpoint/results | References |
|----------------------|-------------------|--------------|--------------------------|------------|
| ARIA; Erlangen, Germany | ACPA(+) arthralgia and MRI synovitis or osteitis in hand. | Weekly injection of CTLA-4-Ig (abatacept) or placebo for six months. 18 months follow up. | Reversing subclinical MRI inflammation. Ongoing. | ClinicalTrials.gov identifier NCT02778906. |
| APIPPRA; UK, Netherlands | ACPA(+) or RF(+) arthralgia. | Weekly injection of CTLA-4-Ig (abatacept) or placebo for one year. Two years follow up. | Time to development of swollen joints or RA. Ongoing. | [93] |
| Dexamethasone-study; Amsterdam, Netherlands | ACPA(+) or RF(+) arthralgia and genetic risk factor SE. | Twice dexamethasone intramuscular injection or placebo. | Reduced ACPA concentration but no decrease in arthritis. | [96] |
| PRAIRI; Netherlands | ACPA(+) and RF(+) with CRP levels ≥0.6 mg/L. | One-time point infusion with anti-CD20 drug (rituximab) or placebo. Follow-up in mean 29 months. | Average 5 months inhibition of arthritis onset and numeric decreased risk at 12 and 18 months. | [92] |
| PreventRA; Stockholm, Sweden | ACPA(+) arthralgia without subclinical ultrasound detected arthritis and VAS pain ≥ 20. | One time-point infusion of osteoclast inhibiting drug (Zolendronic acid) or placebo. Two years follow-up. | Decrease of pain. Increased time to arthritis. Ongoing. | EudraCT-nr 2019-002673-62 |
| STAPRA; Netherlands | ACPA(+) high or ACPA(+) and RF(+) arthralgia. | Daily Atorvastatin 40 mg or placebo for 3 years. | No significant delayed onset of arthritis onset. Preterm closed due to low inclusion rate. | [94] |
| StopRA; USA | ACPA(+) high with arthralgia | Daily hydroxychloroquine or placebo for 1 year. Three years follow-up. | Number of RA cases by month 36. Ongoing. | ClinicalTrials.gov identifier NCT02603146 |
| TREAT EARLIER; Netherlands | CSA and arthralgia (<1 year) and subclinical MRI inflammation of the hand or foot joints. | One dose of intramuscular methylprednisolon and weekly methotrexate (25 mg) or placebo for 1 year. Two years follow-up. | Development of clinically detectable arthritis. Ongoing. | [95] |
Prevention from addressing mechanisms downstream of ACPA effects on target cells in the synovium

A potentially attractive way of preventing RA is to interfere specifically with effector functions of disease-inducing immunity (see Fig. 2).

One effort that is already being translated from mice to humans is use of bisphosphonates. Here, one study has been initiated in our own research and clinical units (PI Prof. Anca Catrina and MD PhD Aase Hensvold) where parental administration of zoledronic acid is used to target osteoclast activity in an effort to ameliorate effects that ACPA may have on pain and osteoclast-mediated bone loss, and possibly also on arthritis development. This therapeutic effort is based on the successful treatment with zoledronic acid on pain-behavior and bone loss in mice given infusions of ACPA [97]. The rationale is that this treatment aimed at reduction of osteoclast activity may reduce both local production of IL-8 and potential other mediators of bone destruction and pain behavior, and possibly also influence other downstream and disease-associated events such as phenotype changes in synovial fibroblasts and NETosis. Other strategies that may be tested in the future include efforts to interfere with local production and/or effector function of IL-8 on osteoclasts, neutrophils, or synovial fibroblasts. Still other attractive options are to interfere with the enzymatic activity of one or several PADS. Efforts to generate and test such PAD inhibitors are ongoing in both pharma companies and in academic units.

Towards the future: What is the prospect for tolerizing therapies?

Development of tolerization therapies addressing potentially pathogenic immunity has for long been an ultimate aim in immune-mediated diseases, and as described above, seropositive RA offers some interesting potentials for such efforts. Thus, the knowledge of mucosal sites as places of origins of the disease-associated immunity and the emerging understanding of T- and B-cell immunity in the context of very distinct HLA class II alleles provide cornerstones for efforts to develop antigen-specific tolerization therapies.

Most tolerizing therapies that have proven successful in MHC class II dependent autoimmune disease in experimental animals have used knowledge of the MHC-peptide-TCR complex to eliminate or modify specific T-cell responses to define MHC class II binding peptides from protein autoantigens. Notably, such tolerizing therapies, using either peptides or MHC class II peptide complexes in a tolerizing context (tolerizing DCs, drug-enhanced tolerization schemes, etc.) have been most successful in the "pre-clinical" phases of disease, that is, at or immediately after initiation of the autoimmunity but before onset of inflammation (for recent reviews see [98–100]).

