**The autoimmune response as a potential target for tolerance induction prior to the development of rheumatoid arthritis.**

Rene E. M. Toes1, 2 and Karim Raza3, 4

* 1; Corresponding author; r.e.m.toes@lumc.nl
* 2: Dept. of Rheumatology, Leiden University Medical Center, 2333 ZA, Leiden, The Netherlands
* 3: Research into Inflammatory Arthritis Centre Versus Arthritis, and MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, Institute of Inflammation and Ageing (IIA), University of Birmingham, Queen Elizabeth Hospital, Birmingham, UK. k.raza@bham.ac.uk.
* 4:Department of Rheumatology, Sandwell and West Birmingham NHS Trust, Birmingham,

**Abstract**

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting synovial joints. Although treatment options and treatment efficacy have increased significantly in the last two decades, the disease cannot be cured or prevented. Therefore, RA still has a considerable impact on the quality of life of patients, not only because life-long medication is often required but also because residual disease activity leads to progressive loss of function in the musculoskeletal system and extra-articular morbidity. Key future goals in the management of RA are the ability to induce long-lasting drug-free remission in patients who have developed RA (i.e. to achieve a cure) as well as to prevent disease before it emerges in the first place. To reach these goals, it is pivotal to understand the autoimmune response underlying RA-pathogenesis and to develop ways to permanently silence it (i.e. to induce tolerance). For preventive studies, the identification of markers (of either clinical or immunological/biological origin) predictive of future disease is crucial, as prevention of disease will not be feasible without the identification of relevant ‘at risk’ target populations. Here, we will review the auto-immune response underlying RA, how RA-specific auto-immunity develops and evolves during the transition from health to disease and how “tolerance studies” could be designed to achieve prevention and/or cure of disease.

**Introduction**

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of synovial joints. At present, the disease is incurable in most patients, necessitating life-long medication. Although, clinical symptoms and signs can be controlled in the majority of patients, current evidence suggests that the underlying autoimmune response is not substantially affected by these treatments.1 It is, however, tempting to speculate that long term drug-free remission could be induced with future treatments aimed at silencing and/or tolerizing the autoimmune reaction that plays a role in RA pathogenesis. In order to design such treatments, it is crucial to understand the immune components contributing to disease. T-cells contribute to RA pathology, likely play an important role in the early stages of disease, and are a rational target for tolerance-induction approaches.2,3 Such approaches could aim to delete/silence the T-cell population directly responsible for disease-induction and/or progression. Alternative approaches include the induction of a new T-cell population able to steer the autoimmune response in a non-inflammatory direction.4 While in many preclinical animal studies, pre-emptive silencing of the immune response can be readily achieved, silencing of ongoing immune responses in humans is much more challenging.3 This is, most likely, a consequence of both the long-standing presence of an autoimmune response which started developing years before the onset of clinical symptoms, and also because the pathogenic T-cell population in humans is often ill-defined and variable between individuals with RA. Likewise, methods for the induction of effective T regulatory cell populations that can modulate disease outcomes in an antigen-specific manner in humans are not currently available. Whilst first successes in type I diabetes have been reported using an anti-CD3-blocking antibody,5 the relevance of these approaches to other auto-immune diseases remains unclear. In this paper, as part of a series on therapeutic tolerance induction in rheumatic disease, we will discuss the autoimmune response in RA; the potential drivers of the development of autoimmunity, the transitions taking place in individuals with autoimmunity as they develop musculoskeletal symptoms and then arthritis. It is important to acknowledge that many components of the innate system within the synovium, adjacent bone and lymph nodes, including macrophages, fibroblasts and osteoclasts, are likely to play pivotal roles in the development of joint symptoms and joint swelling. Furthermore, autoantibodies produced as part of the adaptive immune response have been suggested to interact with components of innate immune system for example modulating fibroblast migration6 and possibly osteoclast behaviour to drive erosion and pain.7,8 However, we will focus on the development of the adaptive immune response in RA. In this context, we will also provide an overview on the possibilities to modulate the autoimmune response in RA to prevent disease-onset or disease-progression.

