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Rheumatoid arthritis vaccine therapies: perspectives and lessons from therapeutic ligand epitope antigen presentation system vaccines for models of rheumatoid arthritis

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Abstract

The current status of therapeutic vaccines for autoimmune diseases is reviewed with rheumatoid arthritis as the focus. Therapeutic vaccines for autoimmune diseases must regulate or subdue responses to common self-antigens. Ideally, such a vaccine would initiate an antigen-specific modulation of the T-cell immune response that drives the inflammatory disease. Appropriate animal models and types of T helper cells and signature cytokine responses that drive autoimmune disease are also discussed. Interpretation of these animal models must be done cautiously because the means of initiation, autoantigens, and even the signature cytokine and T helper cell (Th1 or

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Th17) responses that are involved in the disease may differ significantly from those in humans. We describe ligand epitope antigen presentation system vaccine modulation of T-cell autoimmune responses as a strategy for the design of therapeutic vaccines for rheumatoid arthritis, which may also be effective in other autoimmune conditions.

Keywords

arthritis-specific antigens; arthritogenic epitopes; autoimmune disease; bystander effect; epitope spreading; rheumatoid arthritis; signature cytokine; therapeutic vaccines

Chronic autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus, multiple sclerosis (MS), Type I diabetes and psoriasis, among others, affect large numbers of individuals, account for major expenses for therapy and hospitalizations, for lost time from work, and significantly compromise the quality of life. Currently, there is no cure for these diseases and only treatments are available. To date, treatments for autoimmune diseases have been directed at alleviating the symptoms (e.g., NSAIDs), or non-specific elimination of activated immune cells (by corticosteroids), or the inflammatory immune response driving the disease (disease-modifying anti-rheumatic drugs [DMARDs], including biologics). Biologics target the cytokines or cell surface receptors that are responsible for maintaining the autoimmune and inflammatory disease processes. Although somewhat more selective, this is also an ablative therapy that leaves the patient deficient in certain types of immune protection. These therapies are very expensive and must be administered by specialists on a regular schedule. Furthermore, the ineffectiveness of current treatments for 30–50% of RA patients [1–5] demonstrates the need for new approaches to therapy.

An alternate approach to therapy, which we discuss in this review, is to actively modulate the ongoing aberrant immune response with a vaccine, so that the immune response no longer promotes disease. The Institutes of Medicine published a major report on ‘vaccines for the 21st century’ [6] in which they identified, based on economic and health care analysis, the three most frequent autoimmune diseases (RA, MS and systemic lupus erythematosus) to be targeted for vaccine development. However, even after 15 years, there are no vaccines to prevent or provide therapy for autoimmune conditions in the marketplace or even late stage clinical studies. It is, therefore, time to take a careful reexamination of the disease process and how a vaccine could work to treat an autoimmune disease, using RA as the example.

This review will provide an overview of the immune responses that mediate autoimmune diseases, different types of treatments and the vaccine approaches that can modulate T-cell function to provide beneficial effects in autoimmune diseases such as RA.

Autoimmune diseases

Nature of autoimmune responses

Chronic autoimmune diseases result from tissue damaging inflammation that is initiated and reinforced by improperly regulated immune responses to self-antigens (Figure 1). Responses

to antigens, including self-proteins, arise from the genetically random generation of the antigen-binding region of T-cell receptors and B-cell receptors, that is, immunoglobulins, during the development of T cells and B cells. Normally, the immune system is tolerant of self-antigens. The main mechanism of T cell tolerance is negative selection of high-affinity self-reactive clones of T cells in the thymus. Treg cells that also develop in the thymus maintain tolerance to self-antigens in the periphery. Peripheral T-cell tolerance is also maintained by clonal deletion and anergy [7–10] and reviewed in [11]. During B-cell development, antibodies that bind self-antigen are altered by receptor editing, where B cells undergo a second immunoglobulin gene recombination or, if this is not successful, default to clonal deletion in the bone marrow. In the periphery, self-reactive B cells are either anergic or the immunoglobulin gene somatically mutates away from self-reactivity [12,13].

Autoimmune diseases are caused due to an aberrant immune response that overrides the normal mechanisms of immune tolerance and occur more frequently in those with a genetic predisposition as evidenced by correlations with MHC type and other RA susceptibility genes. The triggers for initiating the override of tolerance are not fully known, but may include chemical modification of proteins (e.g., citrullination, glycosylation or carbamylation), exposure of the immune system to microbial antigens that appear similar to host proteins (i.e., molecular mimicry) (e.g., M protein of *Streptococcus pyogenes* and rheumatic fever self-antigen), or inducers of cytokine storm that overrides the Treg cell control of self-reactive responses, such as virus infection or superantigens. Generally, a combination of several of these factors is required for disease development.

