Commentary

Potential Pharmacologic Targets for the Prevention of Rheumatoid Arthritis

John D. Isaacs, BSc [Hons], MBBS, PhD[1,2]; and
Kundan Iqbal, MRes, MBBS[1,2]

[1]*Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, United Kingdom; and* [2]*Musculoskeletal Unit, Newcastle Upon Tyne Hospitals National Health Service (NHS) Foundation Trust, Newcastle Upon Tyne, United Kingdom*

ABSTRACT

Rheumatoid arthritis (RA) immunopathology starts many years before clinical disease manifests. An early event is the breakdown in B-cell tolerance and the emergence of autoantibodies. Some years later, the autoantibody response matures, with epitope spreading and isotype switching suggesting more focused T-cell involvement. Circulating proinflammatory cytokines and chemokines appear concurrently, marking a phase of preclinical inflammation. Eventually individuals develop musculoskeletal symptoms and fatigue, heralding the imminent onset of synovitis. The prevention of RA can be aligned to these disease stages, which may simplistically be viewed as breach of tolerance, maturation of autoimmunity, and subclinical inflammation. At each stage, an "ethical" balance must be struck between the potential for benefit versus harm, in large part determined by the likelihood of progression to RA. In particular, tolerogenic interventions should be favored during asymptomatic disease, providing long-lasting, possibly lifelong, benefit from a short-term intervention. During the breach-of-tolerance phase, tolerogenic interventions might involve the administration of autoantigenic peptides in an attempt to shut down autoreactive B cells. The peptides may be "naked," encapsulated in nanoparticles or loaded into autologous tolerogenic dendritic cells. It may also be possible to interfere with antigen generation and presentation, such as via peptidyl arginine deiminase inhibition. Attempts to interfere with B cells via depletion, differentiation, or intracellular signaling are also logical at this stage, if deemed sufficiently well tolerated. These interventions remain relevant during maturation of autoimmunity, but targeted T-cell interventions also become relevant, such as co-stimulation blockade and, potentially, interventions that target intracellular signaling pathways within T cells. Targeting of interleukin 23 during this phase may interfere with maturation of the autoantibody response by modulating autoantibody glycosylation, as well as by inhibiting T-helper 17 differentiation. During the phase of subclinical inflammation, targeting cytokines and chemokines could conceivably prevent progression to RA. Furthermore, tolerance induction is opposed by systemic inflammation and, during this phase, it may be necessary to consider therapeutic combinations of tolerogenic and antiinflammatory interventions. (*Clin Ther*. 2019;41:1312–1322) © 2019 Published by Elsevier Inc.

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INTRODUCTION

There is a significant preclinical prodrome to rheumatoid arthritis (RA). In brief, 5 phases precede the onset of clinical synovitis.[1,2] In the first 2 phases, asymptomatic individuals carry predisposing genetic risk factors and/or are exposed to environmental risk factors that increase the risk for future RA. The third phase represents the breakdown of immune tolerance

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and the development of systemic autoimmunity, marked by the appearance of autoantibodies such as rheumatoid factor and anti-citrullinated protein antibody (ACPA). Symptoms develop in phase 4, although without clinical arthritis, and phase 5 is unclassified arthritis. Individuals in phase 4 may have so-called 'clinically suspect arthralgia', with a high probability of progression to RA, and imaging may reveal synovial thickening, increased synovial vascularity, and bone marrow edema.[3] Prior to the development of classifiable RA, the autoimmune response matures, with diversification of specificities and isotype switching of autoantibodies.

Attention to lifestyle may reduce the risk for progression to RA in healthy individuals with genetic or environmental risk factors, as may the targeting of mucosal surfaces in an attempt to intercept potential triggers of tolerance breakdown.[4,5] This commentary, however, focuses specifically on pharmacologic interventions for the possible prevention of RA, as illustrated in the Figure. The main targets are the cells and products of the innate and acquired immune response. In terms of the strategies that could be adopted, these will depend on the phase of preclinical disease, including the presence or absence of symptoms and, critically, the risk for progression to clinical RA. The lower the likelihood of progression, the greater the emphasis on safety, particularly in the absence of symptoms.[6] The Table classifies possible interventions in terms of theoretical risk. The most well tolerated are likely to be those that are antigen specific, providing highly targeted interventions focused on the autoreactive immune response; autologous adoptive cellular therapies should also prove well tolerated, as a boost to normal immune homeostasis. Some adoptive cellular therapies can be rendered antigen specific, combining both of these properties.