**Not all RA is the same.**

Auto-antibodies are detected in up to 70% of RA patients at the time of diagnosis; however, a sizeable proportion is seronegative. In cohorts of patients with longstanding active disease, the proportion of seropositive patients increases because longstanding drug-free remission is more common in seronegative patients.9 These observations illustrate the fact that RA is a heterogeneous disease consisting of different endotypes with distinct pathobiological mechanisms driving disease induction and progression. Seronegative RA lacks most of the typical hallmarks defining an autoimmune disease - no disease-specific auto-immune response has been identified, there is no strong association with the Human Leucocyte Antigen (HLA)-system and whole genome-wide association studies (GWAs) have not revealed consistent associations with genetic regions involved in controlling the adaptive immune response.10-13 Therefore, the immunological components contributing to seronegative RA most likely differ substantially from those underlying seropositive disease. The triggers for the immune response in seronegative RA are still ill-defined although a prominent role for innate immune cells is likely. The insights that RA comprises at least two different endotypes, as defined by different immunological- and genetic associations, are important for efforts aiming to silence the pathoimmunological mechanisms underlying these two disease entities as they likely require different therapeutic approaches.14 In the context of this Series, focus will be given to seropositive RA as this disease-endotype is hallmarked by a clearly defined humoral immune response that could, potentially, be modulated by “tolerance” inducing strategies.

**The autoimmune response in RA**

Seropositive RA is, by definition, characterized by the presence of auto-antibodies. Rheumatoid factors (RF) and anti-citrullinated protein/peptide antibody (ACPA) responses have been studied in most depth and the presence of these antibodies is relevant for disease classification and prognostication.15

Rheumatoid factors were first identified in the 1940’s and recognize the Fragment crystallizable (Fc)-part of human IgGs. The observation that sera from RA-patients could agglutinate red bloods cells coated with IgG from sheep, via RF mediated cross linking of Fc-tails, led to their initial identification16 and highlighted that RFs can bind immune-complexed antigens, i.e. antigens bound by antigen-specific antibodies, to form antigen-antibody complexes. Most likely, the binding of antigen by IgG induces a conformational change in the IgG’s Fc-tail, exposing epitopes in the tail for recognition by RFs.17,18 In doing so, RFs can drive the formation of larger immune complexes, and potentially enhance and exacerbate inflammatory responses through additional recruitment of Fc-receptor- and complement system-mediated effector mechanisms. RFs may contribute to the pathogenesis of RA via this mechanism,19,20 although formal proof is still lacking.

The molecular identity of the antigens recognized by ACPA was defined 20 years ago. In those early studies, the antigens recognized by the RA-specific anti-keratine antibodies or anti-perinuclear factors were shown to contain citrulline, an amino acid formed by the post-translational modification (PTM) of arginine.21 ACPA display a higher specificity for RA than do RFs and can be identified by commercially available tests using cyclic citrullinated peptides (CCP) or a model protein antigen, mutated citrullinated vimentin (MCV).22 While the value of ACPA as biomarker in RA is undisputed, its possible contribution to disease pathogenesis remains a topic of intensive ongoing investigation and debate.23,24 Nonetheless, although the underlying mechanism is unclear, several lines of evidence support the notion that either ACPA and/or the underlying B- and T-cell responses directly contribute to the pathogenesis of ACPA-positive RA25. For example, the most prominent genetic risk factor for RA, encoded by the HLA-region, specifically predisposes to ACPA-positive disease but not to ACPA-negative or “RF-only positive” disease.11 Likewise, one of the RA-susceptibility loci identified by genome-wide association studies in the last decade encodes for peptidylarginine-deiminase, the enzyme responsible for the post-translational conversion of arginine into citrulline.26 Although, a highly useful diagnostic and prognostic tool, the role of ACPA in the pathogenesis of articular and extra-articular features of RA need further clarification.25 Nonetheless, the specificity of the ACPA-response for RA, combined with the observations that both the genetic region encoding an enzyme responsible for the generation of antigens recognized by these antibodies and also the HLA-region, specifically predisposes to ACPA-positive disease, make it plausible that citrulline-reactive immune responses (antibodies, T-cells and/or B-cells) are involved in disease pathogenesis. Indeed, it has recently been shown that in patients with RA, B cells directed against citrullinated proteins display a more activated and proliferative phenotype27 than B cells against other antigens such as e.g. tetanus toxoid. Moreover, these citrulline reactive B cells secrete increased amounts of IL-8, an important chemoattractant for neutrophils, the most abundant cell type in RA synovial fluid. Thus, since the discovery of citrulline as antigenic target for ACPA, several lines of evidence support the notion that citrullinated protein-reactive immune responses contribute to (the onset of) the signs and symptoms of RA, although further experimental support is still needed to define its precise contribution.11,28,29