Although antibodies may be the earliest indicators of an autoimmune disease, $CD4^+$ T helper (Th) cells are ultimately responsible for helping B-cell antibody production and maintaining disease chronicity. These Th cells are induced by different triggers and the subsequent response largely depends upon the cytokine repertoire that they produce. As shown in Figure 1, several T-cell types can be involved, including Th1, Th2 and Th17 [14], Treg [15–17], perhaps even follicular T helper cells [18]. Th2 responses mediated by the cytokines IL-4, IL-5, IL-10 and IL-13 may promote the initial antibody response. During an acute inflammation and in the presence of antigen, dendritic cells (DCs) can direct Th17 differentiation by the production of TGF-β and IL-6, whereas IL-23 is important in maintaining the Th17 phenotype. The Th17 response is characterized by the production of IL-17 and TNF-α which activate epithelial/synovial cells and trigger the recruitment of neutrophils into an inflammatory site. Neutrophils play a prominent role in RA by releasing degradative enzymes (including various matrix metalloproteinases), reactive oxygen species, chemokines and cytokines, as demonstrated in animal models [19]. DCs producing IL-12 promote a Th1 response characterized by the production of IFN-γ, IL-2, TNF-β and TNF-α, which activate inflammatory responses in macrophages including production of cytokines IL-1, TNF-α and IL-6, as well as reactive oxygen species and matrix-degrading enzymes (for more details, see legend to Figure 1). Treg cells produce TGF-β, IL-10 and IL-35 [20], which suppress the activation of T cells, macrophages and DCs. Treg cells can be converted to Th17 cells in the presence of IL-6 and other cytokines [21].

The chronic maintenance of the autoimmune response requires a self-sustaining cycle of cell activation which is fed by the tissue damage caused by inflammation. According to

Holmdahl *et al.*, the process of an autoimmune disease can be divided into three stages: innate/adaptive/inductive immune stage involving environmental factors, T- and B-cell responses and antibody production; the tissue inflammatory stage characterized by acute inflammation; and the chronic clinical phase where widespread tissue inflammation with destruction and remodeling are dominant. Therapy is normally initiated at the third stage [22], but should be initiated earlier as irreversible damage to joints, tissue, neurons, etc. often occurs during this chronic clinical phase. Macrophages and DCs phagocytose tissue debris and the proteins are processed into peptides that bind to MHC class II molecules. Whether a peptide can bind and which peptides can bind are determined by the structure of the MHC class II molecules (i.e., genetic predisposition). These antigen presenting cells (APCs) then activate $CD4^+$ T cells via engagement of the T-cell receptors and co-stimulatory molecules. The APCs (particularly macrophages) and T cells produce cytokines that can promote activation and recruitment of other cells to the site of inflammation and induce tissue damage. The repertoire of cytokines produced by these cells determines the nature of the subsequent response. The activation signals override regulatory cytokines (TGF-β, IL-35 and IL-10) and antagonists, for example, IL-1 receptor antagonist produced by Treg cells and other cells. Ultimately, the tissue damage contributes to the cycle of activation resulting in further tissue destruction and chronic disease.

The cells and cytokines that are involved in the RA disease process and that offer potential targets and markers of disease and therapy are reviewed by Burmester *et al.* [23]. The importance of $CD4^+CD25^+Foxp3^+$ Treg cells is demonstrated in experimental animals by the development of autoimmunity in the absence of Treg cells, which can be reversed by adoptive transfer of such cells [24]. Antigen-specific Treg cells elicit cytokines (IL-10 and TGF-β) that suppress the activation of lymphocytes and inflammatory cells [15,23]. The mechanisms by which Treg cells are impaired or deficient and how to correct the regulatory defect should be considered in design of therapeutic vaccines for RA [25].

Antigens & epitopes

The specificity of the autoimmune response largely determines which tissues and organs will be attacked and, hence, the type of resulting disease. For example, in RA, the affected tissue and the resultant disease involve articular cartilage, synovium and bone in the joints. As for RA, more than one self-antigen may be responsible for triggering and/or maintaining the autoimmunity, and self-antigens may differ in different individuals. Depending upon the antigen and how the autoimmune response was initiated, the type of inflammatory response may also be different. Furthermore, chronic autoimmunity involves continuous acquisition of new self-recognition targets, a process referred to as epitope spreading [26,27].

Arthritogenic epitopes including neoepitopes created by post-translational modification—For autoimmune diseases, the disease-related antigen may consist of quite large, polymeric, often insoluble proteins or complexes, or chemically or enzymatically modified proteins. Single epitopes for T cells may be as small as eight amino acids, based on their ability to bind to both MHC and the T-cell receptors [28]. Some proteins can also be chemically or enzymatically modified *in situ*, a process called post-translational modification (PTM) [29], including citrullination (conversion of an arginine to a citrulline

residue in the protein), carbamylation of lysine residues to form homocitrulline (both of which may elicit anti-citrulline protein antibodies (ACPA) in RA patients and galactosylation of serine and threonine residues [30,31]. PTM of arginine is the result of the catalytic activity of endogenous enzymes called peptidylarginine deiminases [32,33] of which there are several isoforms and certain peptidylarginine deiminase isoforms are associated with RA. PTM of lysine is due to the activity of myeloperoxidase found in neutrophils [34]. Peptidylarginine deiminase, myeloperoxidase and other enzymes mediating PTM are involved in neoepitope formation and subsequent epitope spreading in RA [35–39].

