

Review

Restoring Balance: Immune Tolerance in Rheumatoid Arthritis

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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

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(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 pro-inflammatory 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 responses²⁰ and autoantibody production²¹ 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} AHCAPAs,^{8,10,12,29} and various cytokines.³⁰⁻³² Inflammatory T helper (Th) 1 (IL-1 β , 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-1 β , 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 plasticity³⁶ and may skew toward a Th1/Th17 phenotype³⁹ (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

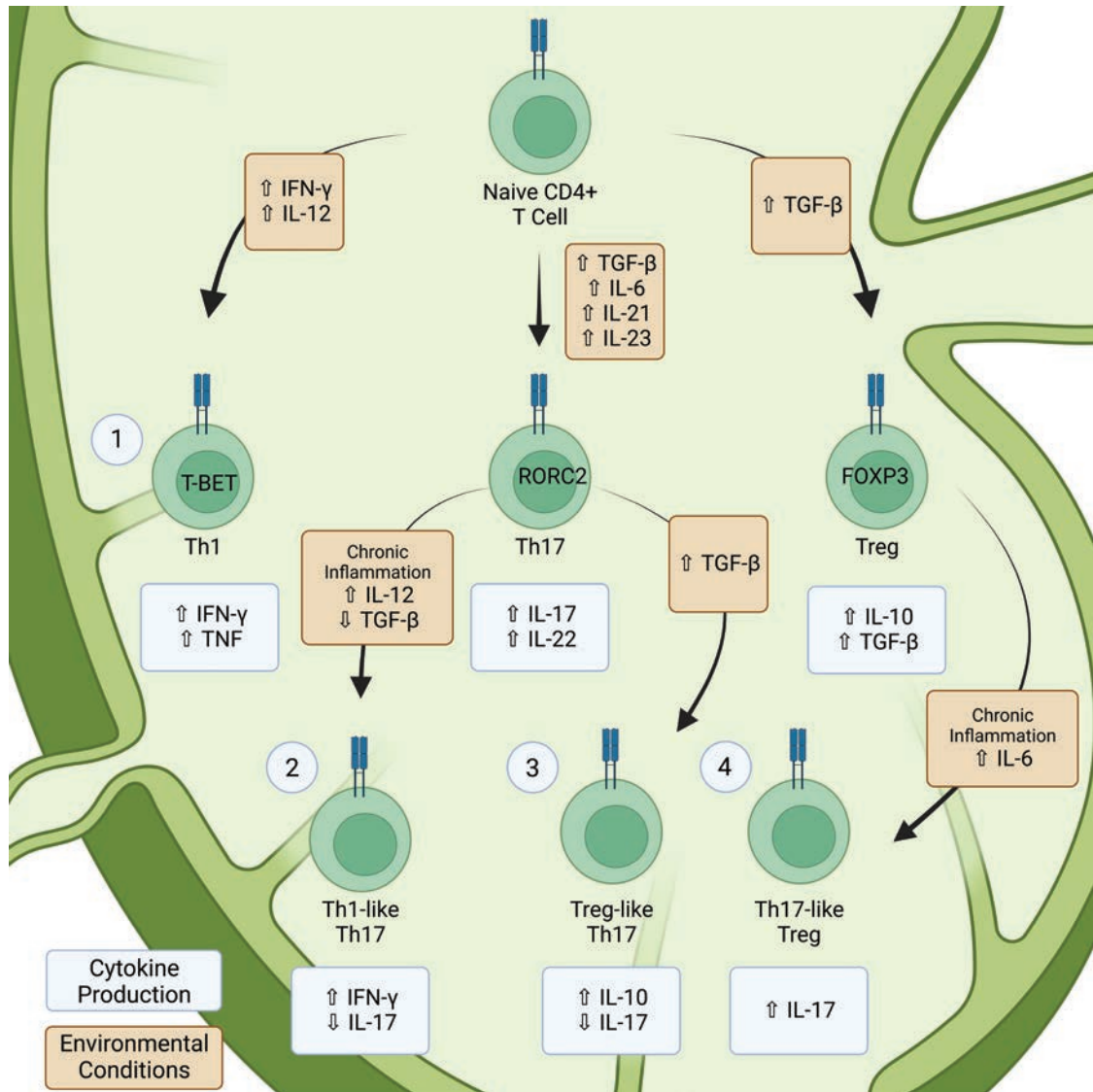


Figure 1. CD4+ T cell plasticity. Under homeostatic conditions, naïve CD4+ T cells are capable of skewing towards any effector subtype, an ability defined as plasticity. Typically, plasticity is progressively lost through differentiation as a CD4+ T cell skews toward Th1, Th2, Th17, or Treg subsets. 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 inflammation⁴⁶ (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 ST6GALL, 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 complement⁶⁷ 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