**The evolution of the RA-associated autoimmune response.**

The characterisation of the auto-antibody responses that define seropositive RA also provided fresh impetus for studies unravelling the induction and evolution of these auto-immune responses. It is now clear that both the RF- and ACPA-response can be present years before subjects develop RA.30-32 Furthermore, both RF and ACPA can also be present in healthy individuals who, even after several years of follow up, do not progress to RA. In a substantial proportion of these individuals, the auto-antibody response stays at relatively low level, or can even disappear over time.33 The latter observation is interesting; whilst the factors that influence the longevity of this ACPA response remain undefined, disappearance of the response raises the possibility that these autoantibodies are part of conventional immune responses against microbes encountered by the immune system.

In contrast, the ACPA- and RF-response does not disappear in subjects who transition to RA. Indeed, several lines of evidence indicate that the auto-immune response undergoes an expansion before the development of RA. For example, isotype-usage, autoantibody-levels and the citrullinated epitope recognition profile of the ACPA-response all increase before onset of RA.34-36 Nonetheless, although it is generally accepted that the broadening of the auto-immune response takes place before the onset of RA, the pre-RA phases in which the expansion of auto-immunity occurs are less well defined. In some individuals, high levels of ACPA can be found years before disease-onset in the absence of apparent musculoskeletal symptoms, whereas in others a broadening of auto-immune responses is found relatively close to the time of joint swelling.30,31,33 Intriguingly, a rather small increase in the avidity of the ACPA-response is observed over time, indicating that isotype-switching and avidity maturation are uncoupled in this auto-immune response.37 Instead, it has been proposed that another feature of ACPA, the acquisition of N-linked glycans in the variable domain, is involved in the maturation and expansion of the ACPA-response.29,38,39 Such glycans are absent from most antibodies, but are found on approximately 90% of ACPA molecules from RA patients.40 The abundance of variable domain glycans on ACPA is a unique feature of the RA-specific ACPA-response. In contrast, however, in ACPA-positive healthy individuals who do not transition to RA, no such abundance of glycans is found, suggesting that the presence of these glycans could distinguish an “unhealthy” ACPA-response from a “healthy” one. Indeed, in a cohort of first degree individuals (FDRs) from RA-patients, it was observed that FDRs who later developed RA showed extensive variable domain glycosylation before the onset of arthritis and that IgG ACPA variable domain glycosylation was strongly associated with future development of RA.39 As the acquisition of variable domain glycans results from a selective introduction of N-linked glycosylation-sites by somatic hypermutation, it is tempting to speculate that the acquisition of variable domain-glycans by ACPA-producing B-cells allows the B-cell response to expand, thereby contributing the development of clinically manifest RA. In this respect, it is noteworthy that ACPA-IgG variable domain glycosylation increases closer to symptom onset and associates with anti-CCP2 antibody levels pre-disease, but not after disease onset, in line with the notion that these glycans facilitate the expansion of the ACPA-response.38 Nonetheless, recent data obtained by analysing pre-RA samples also indicate that ACPA variable domain glycosylation can take place years before RA-development, suggesting that additional immunological biomarkers are needed to describe the pre-RA-phases characterised by musculoskeletal symptoms and then by arthritis not yet fulfilling criteria for RA.38,39

