The Journal of Rheumatology 2023;xx:xxxx
doi:10.3899/jrheum.220881
First Release February 15 2023

The Journal of Rheumatology

Review

Restoring Balance: Immune Tolerance in Rheumatoid Arthritis

Jaspreet Kaur[1], Ewa Cairns[2], and Lillian Barra[3]

ABSTRACT. Rheumatoid arthritis (RA) is a systemic musculoskeletal disease where immune dysregulation and subsequent autoimmunity induce significant synovial joint inflammation and damage, causing pain and disability. RA disease onset is promoted through multifaceted interactions between genetic and environmental risk factors. However, the mechanisms of disease onset are not completely understood and disease-specific treatments are yet to be developed. Current RA treatments include nonspecific disease-modifying antirheumatic drugs (DMARDs) that suppress destructive immune responses and prevent damage. However, DMARDs are not curative, and relapses are common, necessitating lifelong therapy in most patients. Additionally, DMARD-induced systemic immunosuppression increases the risk of serious infections and malignancies. Herein, we review the current understanding of RA disease pathogenesis, with a focus on T and B cell immune tolerance breakdown, and discuss the development of antigen-specific RA therapeutics that aim to restore a state of immune tolerance, with the potential for disease prevention and reduction of treatment-associated adverse effects.

Key Indexing Terms: autoantibodies, autoimmunity, immune tolerance, rheumatoid arthritis, therapeutics

Rheumatoid arthritis (RA) is a common autoimmune disease with a worldwide prevalence of 0.25%.[1] RA disproportionately affects females (sex ratio of 3:1).[1] The heritability of RA is around 40%,[2] and prevalence varies widely by region and ethnicity, with Western European, North American, and Caribbean countries having the highest rates.[1] Elevated prevalence rates are also reported in Indigenous populations: for example, in Manitoba, Canada, Indigenous women have an adjusted prevalence rate of 1.64%, whereas non-Indigenous women have a rate of 0.59%.[3] The prevalence of RA has been steadily increasing worldwide,[1] likely due to multiple factors, including environmental exposures, aging populations, and improvements in diagnostics and patient survival.

The cause of RA is unknown, and although a loss of immune tolerance precedes clinical disease (characterized by joint pain, swelling, and damage), the initial tolerance-breaking antigen has not yet been identified. Nevertheless, there is evidence of immune tolerance breakdown against a wide range of proteins, including those posttranslationally modified through citrullination and homocitrullination.[4,5] Citrullination is a calcium-dependent process catalyzed by peptidylarginine deiminase (PAD) enzymes that convert arginine into citrulline. In homocitrullination, lysine is converted into homocitrulline through a chemical process in the presence of cyanate or by the action of myeloperoxidase.[5] These modifications produce amino acids that are structurally similar, with homocitrulline possessing an additional carbon.

The mechanism by which patients with RA lose tolerance to citrullinated and homocitrullinated antigens involves a complex interplay between various environmental risk factors, such as smoking and infection, and genetic risk factors, primarily *HLA-DRB1* alleles.[4] The resulting tolerance breakdown promotes T and B cell activation and the production of anticitrullinated peptide/protein autoantibodies (ACPAs) and antihomocitrullinated peptide/protein autoantibodies (AHCPAs).[4,6-9] These autoantibodies target many posttranslationally modified proteins, including those found within synovial joints, such as type II collagen (CII), vimentin, and fibrinogen.[4,6,10] Both ACPA and AHCPA can be detected months to years before an individual progresses to clinical disease.[10-12] Therefore, there is a therapeutic window of opportunity where RA progression may be halted through the restoration of immune tolerance before significant tissue damage occurs.

RA Risk Factors

Environmental risks: smoking and infection. The current paradigm is that the loss of immune tolerance in RA first occurs at mucosal surfaces, such as the lungs, mouth, or gastrointestinal

The salaries of EC and LB are supported in part by the Calder Foundation. JK is supported by the Dean's Scholarship, Western University.

[1]J. Kaur, BMSc, Department of Microbiology and Immunology, Western University; [2]E. Cairns, PhD, Department of Microbiology and Immunology, and Department of Medicine, Division of Rheumatology, Western University; [3]L. Barra, MD, MPH, Department of Microbiology and Immunology, Department of Medicine, Division of Rheumatology, Department of Epidemiology and Biostatistics, Western University, London, Ontario, Canada.

The authors declare no conflict of interest relevant to this article.

Address correspondence to Dr. L. Barra, Division of Rheumatology, Department of Medicine, St. Joseph's Health Care London, 268 Grosvenor St., Suite D2-160, London, ON N6A 4V2, Canada.
Email: lillian.barra@sjhc.london.on.ca.

Accepted for publication January 13, 2023.

(GI) tract.[4] For example, cigarette smoking increases PAD expression and activation in the lungs, contributing to immune dysregulation through increased citrullination of self-antigens.[13] Smoking increases RA risk in a dose-dependent manner, especially in individuals expressing RA genetic risk factors.[14] Alterations in the oral and gut microbiota due to environmental exposures and other poorly understood factors are also associated with RA and may prime autoimmunity through molecular mimicry. Periodontal disease has been associated with RA, with a hazard ratio of 1.91,[15] and research in this area has focused on *Porphyromonas gingivalis*, which produces its own PAD enzyme that aberrantly citrullinates proteins,[16] increasing the possibility of cross reactivity between citrullinated bacterial and self-proteins. Oral administration of *P. gingivalis* in a mouse model of RA led to dysbiosis and increased levels of proinflammatory interleukin (IL)-17 in the gut, corresponding with increased arthritis severity.[17] In another study, mice transgenic for an RA-susceptible *HLA-DRB1* gene were immunized with bacterial citrullinated enolase, inducing arthritis and immune responses to human citrullinated alpha-enolase, an antigen present in RA joints.[18]

Genetic risk: HLA-DRB1 shared epitope. Autoimmune T and B cell responses in RA are promoted by specific *HLA-DRB1* allomorphs that share a consensus sequence termed the "shared epitope (SE)" within the peptide binding pocket of the major histocompatibility complex II (MHC-II) molecule.[19] The SE confers the greatest genetic risk for RA and explains up to 60% of the RA heritability risk.[2] Found at positions 70 to 74 of the MHC-II beta chain, the SE influences peptide binding affinity by its overall positive charge in the P4 pocket, enabling neutral citrullinated and/or homocitrullinated peptides to be better accommodated within this pocket than their counterpart peptides containing positively charged arginine and lysine.[6,19,20] Crystal structure analysis of SE-containing MHC-II has shown that peptides with citrulline at position P4 have increased peptide binding affinity compared to noncitrullinated peptides, whereas citrulline in other positions, such as P2 or P3, has minimal influence on affinity.[19] Moreover, in studies of SE-transgenic mice, peptide citrullination increased SE binding affinity 90-fold, whereas citrullination did not change peptide affinities of MHC-II lacking the SE.[20] Additionally, immunization with citrullinated peptides, but not native peptides, promoted T cell responses[20] and autoantibody production[21] in SE-transgenic mice and not the background C57BL/6 strain.

Other roles for the SE, besides antigen presentation, have been proposed. The hapten carrier hypothesis has emerged from association studies linking the SE with improved binding of PAD enzymes, rather than citrullinated peptides.[22] In this model, citrullinated peptides ("haptens") only induce antibody production when bound to PADs ("carriers"). Studies in SE-transgenic mice demonstrate that immunization with PAD induces ACPA production, providing support for this theory.[23] Concurrently, a role for the SE in signal transduction has been considered based on similarities between SE-containing MHC-II molecules and the kink region of MHC-I.[24-26] Certain MHC-I molecules contain a kink region with signal transduction functions that protrudes above the peptide binding pocket, and a similar kink has been detected in SE-containing MHC-II, suggesting that the SE may function as a signal transducing ligand as well.[24] Further investigation has identified a SE binding site on calreticulin (CRT), an innate immunity receptor.[24] This site allows the SE to simultaneously interact with the CRT P domain and the peptide within its groove without steric hindrance, which has many implications in RA pathogenesis.[24] First, this interaction induces intracellular calcium signaling that activates PADs and increases protein citrullination.[25] Second, interactions between the SE and CRT can activate nitric oxide (NO) signaling in cell types such as dendritic cells (DCs).[26] NO production reduces DC-mediated immune regulation through inhibition of indoleamine 2,3 dioxygenase (IDO), a DC mediator of immune tolerance and T cell regulation.[26] These results demonstrate how the SE can induce aberrant citrullination and immune dysregulation, thereby promoting disease onset.