PREVENTING AUTOANTIGEN GENERATION

Major targets of the autoimmune response in RA are citrullinated proteins, such as citrullinated fibrinogen, enolase, vimentin, and histones. Although the mechanism underpinning citrulline autoreactivity in RA is not known for certain, disruption of the process could prevent disease development. On the other hand, citrullination (the deimination of arginine to citrulline) can be both physiologic and pathologic, and it is possible that citrullination in RA is a consequence of inflammation rather than an underlying cause.[7] The enzymes that are involved in citrullination in RA are the peptidyl arginine deiminases (PADs). *Pad4* knockout mice have a reduced severity of collagen-induced arthritis, although the disease is not prevented altogether.[8] Furthermore, while similarly reducing the severity of collagen-induced arthritis, Pad4-specific inhibitors did not reduce joint citrullination or the quantity of ACPA produced. However, there was a modest reduction in epitope spreading, as well as an apparent deviation of the autoimmune response to a less pathogenic T-helper (TH)-2 type.[9,10] While these experiments do not provide conclusive evidence of the value of targeting citrullination, collagen-induced arthritis is a response to immunization with collagen. ACPAs can be detected in this model, but they are not the "driving" antigen, and their pathogenic role has not been defined. Additionally, PAD4 is not the only relevant enzyme, with PAD2 also expressed in RA synovium.[7] Furthermore, citrullinated peptides are not the only autoantigenic specificities in RA, with emerging evidence of autoreactivity against a range of other post-translationally modified targets, such as carbamylated and acetylated peptides.[11] Consequently, even if effective, targeting ACPA production alone may not be sufficient for preventing disease onset. Nonetheless, PAD inhibitors are being developed for assessment in clinical trials.[12]

PEPTIDE THERAPY

Antigen-specific tolerance can be induced by the administration of peptides derived from the autoantigen. The underlying principle is that, by delivering peptide to the immune system in a "nonimmune" or "hypoimmune" form, autoreactive T cells are deleted, anergized, or converted into regulators. A number of strategies are currently being investigated. For example, "naked" peptides can be administered subcutaneously or intradermally. Proof of concept for this approach in type 1 diabetes and multiple sclerosis was recently generated.[13,14] In type 1 diabetes, intradermal injection of a single proinsulin peptide administered over the course of 6 months was demonstrably well tolerated. Furthermore, while that study was not powered for efficacy, patients receiving peptide required less insulin at the end of the study than did placebo recipients, and C-peptide levels remained stable compared to a fall with placebo. Responders to peptide therapy demonstrated higher levels of