The progress made in understanding the ACPA-response in the last two decades has also led to the realization that the ACPA-response is diverse and targets a plethora of citrullinated antigens. The latter is explained by the observation that ACPA are cross-reactive to many different citrullinated proteins, of self- and non-self-origins, both at the polyclonal as well as the monoclonal level.41-43. These findings are important for approaches aiming to silence, or “tolerize”, citrullinated protein-directed immune responses as it is challenging to define the antigen(s) responsible for inducing, sustaining or propagating anti-citrullinated protein immune responses. This is likely to be even more the case in the context of the T-cell response underlying the citrullinated protein-directed B-cell response, as this T-cell response does not have to recognize citrullinated (self)epitopes. For example, because ACPA-expressing B-cells can recognize multiple citrullinated antigens, it is likely that they are cross-reactive to both citrullinated self- and microbe-derived proteins. Consequently, an ACPA-expressing B-cell could attract T-helper cell-activity, required for its growth and differentiation, by recruiting a T-cell response directed against a microbe-derived antigen that has been citrullinated by peptidyl arginine deiminases released from netosing neutrophils attacking the microbe. In this scenario, the ACPA-expressing B cell recognizing the citrullinated foreign protein could present a non-citrullinated epitope from this foreign protein to responding T-cells. Hence, the autoreactive B-cell response does not have to be supported by an autoreactive T-cell reaction.

More recently, it has been shown that ACPA not only recognize citrullinated antigens, but also interact with carbamylated and acetylated proteins, adding another dimension to the complexity of the antigens targeted by the RA-specific auto-immune response.44-46 The finding that the pool of possible candidate T-cell antigens amenable for targeting is large, diverse, and differs between individuals, presents a challenge for those developing strategies to silence the T-cell response underlying RA in an antigen-specific manner.

**What drives the development of autoimmunity in RA?**

The initial antigenic drivers of the induction of auto-immune responses are, in general, unknown. Nonetheless, it is widely held that microbial components in mucosal tissues may be important drivers that could activate autoreactive B cells.47-50 In RA, it has been shown that ACPA can react towards both modified self- and non-self-antigens.51 As many autoreactive B cell responses are isotype-switched and are found long before disease onset, these B cells must have received T-cell help – potentially from microbe-specific T-cells. Indeed, it has been shown that ACPA-producing B cells can receive T-cell help from microbe-directed T-cells, a phenomenon that has also been observed for other auto-antibody systems in other autoimmune diseases.49,52-54 As most microbe-directed T-cell responses are broad and present in all healthy individuals, it is unlikely that this T-cell response is restricted by only a few HLA-alleles. Indeed, the ACPA IgG-response as found in unaffected healthy individuals does not appear to associate with the presence of the specific HLA-alleles predisposing to RA, pointing to the presence of extensive T-cell responses restricted by many different HLA-molecules.55,56 Therefore, it is likely that the B cells fuelling the initial ACPA-response receive helper activity from T-cell responses that are not restricted to the predisposing HLA-molecules, but to other HLA-molecules as well. Instead, other T-cells, restricted to the HLA-molecules predisposing to RA, are likely involved in the subsequent expansion of the initial ACPA-response occurring before disease onset. This notion is based upon the findings that, in contract to ACPA-positivity in unaffected subjects, ACPA-positive RA is hallmarked by a clear association with defined HLA-molecules.11,55,56 Thus, current evidence indicates that different T-cell responses underlie the initial induction and the expansion of the ACPA-response. At present, the identity of the antigens recognized by the T-cells responsible for either the initial induction or the “second expansion” of the ACPA-response is unknown and could, potentially, be of (modified) self- and non-self-origin.