Disease progression: From Pre-RA to clinical RA

Gene-environment interactions during the pre-RA phase of disease pathogenesis result in immune dysregulation and tolerance breakdown.[4] Compared to healthy controls (HCs), patients with pre-RA exhibit signs of immune dysfunction such as increased production of ACPAs,[10,11,27,28] AHCPAs,[8,10,12,29] and various cytokines.[30-32] Inflammatory T helper (Th) 1 (IL-1β, TNF [tumor necrosis factor], interferon (IFN)-γ, IL-6, and IL-17) and regulatory T cell (IL-10) cytokine levels continue to increase until the onset of joint disease.[30-32] Currently, what triggers the progression from systemic autoimmunity to clinical symptoms is not completely understood.[4] However, patients with arthralgia who express RA-associated autoantibodies, but display no clinical or radiographic evidence of inflammatory arthritis, have a high likelihood of developing RA.[4,29,33]

The role of T cells in RA. T cells have long been implicated as crucial drivers of RA pathogenesis.[4,34] Studies using HLA-DR tetramers and multiparameter flow cytometry have shown that citrulline-specific, but not influenza-specific, CD4+ T cells can be detected in RA patients at significantly higher frequencies than HCs.[35] These citrulline-specific CD4+ T cells are skewed toward a Th1 or Th1/Th17 memory (CD45RO+) phenotype, indicative of previous antigen experience.[35,36] Th1 cells maintain inflammation and promote effector cell activation through IFN-γ, IL-1β, IL-6, and TNF, cytokines that predict onset of RA joint disease.[30] IL-17 (produced by Th17 cells) is elevated in patients with RA compared to HCs, correlates with disease severity,[37] and induces tissue-resident cell activation.[38] Together, CD4+ Th1 and Th17 cells help establish a proinflammatory joint microenvironment that directly influences their effector functions. Th1 cells are stable under these chronic inflammatory conditions; however, Th17 cells demonstrate plasticity[36] and may skew toward a Th1/Th17 phenotype[39] (Figure 1). Moreover, these conditions allow T effector (Teff) cells to resist suppression through tumor necrosis factor-related apoptosis-inducing ligand, which limits regulatory T cell numbers.[40]

Single-cell (sc) analysis studies have confirmed and extended these previous findings. A mixed-effects association study

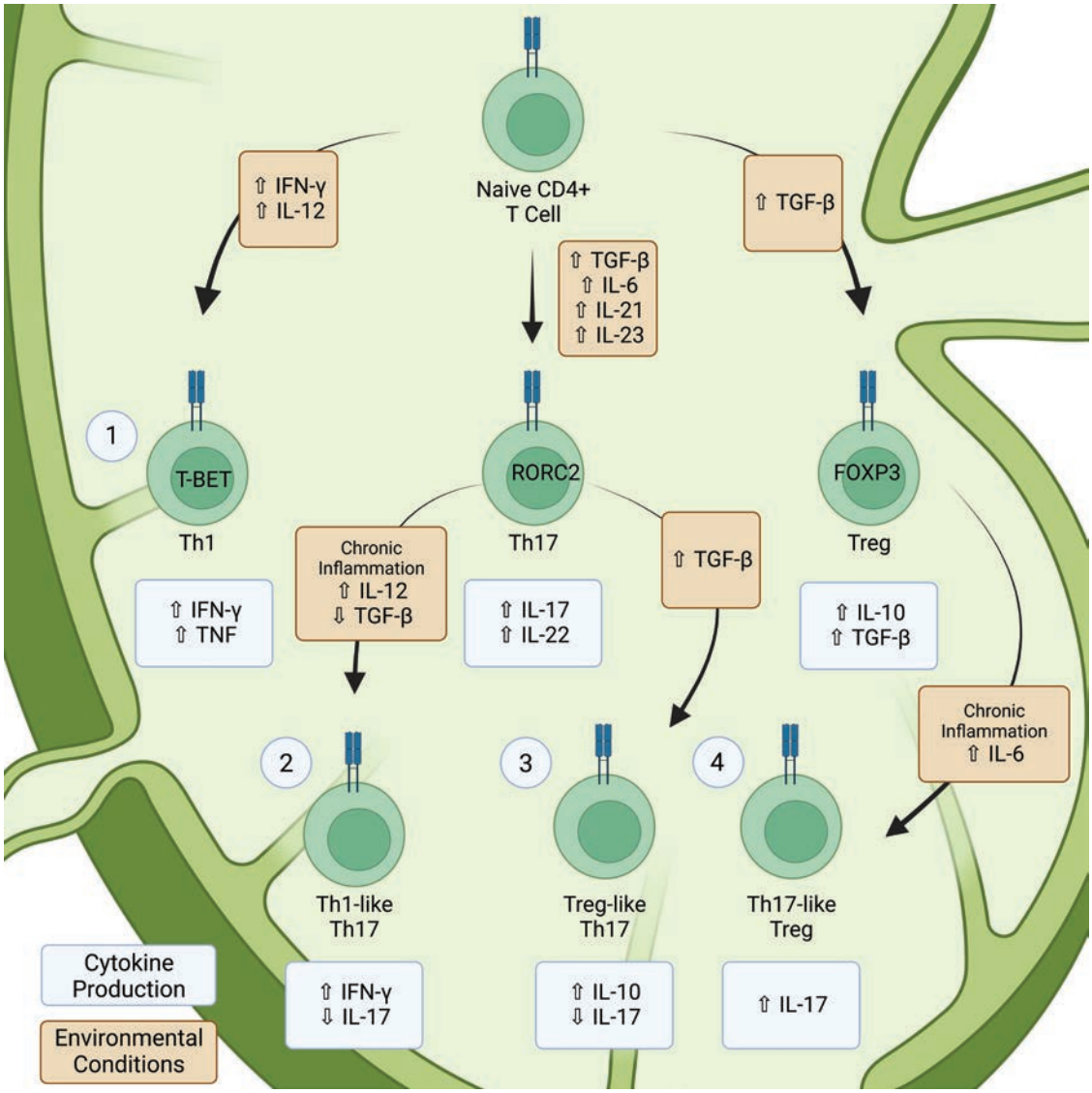

Figure 1. CD4+ T cell plasticity. Under homeostatic conditions, naïve CD4+ T cells are capable of skewing towards any effector subtype, an ability defined as plasticity. Typically, plasticity is progressively lost through differentiation as a CD4+ T cell skews toward Th1, Th2, Th17, or Treg subsets. This is not necessarily the case under chronic inflammatory conditions such as RA, where some subsets are able to retain plasticity. Th1 cells stably express the T-bet transcription factor and produce proinflammatory cytokines (1). In contrast, low levels of TGF-β during inflammation cause Th17 cells, characterized by RORC2 expression, to skew toward Th1. These Th1-like Th17 cells upregulate T-bet and produce higher levels of IFN-γ while reducing IL-17 expression (2). Conversely, Th17 cells can become regulatory (Treg) through downregulation of RORC2 during high levels of TGF-β. These Treg-like Th17 cells decrease IL-17 expression and increase production of IL-10, a common regulatory cytokine (3). Under inflammatory conditions and high IL-6 levels, Treg cells may convert into Th17-like cells by downregulating FOXP3, the master transcription factor of regulatory T cells. This conversion increases the production of inflammatory cytokines like IL-17, changing the role of these cells from regulatory to inflammatory (4). FOXP3: forkhead box P3; IFN: interferon; IL: interleukin; TGF: transforming growth factor; Th: T helper; Treg: regulatory T cell; RORC2: retinoic acid-related orphan receptor gamma; T-bet: T-box transcription factor TBX21.