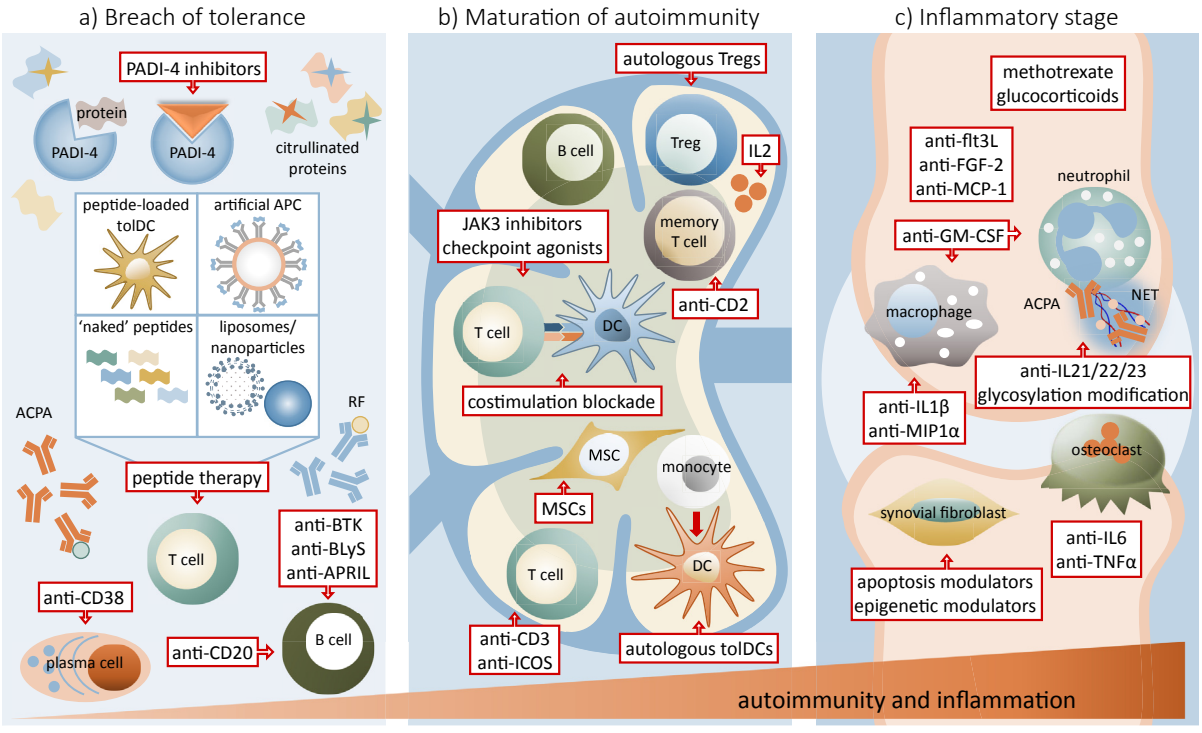

Figure. Potential pharmacologic interventions for the possible prevention of rheumatoid arthritis (RA). Preclinical RA can be considered in distinct stages: breach of tolerance, maturation of autoimmunity, and subclinical inflammation. For illustrative purposes, therapies have been targeted at a specific stage, although several could be utilized more widely. Breach of tolerance results in the emergence of autoantibodies. Autoreactive B cells could be indirectly targeted with autoantigenic peptides—either "naked," loaded into tolerogenic cells, or associated with artificial particles. Less specific therapies could inhibit B-cell differentiation, deplete B cells, or interfere with signaling pathways. In contrast, peptidyl arginine deiminase (PADI)-4 inhibition might interfere with antigen generation. It is assumed that T cells are particularly important during maturation of the autoantibody response, when epitope spreading and isotype switching occur. Targeted T-cell interventions such as co-stimulation blockade, immune checkpoint agonists, and signaling inhibitors might then be considered. Cell therapies such as tolerogenic dendritic cells (DCs), regulatory T cells, and mesenchymal stem cells could be used to reinforce endogenous tolerance pathways, and peptide therapies remain relevant. The emergence of subclinical inflammation may inhibit tolerance induction. Methotrexate, via adenosine upregulation, may provide useful background for tolerogenic therapies during this phase. Anti—granulocyte macrophage colony-stimulating factor (GM-CSF) should interfere with macrophage and neutrophil activation and maturation, with reduced neutrophil extracellular traps (NETs) and citrullinated histone presentation. Blocking soluble mediators of inflammation and leukocyte trafficking and activation could also interfere with the late preclinical phase of RA. Targeting the enzymes that modify anti-citrullinated protein antibody (ACPA) glycosylation, either directly or via the interleukin (IL)-23—T-helper 17 axis, may reduce autoantibody pathogenicity. APC = antigen presenting cell; BLyS = B-lymphocyte stimulator; BTK = Bruton tyrosine kinase; FGF = fibroblast growth factor; Flt = fms-like tyrosine kinase receptor; ICOS = inducible T-cell co-stimulator; JAK = janus tyrosine kinase; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; MSC = mesenchymal stem cells; RF = rheumatoid factor; TNF = tumor necrosis factor; tolDC = tolerogenic dendritic cell; Tregs = regulatory T cells.