The self-proteins implicated in RA include type II collagen, the proteoglycans (PG) aggrecan, vimentin, fibrinogen and filaggrin, which are abundant in the joints, as well as joint-unrelated proteins such as self-IgG (a target of rheumatoid factor [RF]). Likewise, while the prime proteins for other autoimmune conditions may be specific to the target organ, other molecules (e.g., DNA) can trigger autoreactive responses.

Arthritogenic immunodominant epitopes have been identified within collagen type II [40,41] and PG [42,43], especially the first globular (G1) domain of PG [38,44,45]. They have been defined as arthritogenic epitopes in animal models of RA and in the human disease, as evidenced by the specific cellular and humoral responses elicited by them [22,46,47].

Animal models of autoimmune diseases

Although it is possible to analyze the maintenance phase of an autoimmune disease in humans who already have the disease, it is usually not possible to ascertain the patient's past history and determine the origin and causes of the disease as is possible in animals. Animal models allow establishment of a disease which generates a similar phenotype (e.g., symptoms, histopathology, serology, etc.) as the human disease. The mechanism of initiation, antigen, time course and the nature of the $CD4^+$ T-cell response driving the autoimmune inflammation can be determined in animal models. Even so, the model may not fully represent what occurs in humans. Besides RA, animal models exist for other autoimmune diseases (not discussed in this review).

Animal models of arthritis—There are several excellent recent reviews on animal models for arthritis [10,19,23,48,49]. Inflammatory/autoimmune arthritis can be induced in defined strains of inbred mice which are genetically homogenous with very well-defined and controlled means of disease induction. It is also possible to dissect the nature of the autoimmune response using purpose bred mice with defined genetic deficiencies, or those that overexpress cytokines or cytokine receptors, or express the relevant human protein(s), to study their role in the disease process. The so-called humanized mouse can express human cell surface molecules including MHC antigens and toll-like receptors (TLR). Animal models allow evaluation of treatments with a variety of therapeutic agents before introduction of these agents in human clinical trials. Ultimately, however, a rodent (mouse or rat) is not a human and the inbred nature of research animals can be a disadvantage because it makes it difficult to translate findings to the genetically much more heterogeneous human population. Also, many models only possess some of the traits of human disease, are often manipulated for accelerated disease progression and are examined for only the early or

initial disease process phases as opposed to what normally happens in humans, and may therefore not be as reflective of the human condition as desired.

For each of the autoimmune models of arthritis, it is important to keep in mind how the model is initiated (antigen, adjuvants, how many applications, route of administration), the time course of disease progression, effector phase symptomatology, key markers of the disease process and the nature of the dominant immune response. RF and ACPA [50] are the key elements present in human RA, but are detectable only in a few of the models. Table 1 presents some of the key features for induction of arthritis in the different mouse models, including antigens, adjuvants, methods of immunization, time course of the disease, cytokines that exacerbate or ameliorate arthritis, as well as other key factors and features of each model.

Arthritis models induced in rodents are usually defined by the inducing agent(s), and include: type II collagen-induced arthritis (CIA), PG-induced arthritis (PGIA), pristine-induced arthritis, adjuvant arthritis and anti-collagen antibody–induced arthritis [47,51]. There are a number of other arthritis models (e.g., antigen-induced arthritis or spontaneous arthritis models in genetically manipulated animals such as K/BxN, SKG or TNF-α transgenic mice), which develop some form of joint inflammation, but the discussion of these models is beyond the scope of this review.

CIA is induced most often with bovine type II collagen in young male DBA/1 mice [52] which are MHC class II A^q-expressing mice. CIA can be induced in C57BL/6 mice [53], which express the b haplotype of MHC class II using chick type II collagen and a different immunization protocol [54], but the T-cell response in these models may not be initiated against collagen. For the CIA model, some disease markers, such as ACPA, are detected at low levels, but ACPA levels may increase with more aggressive immunization resulting in more severe disease [32,33,55]. Arthritis induced with cartilage PG [56,57] or with the recombinant N-terminal G1 domain of PG (GIA) [58] in aging BALB/c females more closely resembles human RA than the arthritis induced in other animal models. Similarities with RA include high susceptibility of older female mice to PGIA or GIA, the recessive mode of disease inheritance and the presence of genetic susceptibility loci that are also found in RA patients [58,59]. As in humans, mice with PGIA or GIA also produce both RF and ACPA, and exhibit spondylitis not usually seen in most other rodent models (Table 1) [58].

In each of these animal models, one of the most important questions to be answered is which Th cytokine repertoire drives the disease, Th1 or Th17. The cytokine signature is a key determining factor for developing immunomodulatory therapies for RA or other autoimmune diseases, as discussed in [60]. Determination of the Th cytokine repertoire may be complicated because different inducers and mechanisms of induction elicit different responses, and different strains of mice and even mice of the same strain from different vendors may exhibit different responses [61]. In addition, more recent literature suggests that the Th phenotype is plastic and Treg cells can convert to Th17 cells [21] and in man, Th17 cells can convert to Th1 cells, as seen at inflammatory sites such as in the synovial fluid of patients with juvenile idiopathic arthritis [62].