of RA synovial single cells identified an effector memory CD4+ Th1 population (CD27-HLA-DR+CD45RO+) that is highly differentiated and readily produces proinflammatory granzyme A and IFN-γ.[41] A similar population (CD27–HLA-DR+CD45RO+) was found to be significantly expanded in RA joint tissue analyzed by scRNA sequencing (seq) and mass cytometry.[42,43] This subset was further characterized as nonexhausted, PD-1^{hi}CXCR5-T peripheral helper (Tph) cells that express factors associated with B cell help (IL-21, CXCL13, ICOS, MAF).[43] Tph cells are not simply T follicular helper (Tfh) cells; instead, they possess a unique chemokine receptor expression profile (CCR2, CX3CR1, CCR5) that promotes B cell migration and antibody production within chronically inflamed nonlymphoid tissue.[43]

Emerging scRNAseq profiles of RA synovium show that CD8+ T cells are enriched in joints.[42,44] This subset, previously

understudied in RA, is a major source of proinflammatory cytokines. Jonsson et al demonstrated that traditional CD8+ granzyme B+ cytotoxic lymphocytes (CTLs) are minimal in RA tissue, but a new CD8+ effector population with high expression of granzyme K is markedly expanded.[44] Unlike CTLs, granzyme K+ T cells have limited cytotoxic potential but produce inflammatory cytokines at greater than or equal amounts as CD4+ T cells.[44] Indeed, Zhang et al confirmed that CD8+ T cells are the dominant source of IFN-γ in RA tissue.[42] These data suggest that CD8+ T cells perpetuate inflammation alongside CD4+ T cells in RA, warranting further investigation of this subset.

Downregulation of inflammation is typically executed by regulatory T cells (Tregs). In RA, T cell–driven elevations in TNF significantly alter Treg function through various mechanisms, including impairment of the crucial synapse formation step required for contact-dependent immunosuppression.[45] Further, chronic inflammation can induce natural Tregs (nTregs) produced in the thymus to skew toward a Th17-like phenotype, disrupting their regulatory function while promoting further inflammation[46] (Figure 1). In some patients, anti-TNF drugs have been effective in reducing joint swelling and damage through generation of induced Tregs (iTregs) in the periphery that retain their phenotype and suppressive abilities during chronic inflammation.[46] Paradoxically, TNF inhibition in nonresponders may inhibit Treg suppression and increase Th17 numbers,[47] suggesting TNF plays a role in immunosuppression.[45] These contrasting responses may be due to polymorphisms related to TNF signaling and differential expression of TNF receptors.[47] Thus, additional research is required to clarify which patient subsets would benefit from TNF inhibition.

B cells and the autoantibody response. The humoral response in RA is thought to be driven by CD4+ Tfh cells that are elevated during disease and correlate with disease activity.[42,48] However, new evidence suggests that Tph cells may also provide help to B cells in inflamed tissue,[42,43] such as in RA joints where germinal center–like structures consisting of Th cells and B cells develop and support B cell activation and class switching.[49,50] Activated B cells contribute to pathogenesis through various mechanisms including antigen presentation, cytokine secretion, and autoantibody production.[34] Sc-transcriptomics has revealed an autoimmune-associated B cell (ABC) phenotype characterized by high CD11c (DC marker), T-bet (Th1 marker), and HLA-DR expression that is elevated in RA tissue and shows evidence of recent activation.[42] Functional characterization of ABCs is warranted as their transcriptional profile suggests they are germinal center B cells (activation-induced cytidine deaminase) differentiating toward a plasma cell phenotype (signaling lymphocytic activation molecule [SLAM] family member 7).[42]

Currently, autoantibodies are used as diagnostic and prognostic biomarkers in RA.[9,10,51,52] The specificities of these antibodies for RA vary; rheumatoid factor that targets IgG antibodies are the least specific (~85%),[53] whereas ACPAs and AHCPAs (most commonly, anticyclic citrullinated peptide 2 antibodies and anticarbamylated protein antibodies, respectively) are highly specific (~95%).[53,54] ACPAs have been extensively studied and consist of differentially expressed IgM < IgA < IgG isotypes.[55] The presence of mucosa-associated IgA-ACPAs supports the prevailing theory that the initial loss of immune tolerance occurs at mucosal surfaces.[8,28,55] Despite clear disease associations, there are inconsistent findings regarding the arthritogenic potential of ACPA.[56-59] In mice, monoclonal ACPAs enhanced collagen-induced arthritis (CIA),[56] whereas intravenous injection of 2 human monoclonal ACPAs caused pain, inflammation, and bone loss, without synovitis.[57,58] In humans, healthy relatives of patients with RA were found to express ACPAs, but only a small proportion developed disease,[59] indicating that the presence of ACPAs is not enough to induce RA.

The pathogenicity of autoantibodies appears to occur immediately prior to clinical RA when they undergo specific maturation processes, including epitope spreading, and glycosylation pattern modifications.[59,60] Epitope spreading occurs during ongoing immune responses and diversifies antibody specificity, resulting in cross reactivity of autoantibodies.[7,59,60] Changes in glycosylation of both the variable (FV) and constant (FC) domains also occur during RA progression.[27,61-63] N-glycosylation sites within the ACPA FV domain, introduced through somatic hypermutation, have elevated N-linked glycans compared to HCs,[27,61,62] whereas FC domain galactosylation and sialylation levels of IgG ACPAs decrease right before the onset of RA symptoms.[63] These glycosylation patterns are specific to IgG ACPAs, as ACPA-depleted IgG antibodies do not undergo similar changes.[63] Desialylated ACPAs cause increased osteoclast differentiation and activation.[64] Importantly, this pro-osteoclastogenic effect can be abolished through the reintroduction of sialic acid to ACPAs, as demonstrated by Harre et al, who showed that feeding mice a sialic acid precursor decreased arthritis severity and bone destruction.[65] Antibody sialylation may be regulated by the IL-23/Th17 axis, whereby IL-23–producing DCs induce Th17 cell activation and IL-21/IL-22 production, ultimately resulting in pathogenic ACPA through the suppression of ST6GAL1, an enzyme responsible for the addition of sialic acid to terminal galactose residues in B cells.[66] These data suggest that autoantibody pathogenicity may be dictated by their glycosylation profile, which is influenced by the microenvironment and other immune cells.

Immune mechanisms within the RA joint. The mechanisms by which systemic autoimmunity leads to clinical joint symptoms are incompletely understood. The current paradigm is that lymphocyte infiltration of synovial joints creates a proinflammatory microenvironment by secreting cytokines such as TNF, IFN-γ, IL-6, and IL-17 that activate tissue-resident cells within the joint and lead to further immune cell infiltration and other processes that ultimately damage the joint[4,34] (Figure 2). The initial trigger for lymphocyte infiltration is unknown. However, infiltrating B cells within RA joints produce autoantibodies targeting posttranslationally modified joint antigens. These autoantibodies mediate joint damage by immune complex formation, causing complement[67] and macrophage activation.[68] Macrophages contribute to pathogenesis through production of cytokines, matrix-degrading enzymes, leukotrienes, and NO, all of which perpetuate inflammation, activate/recruit other effector