Table. Potential therapeutic targets for the prevention of rheumatoid arthritis.*

Modality	Comments
Peptide-based tolerogenic strategies, including naked peptides, autologous tolerogenic APCs, and nanoparticles coated with or containing peptides	If effective, there should be minimal dysregulation of the healthy immune response; main theoretical risk is unintended stimulation of autoreactivity
Expanded autologous Tregs	Autoantigen specific would be preferable to polyclonal, particularly from a safety perspective; a theoretical risk is plasticity, and differentiation into pathogenic effector cells
Low-dose IL-2 to expand Tregs	Potential to also expand pathogenic effector T cells and NK cells; pharmacokinetics likely to be complex
Co-stimulation blockade and other nonspecific T-cell therapies, including CTLA4-Ig, anti-CD3, anti-CD28, anti-CD2, anti-CD40L, anti-ICOS, JAK1−3 and TYK2 inhibition, anti−IL-12 and anti−IL-23, PD-1 agonist, and TIGIT agonist	Short courses of therapy may be acceptable, particularly if nondepleting; main risk is likely to be enhanced infectious susceptibility for the duration of treatment
B-cell and plasma cell therapies, including anti-CD20, anti-BLyS, BTK inhibitor, and anti-CD38	Comments as for T cells, although rituximab is depleting; targeting of plasma cells, particularly with a depleting agent, is more likely to reduce existing vaccine responses (eg, tetanus)
Mesenchymal stem cells	Allogeneic product; complex biology that is not fully understood
Interference with ACPA glycosylation, anti−IL-23, anti−IL-21, anti−IL-22, and β-galactoside α2,6-sialyltransferase 1	Potentially effective at a late preclinical stage of RA development; risk−benefit may depend on the need for long-term treatment; the consequences of altering glycosylation status on the healthy serologic immune response is not adequately investigated
Targeting inflammation, such as antibodies against IL-1β, IL-6, GM-CSF, TNF, MCP-1, MIP-1α, FGF-2, Flt3L	Targeting inflammation is well established (TNF, IL6R, IL1β blockade) but is associated with infectious morbidity and other adverse effects; therefore, unless tolerogenic and effective over short course(s), treatment would likely be reserved for late preclinical disease with a high likelihood of progression to RA
PAD inhibitors	May not "switch off" all autoreactivity; could also interfere with physiologic citrullination; animal studies suggest mode of action not fully understood

ACPAs = anti-citrullinated protein antibodies; APC = Antigen presenting celli; BLyS = B-lymphocyte stimulator; BTK = Bruton tyrosine kinase; CSF = colony-stimulating factor; CTL = cytotoxic T lymphocyte; FGF = fibroblast growth factor; Flt = fms-like tyrosine kinase receptor; GM-CSF = granulocyte macrophage colony-stimulating factor; ICOS = inducible T-cell co-stimulator; Ig = immunoglobulin; IL = interleukin; JAK = janus tyrosine kinase; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; NK = natural killer; PAD = peptidyl arginine deiminase; PD = programmed cell death protein; RA = rheumatoid arthritis; TIGIT = T-cell immunoreceptor with immunoglobulin and ITIM (immunoreceptor tyrosine-based inhibition motif) domains; TNF = tumor necrosis factor; Tregs = regulatory T cells; TYK = tyrosine kinase.
*The categories are listed in a notional ranking of tolerability (safest at the top). Notional tolerability is based on likely specificity for autoreactivity (with minimal nonspecific immunomodulatory effects), whether the therapy has the potential to be tolerogenic, and a rational and supported mode of action. Therapies toward the top are more likely to be acceptable in asymptomatic disease when the likelihood of progression to RA may be low. In contrast, those toward the bottom are more likely to be reserved for situations in which the likelihood of progression to RA is high (eg, seropositive arthralgia). The order is relative and categories themselves may be subdivided (eg, lymphocytotoxic therapies are likely to be deemed less safe than nondepleting lymphocyte-targeting therapies).

interleukin (IL)-10 release from peripheral blood mononuclear cells stimulated with peptide *ex vivo,* and higher levels of forkhead box P3 (FoxP3) on circulating regulatory T cells (Tregs). In multiple sclerosis, a mixture of 4 peptides derived from myelin basic protein, administered over 20 weeks, was associated with a sustained reduction in gadolinium-enhancing lesions on brain MR imaging, a biomarker of disease activity.