The importance of the route of immunization used for disease induction is clearly illustrated in the PGIA model. Although disease severity (arthritic index and histopathology), the presence of spondylitis, and even serum factors such as RF and anti-PG antibodies are similar, the dominant cytokines indicative of a Th1 or Th17 response differ depending on the route of immunization (intraperitoneal vs intradermal) used for arthritis induction [63]. The potential for Th1 or Th17 signature phenotypes in the same animal model has also been noted for animal models of MS [64] and uveitis [65]. It is also likely for humans that the anatomical location and type of insult (mechanical injury, viral infection or environmental factors such as smoke or exposure to chemicals) may influence the dominant or signature disease-driving cytokine profile.

Current therapies for RA

DMARDs are divided into the categories of small synthetic molecules (drugs) and biologics (also referred to as disease-modifying immunotherapies) [66,67]. The synthetic drugs include immunosuppressants such as methotrexate, leflunomide, tofacitinib and glucocorticoids such as prednisone. Although these function by inhibiting a diverse array of biological pathways, their main effect is on lymphocytes and cytokines. The biologics include cytokine inhibitors and receptor antagonists such as infliximab, etanercept, anakinra, abatacept, tocilizumab and rituximab. The biologics can be monoclonal antibodies against a cytokine, against a cytokine receptor or can be a recombinant soluble cytokine receptor that competes with the cell surface receptor. Biologics neutralize the effects of pro-inflammatory cytokines, such as TNF-α (infliximab, etanercept, adalimumab, golimumab, certolizumab pegol), IL-1β (anakinra), or IL-6 (tocilizumab), and some of them, such as abatacept and rituximab, target cell surface molecules on T and B cells, respectively. It should be noted that a significant proportion of RA patients do not respond to these treatments [1–5].

Although NSAIDs and corticosteroids are still used for treating the symptoms of RA [68], current recommendations for intervention involve early and aggressive treatment in order to suppress disease for as long as possible [69,70]. In addition, corticosteroids and NSAIDs cannot delay clinical disease progression and there are complications associated with long-term use or high dosages.

Some of the negative aspects of DMARDs/biologics and pathway inhibitors (e.g., kinase inhibitors such as tofacitinib) are that they are expensive, difficult to administer, ablate protective immune responses and have significant contraindications. These treatments incapacitate critical immune functions (e.g., TNF-α responses) that are important for controlling infection or cancer [71].

In contrast to DMARDs, immunomodulatory vaccine treatments would be preferable since they should be less expensive per dose, more stable, have less rigid storage requirements (regarding temperature, sensitivity to light, relative humidity and storage time) and may be easier to administer. Vaccines can also be much more disease specific since they can be designed to target the antigen involved in the induction process as well as those that maintain the disease. In order to be therapeutic, the vaccine must be able to activate the appropriate T cells to modulate the production of cytokines responsible for maintaining the

autoimmune disease [60]. By altering the ongoing autoimmune response, the vaccine should be able to break the cycle of immune activation.

Types of vaccines

Immunization may be passive, if a preformed antibody is administered, or active if an antigen is administered to elicit an immune response by the individual. Most vaccines have been developed to prevent microbial disease as a prophylactic treatment and often act via induction of antibodies. These vaccines contain non-self (foreign) antigens. Vaccines for autoimmune diseases, like anti-tumor vaccines, would target the immune cells involved in processing or recognizing self-antigens, but would need to reinforce tolerance or modulate the autoimmune responses to self-antigens or modified self-antigens rather than promote attack responses.

Classical antibody eliciting vaccines

Most anti-viral and anti-bacterial vaccines other than those containing live agents (including attenuated agents) consist of inactivated microbes or a bolus of purified native or recombinant proteins derived from the microbes. These vaccines initiate a Th2 helper T-cell response which is dominated by IL-4, IL-5, IL-10 and IL-13 cytokines. Most of these vaccines are formulated with alum as adjuvant.

Although antibody neutralization of small molecule mediators, such as angiotensin or nicotine, to treat hypertension or to prevent smoking, may be effective as therapeutic vaccines, vaccines eliciting antibodies to autoantigens involved in autoimmune disease are likely to exacerbate the condition rather than be helpful. Antibodies against self-antigens can initiate complement activation and hypersensitivity reactions which cause inflammation and tissue damage, potentially leading to chronic autoimmune responses.

In order to counteract an autoimmune response, it is more likely that a therapeutic vaccine will need to activate only T-cell–mediated immunity and direct that response to suppress the ongoing inflammation. There are relatively few vaccine approaches that activate only T-cell responses.