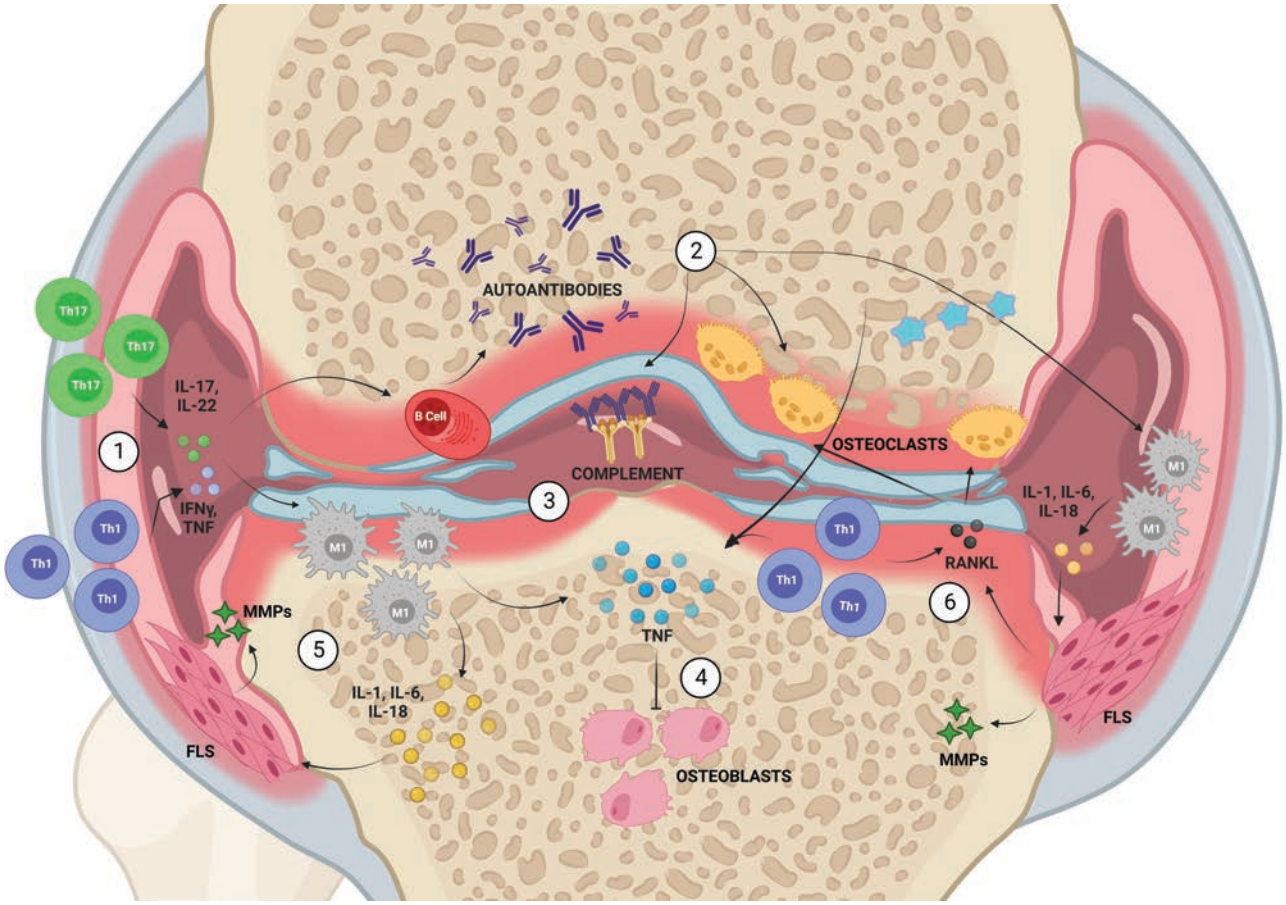

Figure 2. Overview of rheumatoid arthritis (RA) pathogenesis. Inflammatory helper T (Th) cell lineages, Th1 and Th17, induce activation of B cells and macrophages through the release of cytokines such as IFN-γ, TNF, IL-17, and IL-22 (1). B cells produce autoreactive antibodies (2) that mediate tissue damage through immune complex formation and activation of complement, macrophages, and osteoclasts. Complement deposition occurs on the articular cartilage surface (light blue) and attracts phagocytic macrophages that engulf opsonized material (3). Proinflammatory macrophages (M1), along with Th1 cells, release TNF, causing inhibition of bone-producing osteoblasts (4). M1 macrophages also produce additional cytokines (IL-1, IL-6, IL-18) that induce activation of fibroblast-like synoviocytes (FLS) within the hyperplastic synovium (light pink). Active FLS secrete matrix metalloproteinases (MMPs) that cause cartilage destruction (5). Bone damage is caused by the upregulation of RANKL by various cells, which binds to RANK on the surface of osteoclasts, promoting their bone destructive functions (6). FLS: fibroblast-like synoviocyte; IFN: interferon; IL: interleukin; M1: proinflammatory macrophage; MMP: matrix metalloproteinase; RANKL: receptor activator of nuclear factor kappa-B (ligand); Th: T helper; TNF: tumor necrosis factor.

cells, and promote osteoclast activation.[34] Sc-transcriptomic profiles of joint tissue macrophages demonstrate that efferocytic Mer tyrosine kinase (MerTK)+ macrophages, which predominate in HCs and during RA remission,[69] are reduced in RA.[42] Instead, patients with RA have increased populations of MerTK– macrophages expressing SPP1 (osteopontin) or S100A1242,69. Activated SPP1+ macrophages were found to have proinflammatory phenotypes and were capable of degrading bone.[69] The S100A12+ population was also proinflammatory, expressing factors that induce neutrophil migration and IL-6 production in joint fibroblasts.[69] Moreover, activated macrophages can release extracellular traps that introduce PAD enzymes and citrullinated antigens into the joint,[70] a phenomenon typically associated with neutrophils (NET). Besides undergoing NETosis, neutrophils in RA also produce oxygen-derived free radicals that depolymerize matrix hyaluronic acid and inactivate protease inhibitors, contributing to joint damage through cartilage destruction.[4,34,71]

Tissue destruction is also mediated by the synovium, the innermost 2- to 3-cell thick connective tissue lining the joint capsule.[72] Healthy synovium is highly vascularized, provides nutrients to the avascular cartilage, and produces lubricants and structural components to maintain proper joint function.[72] Type B fibroblast-like synoviocytes (FLS) are the predominate nonmyeloid cell type in synovial tissue. The inflammatory RA microenvironment induces FLS to increase cell surface HLA-DR, produce proinflammatory IL-6, and express specific markers (THY1+, CD34+) typically associated with hemopoietic stem cells.[42] Further, these FLS take on tumor-like characteristics including aggressive overproliferation, resistance to apoptosis, and loss of contact inhibition.[72,73] Xu et al demonstrated that RA FLS have increased expression of MDM4, an inhibitor of the transcription factor p53, which enables these cells to bypass cell cycle checkpoints and evade apoptosis.[73] This FLS transformation induces the formation of an invasive pannus

that adheres to articular cartilage, causing tissue damage through cartilage-degrading matrix metalloproteinases (MMPs), and proinflammatory mediators that continuously perpetuate inflammation and joint infiltration.[34,72] Despite the presence of many proangiogenic factors within the joint, the RA synovium and pannus are hypoxic because the nascent vessels have impaired vasoregulation and altered permeability.[74] In hypoxic conditions, FLS have enhanced migrative capacity and upregulate destructive proteases like MMP-2 and MMP-8, leading to further cartilage invasion and degradation.[74]

Bone erosion is also a hallmark of RA, and bone loss is largely mediated by osteoclasts that are activated through various mechanisms including ACPAs[64,65] and RANKL.[34,75] Unlike other IgG antibodies, IgG ACPAs can promote osteoclast differentiation and activation through 2 mechanisms: variable domain interactions with citrullinated epitopes on osteoclast surfaces or constant domain interactions with Fc receptors.[64,65] In addition, elevated levels of TNF and IL-17 within the RA joint induce RANKL secretion by both immune and nonimmune cells, promoting osteoclast activation and osteoclastogenesis.[75] The In Vitro Osteoclast Differentiation in Arthritis study found that patients with RA had higher proportions of circulating CD14+ monocytes capable of differentiating into osteoclasts compared to HCs.[76] Although RA osteoclasts had similar bone erosion capacity as controls, they were resistant to apoptosis.[76] In another study, RA osteoclasts were found to be larger (> 10 nuclei), produced more proteases (MMP-9), and had increased resorptive capacity following IL-1β treatment compared to typical osteoclasts (2-5 nuclei).[77] These results suggest that RA osteoclasts exhibit increased erosive and survival capacity, leading to net loss of bone tissue. Without intervention, the self-perpetuating cycle of joint destruction continues indefinitely, causing severe deformation and disability.