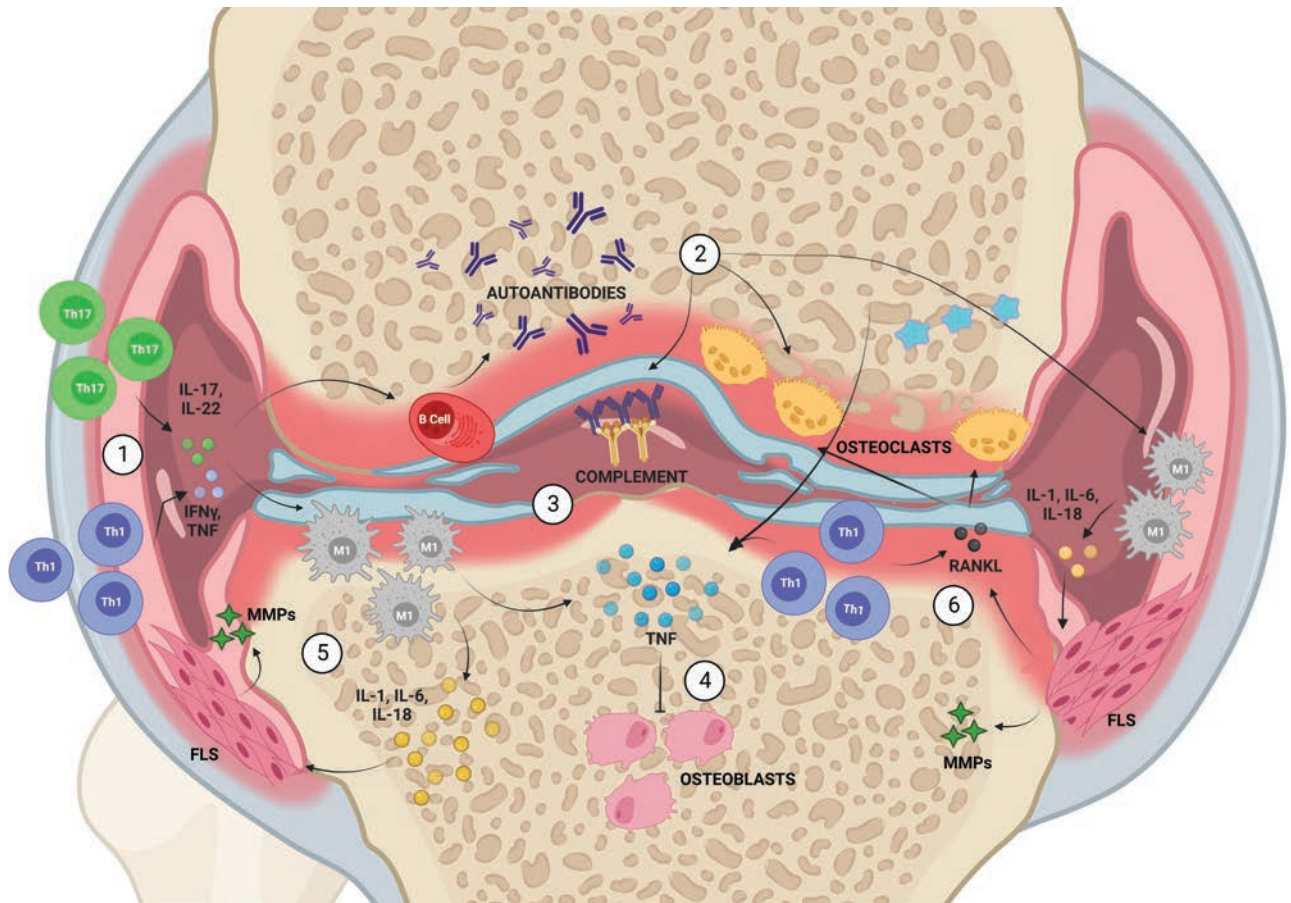


Figure 2. Overview of rheumatoid arthritis (RA) pathogenesis. Inflammatory helper T (Th) cell lineages, Th1 and Th17, induce activation of B cells and macrophages through the release of cytokines such as IFN- γ , TNF, IL-17, and IL-22 (1). B cells produce autoreactive antibodies (2) that mediate tissue 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 predominant 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 hematopoietic 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-1 β 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 induction⁸³ (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 (ToIDC) has also been studied. This approach involves incubation of patient-derived progenitor DCs that are pulsed with RA autoantigen under tolerizing conditions (typically,