Vaccines that induce T-cell–mediated immunity

Classically, vaccines consisting of attenuated viruses or bacteria elicit T-cell–mediated immune responses in addition to antibody. These vaccines initiate an innate response which transitions into the antigen-specific immune response. Similar responses are achieved to hybrid virus or bacteria vaccines which incorporate a gene for the desired antigen. Th1 and Th17 cell-mediated responses to protein vaccines can be obtained in addition to antibody by using adjuvants that mimic the innate response, such as TLR agonists. DNA and RNA vaccines generally stimulate cell-mediated responses without detectable antibody production. After priming with the genomic vaccine, antibody production can be promoted by boosting with the protein antigen [72,73]. Cell-mediated immune responses can also be obtained with peptide vaccines, as discussed subsequently.

Unlike infection-preventing or anti-tumor vaccines which have goals of eliminating a target infectious agent or tumor cell, a therapeutic vaccine for an autoimmune disease should elicit T-cell–mediated immunity that counteracts the inflammatory events associated with the autoimmune response. Like anti-tumor vaccines [74,75], therapeutic vaccines for autoimmune diseases would also augment responses to a self-antigen; but unlike anti-tumor vaccines, the therapeutic vaccine for an auto-immune response should activate or enhance immunomodulatory T cells (e.g., Treg) or generate antagonistic cytokines to block the inflammatory Th1 or Th17 responses involved in the disease [25,76,77]. This can be achieved by a direct effect on APCs or T cells, but may also occur by a bystander mechanism [78–80].

Composition of vaccines

A therapeutic vaccine will consist of the antigenic epitope, protein or its gene that initiates or drives the autoimmune response. The epitope or protein will usually be mixed with an adjuvant that activates APCs (especially DCs) or can induce Treg cells or will interact directly with the patient's autoreactive T cells. Classical adjuvants include alum, monophosphoryl lipid A (MPL) QS21, incomplete Freund's adjuvant, MF59 and ISA51, which enhance antigen uptake and the activation of APCs. Indeed, even cytokines are being used as adjuvants. As for anti-tumor vaccines, vaccines for autoimmune diseases may require the use of special adjuvants that direct the response of $CD4^+$ Th cells and their cytokine repertoires to elicit modulatory or suppressive rather than inflammatory responses [81,82]. As with any drug, the more complex the formulation or means of delivery, the more difficult will be the approval and implementation of the therapeutic vaccine.

Vaccine immunogens

The immunogens for therapeutic vaccines consist of peptides, glycoproteins or lipoproteins. In order to elicit a specific response, a peptide representing an epitope of a disease-related autoantigen can be used and conjugated to an 'immune activator' compound or incorporated into a larger structure, such as a nanoparticle. Various peptide formats and adjuvant formulations have been developed to enhance immunogenicity, but relatively few promote a tolerizing or suppressive effect. In some cases, immunization with a vaccine containing one antigenic epitope will result in immune responses to related epitopes in the process of epitope spreading [26,27] leading to the desired response to an unknown autoimmune disease-driving epitope [26,27]. Alternatively, the vaccine may consist of DNA or RNA sequences encoding the immunogenic protein [72,73]. The DNA or RNA construct can also incorporate genes for cytokines to modulate the subsequent inflammatory response. Although genomic vaccines elicit primarily T-cell responses, DNA and RNA can also elicit responses such as IFN-α and anti-nucleic acid antibodies, which could potentially worsen the autoimmune condition. Most nucleic acid vaccine development is directed at infectious diseases or tumors in which cytotoxic reactions are the desired response rather than immunomodulation.

Conjugate vaccines

Peptide conjugate vaccines allow immunization with peptide epitopes of defined antigens. The antigenic peptides are generally attached to a carrier molecule, a large protein or a special peptide that can enhance its uptake by APCs, promote the activation of APCs and affect the nature of the subsequent immune response.

Large conjugates—Large conjugates are formed by attachment of the antigenic peptide or protein to a carrier protein, such as keyhole limpet hemocyanin (KLH). These conjugates generally elicit antibody responses. TNF-α–KLH conjugates have been proposed for treatment of RA [83] to elicit production of antibodies against TNF-α. The problem with KLH conjugates is that KLH itself is very immunogenic and can cause severe hypersensitivity reactions. Somewhat smaller conjugates are made using cholera toxin or diphtheria toxin [84] to generate antibody responses. MHC molecules have also been used as carriers for peptide epitopes [85].

Small-sized conjugates—Small-sized peptide conjugates include single-chain synthetic long peptides (SLPs), pan DR epitope (PADRE), invariant chain peptide (Ii-Key), TLR and the ligand epitope antigen presentation system (L.E.A.P.S.) platforms. These conjugates can consist of a small disease-associated antigenic peptide (as small as 8–9 amino acids, representing a single T-cell epitope) or multiple or even overlapping epitopes (SLP) attached to a peptide that promotes immunogenicity and perhaps cell binding. Peptides can be readily synthesized under Good Manufacturing Practices conditions and mixtures of peptide conjugates can be administered [86] to ensure that the appropriate response is elicited in individuals with different MHC backgrounds and different compositions of the self-epitope repertoire.