Although peptide immunotherapy primarily induces tolerance to the administered peptide, the phenomena of linked suppression and infectious tolerance facilitate the spread of tolerance to other peptides presented by the same antigen-presenting cell, even if these peptides derive from distinct autoantigenic proteins. These phenomena may be associated with IL-10–producing T cells. Furthermore, in very early and preclinical disease, the autoimmune response is less diverse than in later disease as there has been less opportunity for epitope spreading to occur. In terms of tolerability, peptide therapy could theoretically boost an existing autoimmune response, for example if administered in the presence of immune system danger signals. In this regard, the administration of altered peptide ligand derived from myelin basic protein to patients with multiple sclerosis, designed to anergize autoreactive T cells, was associated with disease exacerbations in a small number of recipients with *ex vivo* evidence of stimulation of disease-associated T cells.[15]

There are a number of other ways by which peptides can be delivered in order to tolerize the autoimmune response. Published methods include: intravenous delivery of nanoparticles coated with disease-relevant peptide–major histocompatibility complex (MHC)-II complexes, which generate Il-10–secreting T cells and regulatory B cells in mice; intravenous or subcutaneous injection of nanoparticles containing peptide (or protein) antigens and rapamycin; synthetic microparticles bearing autoantigenic peptide; and linkage of antigenic peptide to the erythrocyte membrane (so that the linked peptide is cleared tolerogenically when the erythrocyte reaches the end of its lifespan and undergoes apoptosis).[16–19]

Which, if any, of these strategies will provide potent tolerance induction is currently unknown. One other caveat of the use of peptides is that therapy would need to be stratified to patients carrying the relevant MHCII for peptide presentation. Further stratification may require screening for relevant autoantibody specificities or autoreactive T cells. In some ways, such screening would provide an ideal "stratified" therapy although, in this circumstance, several distinct therapies for each disease would need to be generated. Alternatively, a therapy could contain a diverse range of peptides, suitable for patients bearing a range of MHCII types and autoreactivities. In this context, the autoantigen repertoire is considerably more diverse in RA than in, for example, multiple sclerosis or diabetes, and targeting citrullination (or other post-translational modifications) is likely to require a cocktail of peptides even in patients of a single MHC type.

CELLULAR THERAPIES

The other manner by which peptides can be presented in a tolerogenic fashion is by the use of tolerogenic antigen-presenting cells. Tolerogenic dendritic cells (tolDCs) are relatively straightforward to generate, usually from peripheral blood monocytes. Two Phase I studies in RA have been reported. In one of these, the tolDCs, generated by differentiating peripheral blood monocytes into DCs in the presence of an inhibitor of nuclear factor κB, were loaded with a mixture of citrullinated peptides and injected intradermally. The therapy was well tolerated, and peripheral blood immune biomarkers provided an indication of possible immune modulation.[20] In the other study, tolDCs were again differentiated from peripheral blood monocytes, but this time using dexamethasone and vitamin D_3 as "tolerogenic" agents. They were loaded with autologous synovial fluid as a source of autoantigen and injected into an inflamed knee, in order to test stability in an inflamed environment. Again, therapy proved well tolerated, but systemic immune modulation could not be identified.[21] TolDCs have also been administered to patients with type 1 diabetes (no autoantigen, intradermal delivery) or Crohn disease (no autoantigen, intraperitoneal delivery). In both (uncontrolled) studies the therapy appeared safe and there was anecdotal efficacy. There are ongoing studies in multiple sclerosis.[22,23]

Other tolerogenic cell types include Tregs and mesenchymal stem cells (MSCs).[24] Expanded "natural" Tregs have been administered to patients with type 1 diabetes or systemic lupus erythematosus (SLE), and ovalbumin-specific IL-10 secreting type 1 Tregs (Tr1 cells) have been administered to patients

with Crohn disease.[25–28] In one of the diabetes studies, the Tregs were labeled with deuterium, enabling an estimation of their *in vivo* lifespan. Approximately 25% of the administered cells were detectable in the circulation after 12 months. Cells were similarly labeled in the SLE case report, and infused Tregs migrated to an area of skin inflammation. Although current Treg trials are using polyclonally expanded Tregs, there is the potential to render this therapy antigen specific. Antigen specificity could be achieved, for example, by expanding the cells using the relevant autoantigen, or by engineering an autoantigen-specific T-cell receptor onto the Tregs.[29,30] The main potential risk of Treg therapy is plasticity with differentiation into pathogenic cells, a risk that appears to be minimized by expanding $CD45RA^+$ (naïve) rather than $CD45RO^+$ (memory) Tregs.[31] Epigenetic analysis of expanded Tregs can help to predict stability, while a "suicide" gene could be co-inserted into genetically modified Tregs to reduce concerns.[32] When deuterium-labeled Tregs were administered to patients with diabetes, deuterium could not be detected in any other cell subsets up to a year after injection, suggesting stability *in vivo*.[26] The skin-migrating Tregs in the SLE case study expressed IL-17, however, although the presence of IL-17 did not appear to cause disease worsening.[27]