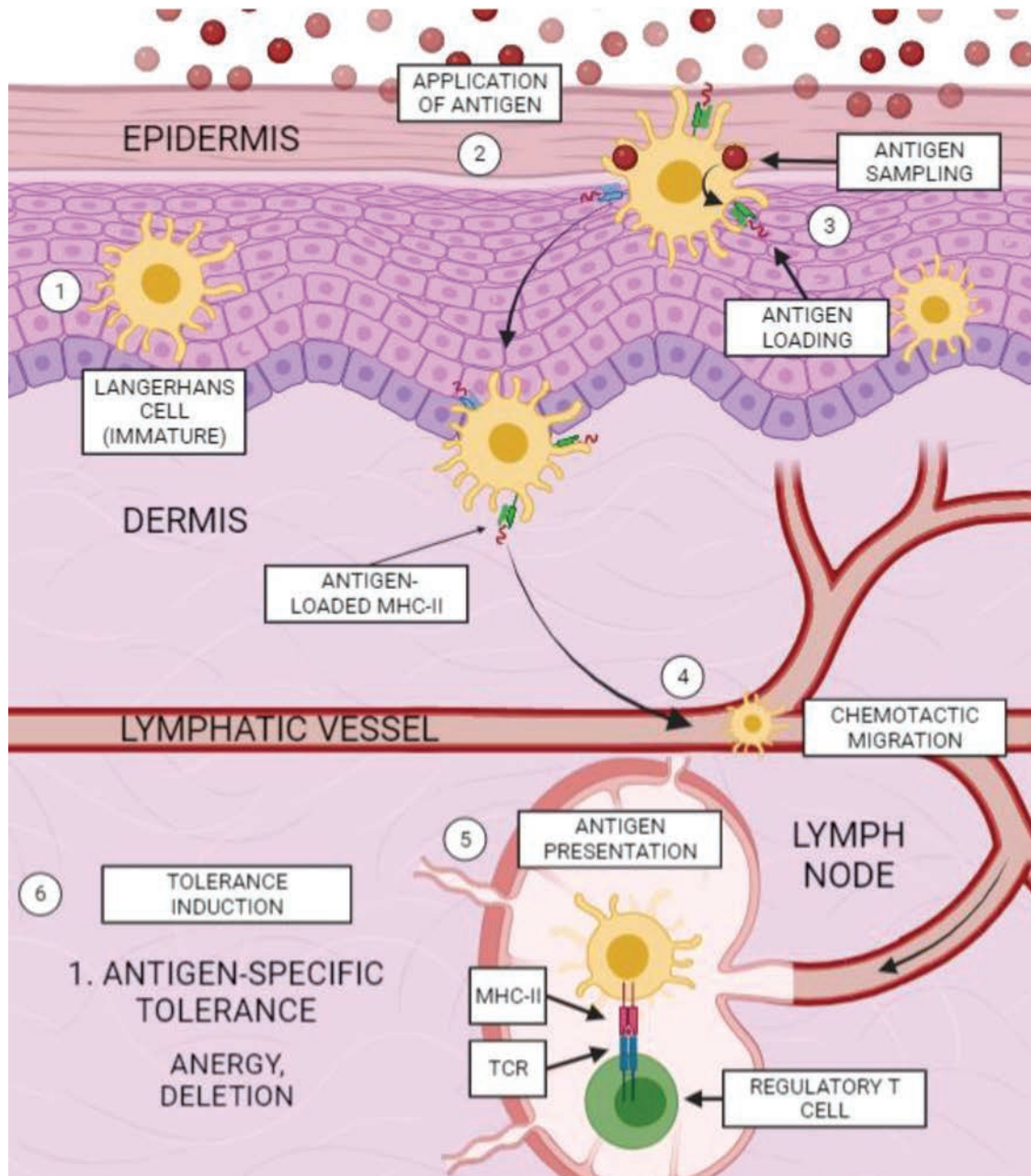


Figure 3. Antigen-specific tolerance induction via transdermal immunotherapy. Langerhans cells (LCs) are tissue resident immune cells that can be harnessed for antigen-specific tolerance induction (1). When an antigen of interest is applied to the epidermis (2), it is taken up by LCs constantly sampling 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- κ B 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 ToIDC treatment, the same group developed CII-loaded liposomes (DEN-181) that can induce CII-specific ToIDC 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.

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