Synthetic long peptide: SLPs contain 20–50 amino acid residues consisting of overlapping, nested or adjacent epitopes derived from the native protein. SLPs have been developed for influenza [87] and for therapeutic treatment of HPV-containing cervical carcinomas. The longer peptides (23–45) of SLPs elicited much more potent T-cell responses in preclinical immunology and tumor therapy experiments than that elicited by short major MHC class I-binding peptides (as paraphrased from [88]). Other SLPs incorporating TLR ligands with the antigenic peptide are reviewed in [89,90].

pan DR epitope: PADRE was developed and studied mainly by Epimmune Corp. The PADRE is derived from a peptide from tetanus toxoid that binds to several of the most common types of human MHC class II molecules (HLA-DR). Conjugation of this PADRE peptide to the antigenic peptide promotes association with the HLA-DR molecule on APCs and activation of $CD4^+$ T cells, and was studied in a rat adjuvant arthritis model using the cytokine B-cell activating factor as antigen [91]. Baleeiro *et al.* developed a PADRE-based vaccine that is particle bound and interacts with and activates DCs, whereas the soluble form does not activate DCs [92].

Ii-Key: Ii-Key utilizes a peptide from the invariant chain of the human MHC class II protein. The invariant chain binds tightly to the antigen-binding cleft of MHC class II in

APCs. Conjugation of the Ii-Key peptide to an antigenic epitope facilitates the binding of the antigen to the MHC class II molecule and presentation to $CD4^+$ T cells [93].

Toll-like receptor: TLR agonists made from flagellin [94] or other molecules (lipid A, β-defensins or chemokines) can be added to antigens to facilitate binding to DCs and other APCs, and at the same time, activate the APCs through the TLR. These TLR agonists can be either large or small, and may contain either complete or only part of the agonist [95]. Although flagellin has been used for influenza vaccines, it is unlikely to be used in RA vaccines as it can exacerbate disease in mice with CIA [96]. Likewise, other TLR agonists such as lipid A may also induce TNF-α and/or IL-17 production [Evans J, Pers. Comm.].

The L.E.A.P.S: The L.E.A.P.S.™ (henceforth referred to as LEAPS) technology creates a heteroconjugate in which an immune cell binding ligand (ICBL) is attached to the antigenic peptide. The two most common ICBLs are the J peptide, a sequence from β-2-microglobulin and derG (or G), a sequence from the β chain of MHC class II. The ICBLs convert small peptides into immunogens, and the J-ICBL directs the immune response toward a Th1 response while the derG ICBL directs the immune response to a Th2 response [97,98]. The immunogenic peptides can be larger than the minimal MHC epitopes and even reach the size of an SLP (15–35, usually 20–30, residues) (as discussed earlier in the section ‘Synthetic long peptide’).

Altered peptide ligand: Altered peptide ligand vaccines utilize peptides in which one or more of the amino acid residues of an antigen are substituted with another amino acid residue to alter the immunogenicity, modify the isoelectric charge, stabilize the peptide or otherwise reduce the possibility of an adverse reaction to the peptide. Ultimately, the altered peptide must serve the original purpose of evoking the desired immunomodulatory response [99,100].

Examples of vaccines proposed for the treatment of RA

Therapeutic vaccines have been developed for RA and tested in various animal models or in clinical trials (Table 2). A vaccine that can control the self-directed T cells which are promoting the autoimmune response would get to the root of the problem. This vaccine would elicit an antigen-specific modulating response. While several technologies, as discussed in the section ‘Composition of vaccines’, have the potential to modulate T-cell activity, other than the LEAPS technology, they do not define the direction of the subsequent antigen-specific response toward Th1, Th2, Th17 or Treg cells. The LEAPS technology can direct the nature of the subsequent antigen-specific T-cell response toward Th1, Th2 or perhaps Treg cells, depending upon whether the J or derG ICBL is attached to the antigenic peptide.

The subsequent discussion of different vaccine approaches will describe their testing in animal models. As indicated above, it is difficult to compare the results between different studies and different labs because of the many variables that can affect the nature of the immune response driving the autoimmune disease in animal (mouse) models. These variables include the mouse strain (even the source of the mouse), the antigen used to induce

the disease and the route/method of delivery of the antigen. Earlier studies did not correctly distinguish the Th1 and Th17 responses or the IL-12 and IL-23 cytokines that induce these responses. In addition, the Th17 response may only be a bystander to the Th1-associated cytokines driving the disease, as in the PGIA model [101], or may be the driving force behind the disease, as described in the CIA model in both DBA/1 and C57BL/6 mice [102].

Many of the vaccines developed for RA focus on blocking TNF-α action. Chackerian *et al.* [103] developed a virus-like particle (on an HPV backbone) of TNF-α conjugates, and others attached TNF-α to KLH and tested the immunogenicity of these vaccines in normal mice [83].

Kochetkova *et al*. [104] used a vaccine composed of a *Salmonella* vector expressing colonization factor antigen delivered orally 7 days before disease induction, which protected DBA/1 mice from CIA. Likewise, Luross *et al.* [105] reported that a heat-labile enterotoxin B–containing vaccine prevented arthritis in the DBA/1 CIA model.