New horizons for RA treatment: Antigen-specific therapies

Progress in the field of RA and the advent of therapies targeting immune mechanisms of disease have drastically improved outcomes. Current therapeutics include synthetic disease-modifying antirheumatic drugs (DMARDs; methotrexate, hydroxychloroquine, leflunomide, sulfasalazine, and Janus kinase inhibitors) and biologic DMARDs (TNF inhibitors, IL-6 inhibitors, abatacept [modulator of T cell co-stimulation] and rituximab [B cell-depleting antibody]).[78] Despite improvements in RA treatments, only 30% to 65% of patients achieve long-term remission,[79] leaving a subset with disease that is resistant to several current treatments. Moreover, DMARDs nonspecifically suppress the immune system, leading to significant adverse effects such as serious infections and malignancy.

To reduce complications of current nonspecific RA treatments, autoimmune responses resulting from tolerance breakdown should be selectively targeted by novel therapies. Thompson and Staines were the first to induce antigen-specific tolerance (AST) in the CIA animal model of RA.[80] They found that feeding CII to rats with CIA delayed disease onset and reduced disease severity.[80] Additional research has demonstrated that this therapy can skew Th1 responses toward Th2 and reduce T cell responses against CII.[81] In clinical trials where AST was induced through oral administration of CII, some clinical improvements were seen; however, response rates did not surpass those of the methotrexate-treated group.[82] Interestingly, patients in the CII group experienced fewer and milder side effects,[82] demonstrating potential benefits of targeted therapeutics.

Besides the oral route, AST may be induced by introducing antigens into the body through the skin, a method referred to here as transdermal immunotherapy (TI). TI is largely mediated by Langerhans cells (LCs), a subset of tissue-resident DCs that shuttle antigens to local lymph nodes for presentation to T cells.[83] In the absence of inflammation, antigen-loaded LCs induce Treg activation, a crucial step in tolerance induction[83] (Figure 3). Following activation, Tregs suppress immune responses selectively through T cell anergy or deletion, and more broadly through the release of antiinflammatory cytokines. Several groups have demonstrated the therapeutic potential of TI in RA models. Marcinska et al demonstrated that transdermal application of CII promoted expansion of CD4+CD8+ suppressor T cells that inhibited CIA progression and reduced disease severity in mice.[84] Likewise, Strid et al demonstrated that in transgenic mice with CIA, CII TI reduced clinical disease scores, swelling, and joint destruction.[85] These benefits were attributed to a shift from Th1 to Th2 responses, indicated by a decrease in IFN-γ and IgG2a isotype antibodies and an increase in IL-4 and IgE isotype antibodies.[85]

In previous studies, tolerance was restored against one specific autoantigen, which may not be sufficient in restoring tolerance against the wide range of posttranslationally modified proteins relevant to RA pathogenesis. Thus, Gertel et al generated a synthetic multiepitope citrullinated peptide (Cit-ME) to represent the various citrullinated proteins against which tolerance is lost in RA.[86] Using the adjuvant-induced arthritis model of RA, they demonstrated that subcutaneous injection of Cit-ME reduced disease severity and Th17 cell numbers while increasing the Treg population.[86] Although TI for RA is still in the preclinical stages, the efficacy of TI in humans has been demonstrated in multiple sclerosis (MS), an autoimmune disease where myelin-related peptides (myelin basic protein, myelin oligodendrocyte glycoprotein, myelin proteolipid protein) have been identified as autoantigens. Glatiramer acetate (GA) is a synthetic peptide similar to MBP and has been approved by the US Food and Drug Administration for MS since 1996.[87] Subcutaneous injection of GA reduces GA-specific T cell frequency, promotes Treg function, and decreases relapse rates by 30% in patients with MS.[87] In another randomized controlled MS trial, TI of all 3 myelin peptides reduced lesions by 66.5% after 12 months and significantly lowered relapse rates compared to placebo.[88] The success seen in patients with MS along with the early results from RA studies suggests that TI may allow for reprogramming of pathogenic responses involved in RA through tolerance restoration.

An alternative approach to tolerance induction using tolerogenic DCs (TolDC) has also been studied. This approach involves incubation of patient-derived progenitor DCs that are pulsed with RA autoantigen under tolerizing conditions (typically,

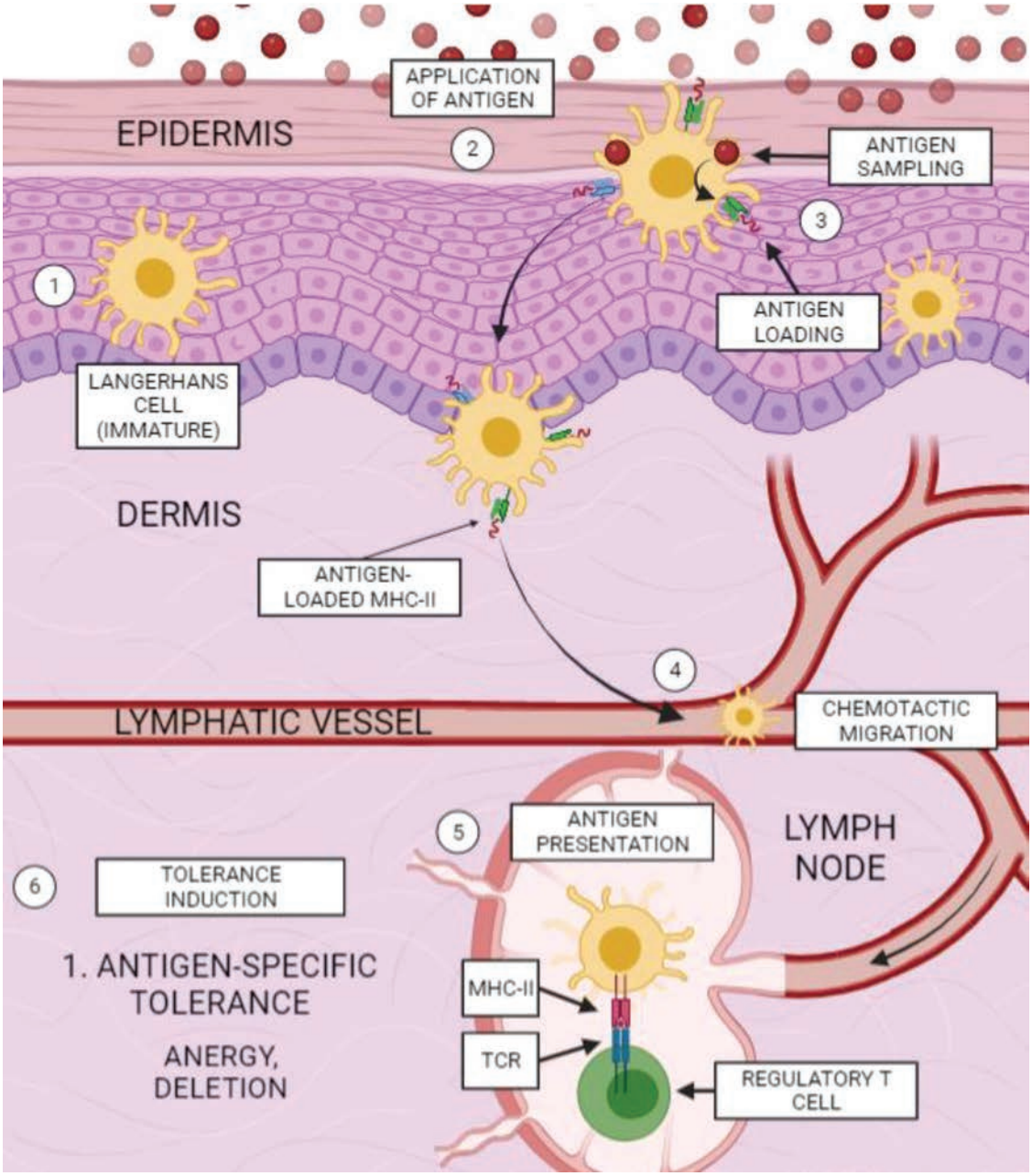

Figure 3. Antigen-specific tolerance induction via transdermal immunotherapy. Langerhans cells (LCs) are tissue resident immune cells that can be harnessed for antigen-specific tolerance induction (1). When an antigen of interest is applied to the epidermis (2), it is taken up by LCs constantly sampling their microenvironment using dendrites that reach the outermost corneal layer of the epidermis. Antigen sampling in the absence of inflammation allows LCs to become antigen-loaded, but remain immature (3). Antigen-loaded LCs move through the lymphatic vessels (4) to reach local lymph nodes where the antigen is presented to T cells (5). T cell interactions with immature LCs lacking sufficient co-stimulatory molecules on their cell surface induce the activation of regulatory T (Treg) cells. Tolerance induction relies on activated Tregs which reduce immune responses through interactions with antigen-specific T cells that promote their anergy or deletion (6). MHC-II: major histocompatibility complex II; TCR: T cell receptor.