Whereas tolDCs and Tregs are usually autologous products derived from the individual to whom they will eventually be administered, MSCs are minimally immunogenic and therefore can be used autologously or allogeneically. They can therefore be cryopreserved as an "off-the-shelf" therapy, which perhaps explains the multiple human studies that have already been reported.[24] Furthermore, MSCs can be derived from umbilical cord blood, Wharton jelly, bone marrow, or adipose tissue. While MSCs appear to hold promise for the management of autoimmunity, their biology remains poorly understood compared with the biology of other cellular therapies. Furthermore, their allogeneic derivation, while convenient, could be perceived as enhancing risk in the setting of disease prevention. Genetically engineered, antigen-specific but MHC-nonrestricted, chimeric antigen receptor Tregs could also provide an "off-the-shelf," allogeneic product.[29]

Regulatory cells essentially reinforce endogenous tolerogenic mechanisms. Therefore, notwithstanding the aforementioned caveats, treatment is expected to be well tolerated even in asymptomatic individuals. Autologous therapies should be particularly safe because any risk for transmission of blood-borne agents from allogeneic products is absent. Furthermore, there is some evidence of defective immune cell function in some autoimmune diseases, particularly of Tregs.[33] Such defects may be reversed when Tregs are expanded *ex vivo*.[26] Tregs can also be expanded *in vivo* by the administration of repeated cycles of low-dose IL-2. While there is the potential to also expand effector T cells and natural killer cells, this therapy has provided beneficial effects in established vasculitis and SLE, and is potentially applicable to a diverse range of conditions.[34–36] Selecting the appropriate dose for each individual patient and condition may, however, prove challenging.[37]

TARGETING B AND T CELLS

It is unclear whether initial ACPA production is T-cell dependent. In the period leading up to clinical presentation, however, the ACPA response matures with diversification of specificities and isotype switching, highly suggestive of T-cell "help."[38] Nonetheless, this does not exclude T-cell involvement earlier in the disease process, when tolerization of autoreactive T cells could prevent subsequent ACPA maturation and its consequences, potentially including pain, osteoporosis, and synovitis.[39] As discussed earlier, peptide therapy would provide a relatively well-tolerated method of tolerizing rare autoreactive T cells, whereas other modalities are nonspecific and could have undesirable consequences such as infection. In this case, treatment would need to be reserved for later phases, when the probability of progression to RA is more certain. For example, co-stimulation blockade with abatacept has been targeted at patients with undifferentiated arthritis (the ADJUST trial; Abatacept study to Determine the effectiveness in preventing the development of rheumatoid arthritis in patients with Undifferentiated inflammatory arthritis and to evaluate Safety and Tolerability[40]), and a current trial is targeting seropositive arthralgia, when the risk for progression to RA is acceptably high (APIPPRA; Arthritis Prevention in the Pre-Clinical Phase of RA With Abatacept[41]). The ADJUST trial included some patients who would now be classified as having RA according to the American College of Rheumatology/

European League Against Rheumatism 2010 classification criteria, perhaps contributing to failure of the trial to meet its primary end point of reduced progression to RA.[42] Nonetheless, fewer patients developed RA within the first 12 months of therapy, and a small number of participants converted from ACPA positive to negative, possibly reflecting true immune modulation.[40]

Other potential T-cell targets include CD3, CD28, CD2 (relatively specific for memory T cells), CD40 ligand (CD40L), and inducible T-cell co-stimulator (ICOS).[43–45] IL-2, -4, -7, -15, and -21 are cytokines important for T-cell activation and homeostasis, and each signals to T cells via janus kinases (JAK)-1 and -3. Consequently, inhibitors of JAK1 and JAK3 may provide a further means of intercepting T-cell activation. Additionally, IL-12 and IL-23 signal via JAK2 and tyrosine kinase 2 (TYK2), which may also prove suitable targets for specific inhibition of TH1 and TH17 differentiation.[46] Anti–IL-12/-23 p40 or anti–IL-23 p19 monoclonal antibodies could similarly target pathogenic T-cell differentiation and stabilization.[45] Agonistic antibodies against immune checkpoint molecules such as programmed cell death protein 1 (PD1) and TIGIT (T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibition motif domains) could provide an alternative strategy to suppress T-cell activation, albeit again nonspecifically.