Antigen-specific T-cell modulating vaccines have been developed using the LEAPS technology. A LEAPS vaccine [106] was shown to prevent and treat autoimmune disease in the CIA model and induce a reduction in the Th17 response that drives inflammation in CIA. Other antigen-specific arthritis vaccines include the CTA1R7K-COL-DD fusion protein vaccine composed of cholera toxin, the type II collagen peptide CII259-274 and Staphylococcus protein A [84], or a gal-CII259-273 peptide complexed with MHC class II [85], all of which were tested in the DBA/1 CIA model and showed initial indications of therapeutic efficacy. Adoptive transfer of DCs incubated (pulsed) with collagen peptides [107] caused a delay in the onset of arthritis and reduced disease severity in the CIA model. Preliminary studies have been conducted and a preliminary report presented [108] for the efficacy of a LEAPS vaccine in the PGIA model [Zimmerman DH, Kurko J, Mikecz K, Glant TT, Unpublished data].

The most advanced peptide (with regard to clinical progression) which has been tested in human Phase I and II studies is the dnaJP1 peptide. Daily oral administration of the dnaJP1 peptide was safe and well tolerated and in the Phase II report on 160 patients, there was a significant reduction in T cells producing TNF-α and an increase in T cells producing IL-10 [109,110].

Factors to be considered for arthritis vaccines using LEAPS as an example

As previously mentioned, the LEAPS technology has been used to develop prophylactic and therapeutic vaccines which were tested in mouse models. LEAPS vaccines appear to interact with and activate DCs or T cells depending upon the ICBL that is attached to the antigen. The J-ICBL interacts with human monocytes and mouse bone marrow DC precursors to promote their maturation into DCs that promote T-cell responses with the Th1 phenotype. The G (and a more stable version of G called derG) ICBLs interact with CD4 molecules on T cells and promote Th2 responses [97,98,111,112].

J–LEAPS conjugates have previously been determined to enhance or focus on Th1 responses, enhance or stimulate IL-12p70 production [86,113,114], target $CD8^+$ T cells [86],

and result in an increased IFN-γ response in mice with reduced production of TNF-α [86,106,113,114], IL-1, IL-4 and IL-6 [86].

The antigen-specific nature of LEAPS conjugates has been demonstrated by reduced morbidity and mortality as well as by induction of favorable immune responses to disease-related peptides (but not to unrelated peptides) upon challenge of LEAPS conjugate-treated mice with HSV [113] or influenza A [85] and in the CIA model [105]. Antigen specificity was also demonstrated in immunogenicity studies for LEAPS conjugates containing HIV or tuberculosis epitopes [97,98,115].

Initiation of the immune responses appears to require $CD8^+$ T cells, but both $CD4^+$ and $CD8^+$ cells are required for the effector phase as shown by ablation studies in the HSV1 challenge and other immunogenicity studies. After immunization with J–LEAPS™ vaccines, no antibody is detectable but an antigen boost elicits Th1-associated antibody responses which favor production of IgG2a isotype antibodies [86,97,98]. Response in several MHC backgrounds was observed for the J–gD LEAPS conjugate, which had a peptide larger than a minimal epitope, as indicated by protection against HSV1 challenge in six inbred and three outbred strains of mice [116].

Treatment of human monocytes or mouse bone marrow DC precursors with J–LEAPS vaccines promotes their development into DCs, as indicated by increased surface expression of CD80, CD86, CD11c and MHC class II molecules [86,113]. These cells are activated, release IL-12 and promote the development of Th1 immune responses *ex vivo*. As for the peptide vaccines, the adoptive transfer of DCs activated by J–LEAPS vaccines for HSV or influenza enhanced or stimulated the production of IL-12p70 [86,113,114] and activated Th1 responses, but unlike in other approaches using TLR agonists, no TNF-α was generated [97,98].

Indication for the immunomodulatory activity of LEAPS vaccines on established immune responses was obtained in mice infected with influenza virus which then received DCs treated with a J–NP (influenza nucleoprotein) peptide conjugate. These DCs homed to the lungs and counteracted the inflammatory cytokine responses while eliciting a functional antiviral response that reduced morbidity and mortality [86]. A combination of two J–LEAPS conjugates against two different epitopes could be used together and were found to be protective. These treatments initiated protective Th1 responses, as indicated by the cytokine repertoire and the production of IgG2a isotype antibodies, and appeared to reduce inflammation.

The derG–LEAPS conjugates enhance Th2 responses. The derG or G peptide binds to the CD4 molecule [111,117] and modulates its function. As with the J-ICBL, no antibody is generated by LEAPS vaccines unless a protein booster is administered after which IgG1 antibody production occurs indicative of a Th2 response. G or derG conjugate vaccines do not protect against and possibly exacerbate HSV infection due to promotion of Th2 responses [97,98].