Similarly, the induction of RF-specific immune responses has been postulated to involve T-cells which recognise microbial antigens.57 Many microbes are recognized by conventional antibodies and the interaction can lead to microbe-IgG immune complexes. RF-expressing B-cells could recognize such immune complexes. This could not only lead to the direct activation of these B-cells by toll-like receptors,58 but also to the concurrent recruitment of microbe-directed T-cell help for their further maturation and expansion. Where these B-cells or the RFs they produce interact with ACPA complexed with citrullinated self-antigens, they could further contribute to the inflammatory response for example in the inflamed synovium. Thus, although still under debate, increasing evidence indicates that components of microbiome, either in situ or after escaping mucosal barriers,59 play a pivotal role in driving the development and/or exacerbation of autoimmunity.

**Transitions from health to RA: clinical windows for preventive intervention**

During the transition from health to seropositive RA, the focus of this review, a number of ‘pre-RA’ phases can be identified.60 Individuals, typically with genetic and environmental risk factors for RA, may develop RA related autoantibodies whilst remaining entirely asymptomatic. Subsequently, an autoantibody positive asymptomatic subject may develop musculoskeletal symptoms suggestive of underlying joint inflammation (e.g. joint pain and morning stiffness) but in the absence of clinically apparent joint swelling; such an individual can be identified as having Clinically Suspect Arthralgia (CSA) with autoantibody positivity.61 Such patients may then develop clinically apparent synovial swelling not fulfilling classification criteria for RA (i.e. an unclassified arthritis (UA)) and then eventually progress to RA. However, not all patients who develop RA progress through these phases in this way. For example, some patients’ initial symptom onset may manifest as UA without a preceding CSA phase and for others it may manifest initially as RA without a preceding UA phase. Likewise, not all seropositive individuals (either asymptomatic or with CSA or UA) will develop RA. Nonetheless, in those individuals in whom they occur, the clinically apparent ‘pre-RA’ phases represent important windows in which therapeutic intervention can be applied to limit the rate of progression to RA.62 At present, several proof-of-concept trials in pre-arthritis (preventive trials) are ongoing to investigate whether onset of RA can be prevented in subjects at risk that show first joint symptoms.63 The design of such interventions should be informed by an understanding of the evolution of the RA associated autoimmune response from the asymptomatic state through to, where relevant, the development of CSA, UA and the eventually RA. Such an understanding might be used to develop actionable biomarkers and also to design (patient-tailored) interventions aiming to specifically target and halt the disease-associated autoimmune response.

**Predicting transition to RA**

In addition to understanding the development and evolution of the autoimmune reaction, it is crucial to develop accurate prediction models in the auto-immune disease field. Without such models, prevention will be difficult to achieve as prediction is vital to identify relevant target populations for prevention approaches. This is not only important for willingness of individuals at risk to accept medications and/or life-style changes,64,65 but also for the design of trials to assess the effectiveness of interventions. Prediction models including various combinations of clinical and serological biomarkers, have been developed66,67 and perform reasonably well, especially in patients with early joint complaints. Refinement of these models is ongoing and the addition of imaging related variables, together with other biomarkers, may improve performance.68-70 As mentioned, several clinical trials are underway aiming to prevent development of chronic arthritis in subjects at risk.71-75 The outcomes of these trials are expected over the next five years and are likely to offer new insights into the design of further interventions in the pre-RA phases aiming to silence the autoimmune response in the long term.