nuclear factor-κB inhibition through vitamin D) to generate antigen-loaded TolDCs that are then reintroduced into the same individual. Martin et al evaluated the therapeutic capacity of TolDCs and showed that subcutaneous DC administration skewed immune responses toward Th2 and reduced histopathological damage in mice.[89] Similarly, other groups have found that intravenous injection of CII-loaded TolDCs delayed CIA onset, and reduced its incidence and severity, coinciding with reductions in Th17 cells.[90,91] A phase I study found that TolDCs exposed to citrullinated antigens was well-tolerated, increased Treg proportions, and reduced Teff cells, proinflammatory cytokine levels, and disease activity score in patients with RA.[92] Another phase I trial validated the safety of TolDCs injected into inflamed RA knees and found this treatment did not cause disease flares.[93]

To increase the feasibility of TolDC treatment, the same group developed CII-loaded liposomes (DEN-181) that can induce CII-specific TolDC formation in vivo.[94] Subcutaneous administration of DEN-181 was well-tolerated and improved RA disease symptoms, with all patients achieving remission by day 57.[94] DEN-181 treatment caused dose-dependent changes in citrullinated vimentin–specific and CII-specific T cell frequencies,[94] demonstrating that treating with 1 type of citrullinated antigen can lead to tolerance against other citrullinated antigens. DEN-181 also affected ACPA V-domain glycosylation,[94] an intriguing finding that necessitates further investigation.

Chimeric antigen receptor (CAR) T cell technology, initially developed for antigen-specific cancer treatment, is also being studied for its utility in autoimmune diseases.[95] The modifiable CAR consists of 4 components (an extracellular antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain) that allows antigen recognition without MHC-restriction and enables CAR-T cells to recognize and kill in an antigen-specific manner.[95] In a proof-of-concept study, Zhang et al recently demonstrated the potential of CAR-T cells to destroy CIA and RA patient-derived autoreactive B cells in vitro.[96] Concurrently, Whittington et al developed CAR-T cells expressing HLA molecules rather than TCRs.[97] When covalently linked to a CII peptide, this CAR can specifically target anti-CII CD4+ T cells.[97] CIA mice treated with HLA-CII CAR-T cells following arthritis induction had reduced disease incidence, onset and severity.[97] Alternatively, CAR Tregs have also been developed to suppress antigen-specific autoimmune responses; Raffin et al designed anticitrullinated vimentin CAR Tregs that become activated when cultured in RA synovial fluid.[98] Although CAR-T cell therapy has been underexplored for RA, it has shown promise in preclinical studies and there are several ongoing clinical trials in patients with other autoimmune diseases.[95] However, its use may be limited by reports of severe adverse events in other conditions, including neurologic symptoms and cytokine release syndrome.[99]

Conclusion

Despite advancements in RA management over the last few decades, outcomes remain suboptimal, and patients often experience adverse events from long-term immunosuppression. Strategies to restore immune tolerance toward RA-specific antigens are currently under development and employ various approaches. TI targeting multiple epitopes either by direct administration of antigens into the skin or using liposomes may be optimal given practical considerations and safety profile. These emerging antigen-specific therapies for RA offer the potential to prevent disease onset and chronic inflammation.

REFERENCES

1. Safiri S, Kolahi AA, Hoy D, et al. Global, regional, and national burden of rheumatoid arthritis 1990-2017: a systematic analysis of the Global Burden of Disease study 2017. Ann Rheum Dis 2019;78:1463-71.
2. Frisell T, Holmqvist M, Källberg H, Klareskog L, Alfredsson L, Askling J. Familial risks and heritability of rheumatoid arthritis: role for rheumatoid factor/anti-citrullinated protein antibody status, number and type of affected relatives, sex, and age. Arthritis Rheum 2013;65:2773-82.
3. Hitchon CA, Khan S, Elias B, Lix LM, Peschken CA. Prevalence and incidence of rheumatoid arthritis in Canadian First Nations and non-First Nations People: a population-based study. J Clin Rheumatol 2020;26:169-75.
4. Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. Nat Rev Immunol 2017;17:60-75.
5. Turunen S, Huhtakangas J, Nousiainen T, et al. Rheumatoid arthritis antigens homocitrulline and citrulline are generated by local myeloperoxidase and peptidyl arginine deiminases 2, 3 and 4 in rheumatoid nodule and synovial tissue. Arthritis Res Ther 2016;18:239.
6. Scinocca M, Bell DA, Racapé M, et al. Antihomocitrullinated fibrinogen antibodies are specific to rheumatoid arthritis and frequently bind citrullinated proteins/peptides. J Rheumatol 2014;41:270-9.
7. Lac P, Racapé M, Barra L, Bell D, Cairns E. Relatedness of antibodies to peptides containing homocitrulline or citrulline in patients with rheumatoid arthritis. J Rheumatol 2018;45:302-9.
8. Bell DA, Elhayek S, Cairns E, Barra L. Anti-homocitrullinated protein antibody isotype usage in rheumatoid arthritis and their unaffected first-degree relatives. Clin Exp Rheumatol 2017; 35:948-53.
9. Zhang B, Lei Y, Li X, et al. Elevated levels of anti-carbamylated protein antibody in patients with rheumatoid arthritis: association with disease activity and bone destruction. J Investig Med 2020;68:1186-92.
10. Brink M, Verheul MK, Ronnelid J, et al. Anti-carbamylated protein antibodies in the pre-symptomatic phase of rheumatoid arthritis, their relationship with multiple anti-citrulline peptide antibodies and association with radiological damage. Arthritis Res Ther 2015;17:25.
11. Nielen MMJ, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004;50:380-6.
12. Gan RW, Trouw LA, Shi J, et al. Anti-carbamylated protein antibodies are present prior to rheumatoid arthritis and are associated with its future diagnosis. J Rheumatol 2015;42:572-9.
13. Makrygiannakis D, Hermansson M, Ulfgreen AK, et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. Ann Rheum Dis 2008;67:1488-92.
14. Kallberg H, Ding B, Padyukov L, et al. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. Ann Rheum Dis 2011;70:508-11.
15. Chou Y, Lai K, Chen D, Lin C, Chen H. Rheumatoid arthritis risk associated with periodontitis exposure: a nationwide, population-based cohort study. PLoS One 2015;10:e0139693.
16. Wegner N, Wait R, Sroka A, et al. Pepidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and α-enolase: Implications for autoimmunity in rheumatoid arthritis. Arthritis Rheum 2010;62:2662-72.
17. Sato K, Takahashi N, Kato T, et al. Aggrevation of collagen-induced arthritis by orally administered Porphyromonas gingivalis through modulation of the gut microbiota and gut immune system. Sci Rep 2017;7:6955.
18. Kinloch AJ, Alzabin S, Brintnell W, et al. Immunization with Porphyromonas gingivalis enolase induces autoimmunity to mammalian α-enolase and arthritis in DR4-IE-transgenic mice. Arthritis Rheum 2011;63:3818-23.
19. Scally SW, Peterson J, Law SC, et al. A molecular basis for the