So far, very limited experience exists in humans where efforts to address specific T-cell immunity in established RA has been reported. Thus, one small study in RA patients from University of Queensland used a set of citrullinated peptides for loading of DCs treated with a NF-κB inhibitor [101] and another study has used DCs modified toward a tolerizing phenotype in vitro and loaded the potentially tolerizing DC with material from synovial fluids or RA patients (AutoDECTRA ClinicalTrials.gov Identifier: NCT01352858). Notably, no side-effects were observed which was a major positive outcome of these studies. These studies, thus, contribute to setting the scene for further development of tolerizing therapies which may initially happen in early RA patients but may later be translated also to prevention studies in individuals at high risk for disease.

Targeting antigen-specific B-cell development and antibody production is yet much less developed in experimental systems than efforts toward T-cell tolerance although potentially
Figure 2. The cartoon illustrates the gradual development of RA-associated immunity to post-translationally modified proteins and peptides and how different interventions may intercept with either the development of potentially pathogenic immunity or with effector functions of such immunity. Notably, several of the potential interventions may be able to affect immunity or effects of immunity before onset of joint inflammation, but not necessarily after onset of this inflammation in established RA. The figure shows the tentative evolution of T-cell dependent immunity from the triggering of T- and B-cell responses against citrullinated and otherwise post-translationally modified proteins in the lungs or in the gums, further during expansion and enlargement of this immunity and towards targeting of cells and molecules in the joint. This evolution of immunity occurs in many cases in parallel with the emergence of symptoms, such as joint pain and bone loss, which may in part be due to these same immune reactions. Below the graphs illustrating different stages of cellular activation and interactions shows tentative interventions aiming at prevention of arthritis by means of lifestyle changes or by pharmacological therapies. 

**Abbreviations:** RA, rheumatoid arthritis; ACPA, anti-citrullinated protein antibodies; RF, rheumatoid factor; PAD, peptidylarginine deiminase; HLA DR SE, human leukocyte antigen DR isotype-shared epitope gene(s); PTPN22, Protein tyrosine phosphatase, non-receptor type 22 gene; CD40L, cluster of differentiation 40 ligand; TCR/MHC II, T-cell receptor/major histocompatibility complex; PAdi, peptidylarginine deiminase interference; CTLA4 Ig, cytotoxic T-lymphocyte-associated protein 4 immunoglobulin; Anti IL-8, antibody targeting interleukin 8; Anti CD20, antibody targeting cluster of differentiation 20.

targetable checkpoints for pathogenic B-cells development in RA have been described [102]. Experimental methods to eliminate antigen-specific B cells and even plasma cells have been described [103], and the pre-RA phase may in the future provide an opportunity to test also such methods.

On balance, the phase of autoimmunity that precedes the development of seropositive RA provides an attractive, but also challenging opportunity for future tolerizing therapies. Strategic efforts to learn more about prediction of onset of RA, about T- and B-cell specificity and regulation before this onset, and about monitoring of molecular and cellular effects of different therapies are, thus, warranted and are in certain aspects ongoing in a focused EU/IMI project in this area (www.RTCure.com).

**Ethical issues concerning personalized prevention**

Obvious ethical concerns exist for any type of effort toward personalized prevention. One concern, valid for all studies, observational or interventional, relates to effects, psychological, and/or legal (insurance, etc.) that may occur from notifying an individual that he/she is a high risk for acquiring a serious disease with as yet limited capacity for prevention. Another concern is the risk associated with testing of a drug with potential side-effects in individuals where development of future disease is substantially lower than 100%. The opportunity to prevent disease by modifying environment or lifestyle, addresses to some extent the first concern. The opportunity to treat symptoms, such as arthralgia and bone loss, that may exist in the “pre-RA” phase with the same therapy as is aimed to prevent arthritis may address the second concern. The main argument, however, remains the wins that may follow from any successful prevention strategy that can be based on the science that requires the contribution from individuals at risk for disease.

**Concluding remarks**

Prevention of common chronic conditions, like autoimmune diseases, is the most attractive way to address the challenges from increasing numbers of patients with these diseases which...
parallel the increasing use of therapies, often expensive, that diminish symptoms of the diseases but provide no cure. Cardiovascular diseases provide a prime example of how a combination of public health measures (such as societal measures to reduce cigarette smoking) and therapies targeting individuals at high risk for disease (such as reduction of blood pressure and high lipids) have diminished morbidity and mortality [104]. We now need similar strategies for autoimmune diseases addressing modifiable environmental and lifestyle risk factors with public health measures and with immunology and immunotherapy at the center for personalized prevention. The argument in the current review is that the seropositive variant of RA provides a very attractive disease entity for such efforts. However, as for most autoimmune diseases, definite evidence is lacking of which immune reactions that exist before disease are disease causative. We argue that such knowledge will not emerge until a therapy is designed that eliminates or modifies distinct parts of this immunity and/or its consequences, and that this therapy prevents the disease.

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Abbreviations: ACPA: anticitrullinated protein antibodies · PTM: post-translationally modified · RA: Rheumatoid Arthritis · RF: Rheumatoid factor · SE: shared epitope