**Is there tolerance in the pre-RA-state and could tolerance be induced?**

As indicated above, the antigens recognized by T-cells involved in the auto-immune response underlying RA have not been well defined. As it is possible that these antigens are different in different patients and can fluctuate over time within a patient during disease as well as in different pre-RA-phases, it might prove very challenging to design T-cell targeted antigen-specific approaches to silence inflammation in RA. Nonetheless, the induction of regulatory T cells able to inhibit the inflammation in patients with autoimmune disease is appealing as these cells might create a milieu that more widely suppresses immune responses locally.76 Historically, several approaches aiming to induce/modulate T-cell mediated immune responses against heat shock proteins (HSPs) have been assessed. HSPs are intracellular proteins involved in the stabilization and (re)folding of proteins, both newly synthesized and those damaged during cellular stress. As cellular stress is a characteristic feature of inflammation, these proteins are overexpressed in inflamed tissues. Likewise, as these proteins are highly conserved across species and also expressed by bacteria, many HSPs have been reported to be recognized by T-cells.77 On the basis of preclinical animals studies suggesting the possibility to induce immune-regulatory circuits able to inhibit autoimmune diseases, several phase I/II studies have been initiated using HSP-derived peptides to control disease activity in diabetes type I and RA.78-81 In these studies, no serious adverse effects were noted, indicating that the interventions were safe. Although signals pointing to immune deviation were described, the efficacy of HSP-directed immune interventions remains to be determined also because initial promising results of a large phase III study in diabetes have been retracted,80 whereas primary clinical endpoints in RA were not met.78

Like the induction of antigen-specific immune deviation by vaccination with HSP-derived agents, also the (permanent) induction or maintenance of T cell tolerance in the (pre-)RA state might represent an attractive option to dampen disease-activity. Current data suggest that in the pre-RA state, T-cell tolerance to the antigens recognized by autoreactive B cells in RA, is likely not present as e.g. isotype-switched and somatically mutated ACPA derived from “helped” B-cells can appear years before disease-onset.38 Therefore, it might prove difficult to design ways to “maintain” a tolerant state before disease onset as this state might have disappeared long before. Also the control of disease activity by *de* novo induction of antigen-specific “tolerance” might be very challenging because it is not known which antigens are involved in disease pathogenesis and/or are relevant for the induction of tolerance. Likewise, the optimal “platforms” allowing the induction of antigen-specific tolerance in humans are, at present, not defined. For example, cellular interventions aiming to induce regulatory circuits mediated by regulatory T cells, such as e.g. the use of (auto)antigen-loaded tolerogenic dendritic cells, should be tailored to (the HLA of) individual patients, making both clinical trials to assess efficacy and subsequent implementation in clinical practice challenging. Likewise, the introduction of Chimerice Antigen-Receptors recognizing auto-antigens in regulatory T cells by gene-transfer would need to be performed in a patient-specific manner. In this context, new approaches are being developed making use of the introduction of chimeric autoantigen-receptors (CAAR) into T cells to target autoreactive B cells.82-84 Although not aiming to induce tolerance, preclinical studies have set the stage for further clinical development of these precision therapies to target pathogenic B cells in B cell mediated autoimmune diseases. Although also this requires a patient-tailored approach, it is not dependent on the processing and HLA-restricted presentation of auto-antigens at the inflamed site.