As with T cells, the potential benefits of targeting B cells must be balanced against potential risks. CD20, B-lymphocyte stimulator (BLyS), and targets on plasma cells such as CD38 each merit consideration. Bruton tyrosine kinase is essential for B-cell activation, and inhibition of this signaling molecule could provide an alternative approach.[47] The PRAIRI study (Prevention of clinically manifest rheumatoid arthritis by B-cell directed therapy in the earliest phase of the disease)[48] targeted B cells with rituximab in late preclinical RA with subclinical synovitis on imaging and an elevated C-reactive protein. In this scenario, the treatment may have delayed, but did not prevent, progression to RA.

The potential risks of B-cell and T-cell targeting would be somewhat mitigated if therapy were short-term and nondepleting. This may be the case with co-stimulation blockade, which, at least in theory, is tolerogenic. Along these lines, a 6-day course of nonactivating anti-CD3 provided long-term disease modulation in patients with very early type 1 diabetes.[43] While not yet proven to be tolerogenic in humans, short intermittent courses of such a therapy may be acceptable if they safely prevented progression to clinical autoimmunity. It may also be worth reconsidering other tolerogenic strategies, such as anti-CD4, for prevention. Although such therapies appeared ineffective in established RA, this finding may in part have reflected the lack of biomarkers with which to monitor treatment.[41] Furthermore, they could prove more effective in very early disease when inflammation is absent or minimal, and should be relatively well tolerated, particularly if nondepleting. Similarly, a short course of anti-CD20, administered before ACPA diversification (and therefore considerably earlier than in the PRAIRI study), could provide long-term benefit if ACPA production were limited to B cells at that disease stage.

TARGETING INFLAMMATION

Whether targeting pivotal cytokines could prevent progression to RA is uncertain, although it seems unlikely that targeting inflammation *per se* would have such a profound effect. Nonetheless, certain cytokines such as tumor necrosis factor and IL-6 also have important immunoregulatory functions. IL-6, for example, plays a central role in the balance between tolerance and immunity via effects on T cells, including TH17 differentiation, and on antigen-presenting cells.[49,50] Given the variety of relevant drugs licensed and in development, it could be of interest to explore their use during earlier phases of preclinical RA.

In the late preclinical phases of RA, a number of cytokines and chemokines appear in the circulation.[51] These include the "usual suspects" such as IL-1β, IL-6, granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor, monocyte chemoattractant protein 1 and macrophage inflammatory protein 1α, but also less commonly discussed factors such as fibroblast growth factor 2 and fms-related tyrosine kinase (FLT)-3 ligand.[51,52] Their source is unclear, but they are presumably involved in the migration and activation of leukocytes, and the development of inflammation that precedes the development of clinical synovitis. Consequently, blocking one or more of these soluble mediators, or indeed the chemokine receptors and adhesion molecules that are also required for

leukocyte trafficking, could provide a further strategy for disrupting progression to clinical RA. For example, one of the first cells to appear in the rheumatoid synovium is the macrophage, and targeting macrophage maturation and activation using an anti−GM-CSF pathway biologic is worthy of consideration.[53] By also interfering with neutrophil differentiation and activation (associated with neutrophil extracellular trap (NET) formation and citrullinated histone exposure), such an approach could also interfere with ACPA generation. As with B-cell and T-cell targeting, cytokine and chemokine inhibition will be immunosuppressive and would need to be considered carefully in this light, including whether the outcome is likely to be a short-term delay in progression versus disease prevention.