association of the HLA-DRB1 locus, citrullination, and rheumatoid arthritis. J Exp Med 2013;210:2569-82.
20. Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E. Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. J Immunol 2003;171:538-41.
21. Hill JA, Bell DA, Brintnell W, et al. Arthritis induced by posttranslationally modified (citrullinated) fibrinogen in DR4-IE transgenic mice. J Exp Med 2008;205:967-79.
22. Balandraud N, Auger I, Roudier J. Do RA associated HLA-DR molecules bind citrullinated peptides or peptides from PAD4 to help the development of RA specific antibodies to citrullinated proteins? J Autoimmun 2021;116:102542.
23. Hemon MF, Lambert NC, Roudier J, Auger I. PAD2 immunization induces ACPA in wild-type and HLA-DR4 humanized mice. Eur J Immunol 2022;52:1464-73.
24. Ling S, Cheng A, Pumpens P, Michalak M, Holoshitz J. Identification of the rheumatoid arthritis shared epitope binding site on calreticulin. PLoS One 2010;5:e11703.
25. van Drongelen V, Ali WH, Holoshitz J. Uncovering a shared epitope-activated protein citrullination pathway. J Immunol 2020;205:579-86.
26. De Almeida DE, Ling S, Pi X, Hartmann-Scruggs AM, Pumpens P, Holoshitz J. Immune dysregulation by the rheumatoid arthritis shared epitope. J Immunol 2010;185:1927-34.
27. Kissel T, Hafkenscheid L, Wesemael TJ, et al. IgG anti-citrullinated protein antibody variable domain glycosylation increases before the onset of rheumatoid arthritis and stabilizes thereafter: a cross-sectional study encompassing ~1,500 samples. Arthritis Rheumatol 2022;74:1147-58.
28. Barra L, Scinocca M, Saunders S, et al. Anti-citrullinated protein antibodies in unaffected first-degree relatives of rheumatoid arthritis patients. Arthritis Rheum 2013;65:1439-47.
29. Shi J, van de Stadt LA, Levarht EW, et al. Anti–carbamylated protein antibodies are present in arthralgia patients and predict the development of rheumatoid arthritis. Arthritis Rheum 2013;65:911-5.
30. Kokkonen H, Soderstrom I, Rocklov J, Hallmans G, Lejon K, Dahlqvist SR. Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis Rheum 2010;62:383-91.
31. Barra L, Summers K, Bell D, Cairns E. Serum cytokine profile of unaffected first-degree relatives of patients with rheumatoid arthritis. J Rheumatol 2014;41:280-5.
32. El-Gabalawy HS, Robinson DB, Smolik I, et al. Familial clustering of the serum cytokine profile in the relatives of rheumatoid arthritis patients. Arthritis Rheum 2012;64:1720-9.
33. Bos WH, Wolbink GJ, Boers M, et al. Arthritis development in patients with arthralgia is strongly associated with anti-citrullinated protein antibody status: a prospective cohort study. Ann Rheum Dis 2010;69:490-4.
34. Fang Q, Zhou C, Nandakumar KS. Molecular and cellular pathways contributing to joint damage in rheumatoid arthritis. Mediators Inflamm 2020;3830212.
35. Gerstner C, Turcinov S, Hensvold AH, et al. Multi-HLA class II tetramer analyses of citrulline-reactive T cells and early treatment response in rheumatoid arthritis. BMC Immunol 2020;21:27.
36. van Hamburg JP, Tas SW. Molecular mechanisms underpinning T helper 17 cell heterogeneity and functions in rheumatoid arthritis. J Autoimmun 2018;87:69-81.
37. Leipe J, Grunke M, Dechant C, et al. Role of Th17 cells in human autoimmune arthritis. Arthritis Rheum 2010;62:2876-85.
38. Hirota K, Hashimoto M, Ito Y, et al. Autoimmune Th17 cells induced synovial stromal and innate lymphoid cell secretion of the cytokine GM-CSF to initiate and augment autoimmune arthritis. Immunity 2018;48:1220-32.
39. Nistala K, Adams S, Cambrook H, et al. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. Proc Natl Acad Sci USA 2010;107:14751-6.
40. Xiao H, Wang S, Miao R, Kan W. TRAIL is associated with impaired regulation of CD4+CD25- T cells by regulatory T cells in patients with rheumatoid arthritis. J Clin Immunol 2011;31:1112-9.
41. Fonseka CY, Rao DA, Teslovich NC, et al. Mixed-effects association of single cells identifies an expanded effector CD4+ T cell subset in rheumatoid arthritis. Sci Transl Med 2019;10:eaaq0305.
42. Zhang F, Wei K, Slowikowski K, et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat Immunol 2019;20:928-42.
43. Rao DA, Gurish MF, Marshall JL, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature 2017;542:110-4.
44. Jonsson AH, Zhang F, Dunlap G, et al. Granzyme K+ CD8 T cells form a core population in inflamed human tissue. Sci Transl Med 2022;14:eabo0686.
45. Farrugia M, Baron B. The role of TNF-α in rheumatoid arthritis: a focus on regulatory T cells. J Clin Transl Res 2016;2:84-90.
46. Ehrenstein MR, Evans JG, Singh A, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFα therapy. J Exp Med 2004;200:277-85.
47. Bystrom J, Clanchy FI, Taher TE, et al. TNFα in the regulation of Treg and Th17 cells in rheumatoid arthritis and other autoimmune inflammatory diseases. Cytokine 2018;101:4-13.
48. Wang J, Shan Y, Jiang Z, et al. High frequencies of activated B cells and T follicular helper cells are correlated with disease activity in patients with new-onset rheumatoid arthritis. Clin Exp Immunol 2013;174:212-20.
49. Kim HJ, Krenn V, Steinhauser G, Berek C. Plasma cell development in synovial germinal centers in patients with rheumatoid and reactive arthritis. J Immunol 1999;162:3053-62.
50. Humby F, Bombardieri M, Manzo A, et al. Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid arthritis. PLoS Med 2009;6:e1.
51. Jilani AA, Mackworth-Young CG. The role of citrullinated protein antibodies in predicting erosive disease in rheumatoid arthritis: a systematic literature review and meta-analysis. Int J Rheumatol 2015;2015:728610.
52. Barra L, Pope JE, Orav JE, et al; CATCH Investigators. Prognosis of seronegative patients in a large prospective cohort of patients with early inflammatory arthritis. J Rheumatol 2014;41:2361-9.
53. Nishimura K, Sugiyama D, Kogata Y, et al. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med 2007:146:797-808.
54. Li L, Deng C, Chen S, et al. Meta-analysis: diagnostic accuracy of anti-carbamylated protein antibody for rheumatoid arthritis. PLoS One 2016;11:e0159000.
55. Sieghart D, Platzer A, Studenic P, et al. Determination of autoantibody isotypes increases the sensitivity of serodiagnostics in rheumatoid arthritis. Front Immunol 2018;9:876.
56. Kuhn KA, Kulik L, Tomooka B, et al. Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. J Clin Invest 2006;116:961-73.
57. Krishnamurthy A, Circiumaru A, Sun J, et al. Combination of two monoclonal ACPAs induced tenosynovitis, pain and bone loss in mice in a peptidyl arginine deiminase-4 dependent manner. Arthritis Rheumatol 2022 Aug 5 (Epub ahead of print).
58. Wigerblad G, Bas DB, Fernades-Cerqueira C, et al. Autoantibodies to citrullinated proteins induce joint pain independent of