Given that antigen-specific interventions targeting regulatory circuits are challenging in RA due to the nature of the disease, several studies are currently ongoing to reset the immune system by other means. As mentioned, the citrullinated protein-directed immune response is dynamic and continuously active, and therefore approaches to modulate the activity of those antigen-presenting cells required to induce and steer T-cell responses, might prove rewarding. An advantage of a more general targeting of regulatory pathways, for example by in vivo modulation of dendritic cells, is that the identity of the antigens presented by the body to sustain and expand the autoimmune response can remain unknown. Approaches focussing on antigen-presenting cells could, for example, entail targeting of immunosuppressive agents by dendritic cell-directed liposomes or nanobodies.85,86 Indeed, in the field of tumor-immunology several approaches to deliver therapeutics specifically to dendritic cells are under development.87,88 Likewise, such methods are being explored in autoimmune diseases. For example, calcitriol-containing liposomes have been shown to modulate human dendritic cells phenotype and function and could, potentially, be used to regulate/dampen ongoing auto-reactive T-cell responses that undergo recurrent activation by dendritic cells.89 Whether continual reactivation of disease-contributing T-cell responses is occurring in established RA needs to be established, but current evidence indicates that this is possible. Similarly, such information is currently not available for pre-RA-phases and needs to be obtained. However, given the expansion and maturation of the auto-antibody response before the onset of arthritis, it is likely that in this phase, RA-related T- and B-cell responses are active and expanding. For this reason this might be a preferred phase for interventions aiming to tolerize and/or silence RA-related auto-immune responses using antigen-independent methodologies, which could be supplemented by antigen-specific approaches when such antigens become available. Such interventions could aim to specifically inhibit ongoing T-cell responses by pharmacological modulation of dendritic cells, but might also entail other approaches such as targeting T-cells by anti-CD3 antibodies.5 Alternatively, regulatory T cell populations might be expanded in patients by the use of low-dose interleukin-2. Several clinical studies indicate that the dose and treatment regimens to expand regulatory T cells by low dose interleukin-2 are safe in patients with RA and other rheumatic diseases, allowing the initiation of larger studies to test clinical efficacy.90-92 Likewise, other cells involved in disease pathogenesis could be targeted in a specific manner including B-cells and potentially even fibroblast-like synoviocytes that are thought to provide the “niche” in which the deranged auto-immune response is embedded.93,94

Another appealing approach to modulate the RA-associated immune response, because of simplicity and safety, might be dietary modification to modulate the microbiome. Multiple species present in the gut-microbiome can, for example, produce short-chain fatty acids such as butyrate that possess immunomodulatory activity. Such short chain fatty acids have been shown to modulate T-cell differentiation, cytokine-production and the promotion of peripheral regulatory T-cell generation.95,96 As the production of such metabolites by gut bacteria could, potentially, be modulated by certain dietary interventions,97 it would be attractive to investigate their impact on the RA-associated autoimmune response and on clinical features in different “pre-RA” phases.98,99

**Concluding remarks**

Despite considerable success in treating signs and symptoms of disease, RA, like most other human autoimmune diseases, remains incurable. Cure and/or prevention remain tantalizing goals. In this overview, as part of a Series on tolerance induction, we have summarized the different phases leading up to the development of RA discussing how autoimmunity could be induced and how the evolution of the autoimmune response could relate to the development of symptoms and joint swelling. Key future goals in the management of RA are the ability to induce long-lasting drug-free remission in patients who have developed RA (i.e. to achieve a cure) as well as to prevent disease before it emerges in the first place. This could, potentially, be achieved by silencing/tolerizing the autoimmune response underlying RA. Although the road ahead appears long and challenging, the unprecedented progress made in our understanding of the clinical- and immunological stages preceding disease-onset, combined with the first successes with tolerizing interventions in other autoimmune diseases, offers great hope for therapeutic approaches aimed at silencing the underlying auto-immune response.

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**Search strategy and selection criteria.**

We conducted an electronic search of Pubmed and web of science for autoantibodies and autoantibody specificities in RA, phases of RA development and tolerance induction. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant/first showed a certain finding. The search was limited to human and English language. The reference cited were chosen on the basis of their relevance to the contents of this review.

**Legends**

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Figure 1: Graphical depiction of the evolution of the autoimmune response in RA related to progression from one clinical phase to another. The extent to which this evolution is gradual or stepwise remains unclear; dissecting this will require the longitudinal and frequent collection of biological material in pre-RA cohorts in which clinical characteristics are carefully defined at the time of sample collection.



Figure 2: Graphical depiction of how the anti-citrullinated protein antibody (ACPA) immune response is hypothesized to emerge and progress in time till development of rheumatoid arthritis (RA).

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