A potentially promising cytokine target is IL-23. Recently, the influence of variable region glycosylation was highlighted as a determinant of the proinflammatory activity of autoantibodies.[54] This process appears to be regulated by the IL-23−TH17 axis, involving IL-21 and IL-22, which themselves regulate the enzyme β-galactoside α2,6-sialyltransferase 1. A reduction in enzyme activity, and therefore immunoglobulin G (IgG) sialylation, accompanies RA clinical onset.[55] Therefore, in addition to inhibiting TH17 differentiation, targeting this pathway may provide a means of interrupting RA onset at a relatively late stage of preclinical disease via its effects on autoantibodies. The effects of such interventions on the healthy immune response await study, but they could theoretically impact the effectiveness of serologic responses against pathogens.

A small number of studies have used glucocorticoids or conventional synthetic disease-modifying antirheumatic drugs in the earliest stages of joint inflammation in attempts to prevent progression to RA. These include PROMPT (Probable Rheumatoid Arthritis: Methotrexate Versus Placebo Treatment),[56] which studied methotrexate in patients with "probable" RA according to the 1958 definition; STIVEA (Steroids in Very Early RA),[57] which examined intramuscular methylprednisolone (3 doses) in undifferentiated arthritis; and SAVE (Stop Arthritis Very Early),[58] which administered a single dose of methylprednisolone to patients in the earliest stages of inflammatory arthritis. PROMPT and STIVEA reported delayed progression to RA, the former particularly in seropositive patients and in those with a high likelihood of progression at baseline.[59] In contrast, SAVE did not demonstrate benefit. Similarly, 2 doses of dexamethasone in individuals with seropositive arthralgia failed to prevent progression to RA.[60] In mechanistic terms, methotrexate could have tolerogenic properties by potentiating the effects of adenosine.[61–63] In contrast, while there is no direct evidence, the myriad effects of glucocorticoids on immune cells could antagonize tolerance induction by interfering with protolerogenic pathways. These observations are also pertinent if contemplating tolerogenic interventions in the final stages of RA development. Inflammation drives immunity and, under these circumstance, it may be appropriate to administer an antiinflammatory therapy while attempting tolerance induction. A combination of tolerogenic therapy with methotrexate may be worth considering under these circumstances.

TARGETING THE FIBROBLAST

The advent of early arthritis clinics has made a huge difference in the outcomes of patients with RA. There appears to be a window of opportunity during which the institution of therapy has lasting effects on disease progression and joint damage. Patients treated early are more likely to enter disease remission, and to achieve drug-free remission, than those treated later. They are also less likely to require biologic therapies. Although the biology underpinning these observations has not been fully elucidated, epigenetic modification of the synovial fibroblast remains a favored mechanism.[64] This modification may occur secondary to persistent, clinical and subclinical, inflammation and results in oncogene upregulation, and a proliferative and invasive phenotype.[65] With current therapeutics it is not possible to reverse this phenotype, which may account for the poor prognosis of patients in whom the treatment of RA is delayed. In the future, however, drugs that modulate fibroblast apoptosis, and epigenetic modulators, may provide novel treatment strategies for RA, both for prevention and for treatment of the later stages.[66] If persistent and destructive RA can be converted to therapeutically responsive, nondamaging disease, such strategies should be considered alongside one another.

CONCLUSIONS

Given the unquestionable benefit of treating RA soon after symptom onset, there is enthusiasm among the

rheumatology community to treat even earlier, to interrupt progression from asymptomatic disease to synovitis. In this commentary we have debated the arguments for preventive interventions, administered from the very first signs of tolerance breakdown to late preclinical disease. The acceptability of such an approach critically depends on the balance of risks and benefits, tempered by the likelihood of progression to established RA. We have considered the various immunomodulatory approaches that might be used to prevent RA development, and attempted to "rank" them in terms of safety. For each treatment, we have notionally judged safety in terms of general versus autoimmunity-specific immune modulation, tolerogenic capacity, a clearly defined mechanism(s) of action, and reversibility of effects. The greater the anticipated safety, the earlier the intervention might be considered. We now look forward to well-designed trials of these and other agents at appropriate disease stages and using relevant outcome measures.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest with regard to the content of this article.

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Address correspondence to: Prof. John D. Isaacs, Institute of Cellular Medicine, Faculty of Medical Sciences, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK. E-mail: john.isaacs@ncl.ac.uk