inflammation via a chemokine-dependent mechanism. Ann Rheum Dis 2016;75:730-8.
59. Ioan-Facsinay A, Willemze A, Robinson DB, et al. Marked differences in fine specificity and isotype usage of the anti-citrullinated protein antibody in health and disease. Arth Rheum 2008;58:3000-8.
60. Falkenburg WJJ, van Schaardenburg D. Evolution of autoantibody responses in individuals at risk of rheumatoid arthritis. Best Pract Res Clin Rheumatol 2017;31:42-52.
61. Hafkenscheid L, de Moel E, Smolik I, et al. N-linked glycans in the variable domain of IgG anti-citrullinated protein antibodies predict the development of rheumatoid arthritis. Arthritis Rheumatol 2019;71:1626-33.
62. Ercan A, Cui J, Chatterton DEW, et al. Aberrant IgG galactosylation precedes disease onset, correlates with disease activity and is prevalent in autoantibodies in rheumatoid arthritis. Arthritis Rheum 2010;62:2239-48.
63. Scherer HU, van der Woude D, Ioan-Facsinay A, et al. Glycan profiling of anti-citrullinated protein antibodies isolated from human serum and synovial fluid. Arthritis Rheum 2010;62:1620-9.
64. Harre U, Lang SC, Pfeifle R, et al. Glycosylation of immunoglobulin G determines osteoclast differentiation and bone loss. Nat Commun 2015;6:6651.
65. Harre U, Georgess D, Bang H, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. J Clin Invest 2012;122:1791-802.
66. Pfeifle R, Rothe T, Ipseiz N, et al. Regulation of autoantibody activity by the IL-23-Th17 axis determines the onset of autoimmune disease. Nat Immunol 2017;18:104-13.
67. Trouw LA, Haisma EM, Levarht EWN, et al. Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. Arthritis Rheum 2009;60:1923-31.
68. Sokolove J, Zhao X, Chandra PE, Robinson WH. Immune complexes containing citrullinated fibrinogen costimulate macrophages via toll-like receptor 4 and Fcγ receptor. Arthritis Rheum 2011;63:53-62.
69. Alivernini S, MacDonald L, Elmesmari A, et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. Nat Med 2020;26:1295-306.
70. Eldin M El Shikh M, El Sayed R, Nerviani A, et al. Extracellular traps and PAD4 released by macrophages induce citrullinated and auto-antibody production in autoimmune arthritis. J Autoimmun 2019;105:102297.
71. Cecchi I, de la Rosa IA, Menegatti E, et al. Neutrophils: Novel key players in rheumatoid arthritis. Current and future therapeutic targets. Autoimmun Rev 2018;17:1138-49.
72. Schuster R, Rockel JS, Kapoor M, Hinz B. The inflammatory speech of fibroblasts. Immunol Rev 2021;302:126-46.
73. Xu N, Wang Y, Li D, et al. MDM4 overexpression contributes to synoviocyte proliferation in patients with rheumatoid arthritis. Biochem Biophys Res Commun 2010;401:417-21.
74. Akhavani MA, Madden L, Buysschaert I, Sivakumar B, Kang N, Paleolog EM. Hypoxia upregulates angiogenesis and synovial cell migration in rheumatoid arthritis. Arthritis Res Ther 2009;11:R64.
75. Theill LE, Boyle WJ, Penninger JM. RANK-L and RANK: T cells, bone loss, and mammalian evolution. Annu Rev Immunol 2002;20:795-823.
76. Allard-Chamard H, Carrier N, Dufort P, et al. Osteoclasts and their circulating precursors in rheumatoid arthritis: Relationships with disease activity and bone erosions. Bone Rep 2020;12:100282.
77. Trebec DP, Chandra D, Gramoun A, Li K, Heersche JNM, Manolson MF. Increased expression of activating factors in large osteoclasts could explain their excessive activity in osteolytic diseases. J Cell Biochem 2007;101:205-20.
78. Bykerk VP, Akhavan P, Hazlewood GS, et al. Canadian Rheumatology Association recommendations for pharmacological management of rheumatoid arthritis with traditional and biologic disease-modifying antirheumatic drugs. J Rheumatol 2012; 39:1559-82.
79. Pokharel G, Deardon R, Barnabe C, et al. Joint estimation of remission and response for Methotrexate-based DMARD options in rheumatoid arthritis: a bivariate network meta-analysis. ACR Open Rheumatol 2019;1:471-9.
80. Thompson HS, Staines NA. Gastric administration of type II collagen delays the onset and severity of collagen-induced arthritis in rats. Clin Exp Immunol 1986;64:581-6.
81. Garcia G, Komagata Y, Slavin AJ, Maron R, Weiner HL. Suppression of collagen-induced arthritis by oral or nasal administration of type II collagen. J Autoimmun 1999;12:315-24.
82. Wei W, Zhang LL, Xu JU, et al. A multicenter, double-blind, randomized, controlled phase III clinical trial of chicken type II collagen in rheumatoid arthritis. Arthritis Res Ther 2009;11:R180.
83. Dioszeghy V, Mondoulet L, Laoubi L, et al. Antigen uptake by Langerhans cells is required for the induction of regulatory T cells and the acquisition of tolerance during epicutaneous immunotherapy in OVA-sensitized mice. Front Immunol 2018;9:1951.
84. Marcinska K, Majewska-Szczepanik M, Maresz KZ, Szczepanik M. Epicutaneous immunization with collagen induced TCRab suppressor T cells that inhibit collagen-induced arthritis. Int Arch Allergy Immunol 2015;166:121-34.
85. Strid J, Tan LA, Strobel S, Londei M, Callard R. Epicutaneous immunization with type II collagen inhibits both onset and progression of chronic collagen-induced arthritis. PLoS One 2007;2:e387.
86. Gertel S, Serre G, Shoenfeld Y, Amital H. Immune tolerance induction with multiepitope peptide derived from citrullinated autoantigens attenuates arthritis manifestations in adjuvant arthritis rats. J Immunol 2015:194:5674-80.
87. Schrempf W, Ziemssen T. Glatiramer acetate: mechanisms of action in multiple sclerosis. Autoimmun Rev 2007;6:469-75.
88. Walczak A, Siger M, Ciach A, Szczepanik M, Selmaj K. Transdermal application of myelin peptides in multiple sclerosis treatment. JAMA Neurol 2013;70:1105-9.
89. Martin E, Capini C, Duggan E, et al. Antigen-specific suppression of established arthritis in mice by dendritic cells deficient in NF-kappaB. Arthritis Rheum 2007;56:2255-66.
90. van Duivenvoorde LM, Louis-Plence P, Apparailly F, et al. Antigen-specific immunomodulation of collagen-induced arthritis with tumor necrosis factor-stimulated dendritic cells. Arthritis Rheum 2004;50:3354-64.
91. Stoop JN, Harry RA, von Delwig A, Isaacs JD, Robinson JH, Hilkens CMU. Therapeutic effect of tolerogenic dendritic cells in established collagen-induced arthritis is associated with a reduction in Th17 responses. Arthritis Rheum 2010;62:3656-65.
92. Benham H, Nel HJ, Law SC, et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. Sci Transl Med 2015;7:290ra87.
93. Bell GM, Anderson AE, Diboll J, et al. Autologous tolerogenic dendritic cells for rheumatoid and inflammatory arthritis. Ann Rheum Dis 2017;76:227-34.
94. Sonigra A, Nel HJ, Wehr P, et al. Randomized phase I trial of antigen-specific tolerizing immunotherapy with peptide/calcitriol liposomes in ACPA+ rheumatoid arthritis. JCI Insight 2022;7:e160964.

95. Orvain C, Boulch M, Bousso P, Allanore Y, Avouac J. Is there a place for chimeric antigen receptor-T cells in the treatment of chronic autoimmune rheumatic diseases? Arthritic Rheumatol 2021;73:1954-65.
96. Zhang B, Wang Y, Yuan Y, et al. In vitro elimination of autoreactive B cells from rheumatoid arthritis patients by universal chimeric antigen receptor T cells. Ann Rheum Dis 2021;80:176-84.
97. Whittington KB, Prislovsky A, Beaty J, Albritton L, Radic M, Rosloniec EF. CD8+ T cells expressing an HLA-DR1 chimeric antigen receptor target autoimmune CD4+ T cells in an antigen-specific manner and inhibit the development of autoimmune arthritis. J Immunol 2022;208:16-26.
98. Raffin C, Zhou Y, Piccoli L, Lanzavecchia A, Sadelain M, Bluestone JA. Development of citrullinated-vimentin-specific CAR for targeting Tregs to treat autoimmune rheumatoid arthritis. J Immunol 2016;196:210-9.
99. Tallantyre EC, Evans NA, Parry-Jones J, Morgan MPG, Jones CH, Ingram W. Neurological updates: neurological complications of CAR-T therapy. J Neurol 2021;268:1544-54.