LEAPS has an advantage over other peptide epitope-based technologies because LEAPS vaccines can be designed to produce an antigen-specific Th1 or Th2/Treg response

depending upon the ICBL incorporated with the antigenic epitope. Protection or modulation of an ongoing immune response occurs by the activated DCs and/or T cells and the cytokines that they produce. In addition, without an additional boost with antigen, LEAPS vaccines elicit only a T-cell response [97,98,115,118–120].

LEAPS vaccines as immunomodulators for arthritis

Vaccines that can be directed to activate or modulate a response to a specific antigen would provide focused therapy for autoimmune diseases with a minimum of immunosuppression. There are relatively few vaccine approaches that can direct the nature of the antigen-specific T-cell response and can provide an antigen-specific inhibition of the autoimmune and inflammatory responses without generating autoantibodies that induce or perpetuate inflammation.

J–LEAPS conjugates proved efficient for the Th17-driven inflammatory conditions in the experimental autoimmune myocarditis and CIA mouse models by generating Th1 cytokines to modulate the ongoing inflammatory Th17 response. In the CIA mouse model, immunization with CEL-2000 (J–collagen peptide conjugate) converted the disease-generating Th17 response, characterized by high blood levels of IL-17, IL-12p40 (presumably associated with IL-23) and TNF-α, to a regulated Th1-like response, characterized by decreases in IL-17 and IL-12p40, with increases in IL-12p70 and IFN-γ [106,121]. IFN-γ is known to regulate IL-17expression [100]. With biweekly immunizations, the CEL-2000 vaccine stopped the progression of disease as efficiently as the TNF-α antagonist, etanercept [106].

No antigen-specific vaccines have been reported for PG, the other common arthritis-associated antigen used to induce PGIA in mice. In many studies, PG elicits a disease-promoting Th1 phenotype [122] in contrast to the Th17 phenotype of CIA in the DBA/1 mouse model. Pilot studies compared the effects of J–PG70 and derG–PG70 LEAPS conjugates on both antigen-specific proliferation and the ratio of Th1:Th2 cytokine responses of spleen cells from BALB/c mice immunized with PG and exhibiting early PGIA with low arthritis scores. As shown in Figure 2, the J–PG70 conjugate enhances a proliferative response and a higher Th1: Th2 (IFN-γ/IL-4) ratio when evaluated in *in vitro* stimulation assays. In contrast, the derG–PG70 conjugate promotes Th2 responses and lowers the Th1: Th2 (IFN-γ:IL-4) ratios, which is a more favorable outcome for treatment of auto-immunity in this model.

Since the J– and the derG–PG70 conjugates demonstrated different effects on spleen cells from PG-immunized arthritic mice, we next conducted a pilot study using these conjugates in BALB/c mice with PGIA, similar to the therapeutic efficacy study conducted on mice with CIA [106]. As shown in Table 3 for the Th1-driven PGIA model, the derG–PG70 conjugate provided protection from progressive arthritis as evidenced by the modest increase in arthritic index scores as compared to the sharp increase in disease severity in mice treated with adjuvant only or the J–PG70 conjugate. In this model, promotion of a Th1-like cytokine response by the J–PG70 conjugate did not elicit a therapeutic effect, whereas a Th2-like response (demonstrated *ex vivo*) induced by the derG–PG70 conjugate provided a

therapeutic effect *in vivo*. It has been previously demonstrated that IL-4 suppresses the disease in the PGIA model [123]. These results suggest that once the nature of the inflammatory autoimmune response in a patient with RA has been identified (e.g., by analyzing prominent serum cytokine levels), then this patient can be treated with the appropriate LEAPS vaccine with either a J-ICBL to counteract a Th17-driven inflammatory response or a derG-ICBL to counteract a Th1-dominated inflammatory response. Several possible immunogens have been suggested for inclusion into LEAPS vaccines by these early studies and such other immunogens or a mixture of LEAPS vaccines may be appropriate for treating RA. The broad controlling nature of regulatory T-cell responses through their immunosuppressive cytokines may make it less important to immunize against all the disease-related epitopes as long as a response to a relevant epitope is initiated.

Expert commentary & five-year view

Looking into the future, immunotherapy for autoimmune diseases should do less harm by focusing on the cause rather than the effect of the autoimmune response. Figure 3 depicts the progression of RA from the initial arthritis symptoms to the advanced stage of a chronic, debilitating disease. The goal for the future is to progress beyond current therapies that target the symptoms or the immune components that maintain the cycle of inflammation to an immunotherapeutic vaccine that modulates the underlying cause of the disease.

Looking into the future, we also see a clinical need and a way to fulfill that need using vaccine technologies that are antigen specific, are able to direct immune responses that correct the aberrant immune and cytokine responses, and allow for earlier intervention in RA and possibly other autoimmune diseases. Vaccination with peptide conjugates should be less expensive and less toxic than many of the current RA medications, should not be contraindicated in the presence of cancers or infections and would not require intravenous administration in a clinical setting.

The LEAPS vaccines described herein have the potential to act on early events in the autoimmune disease as shown for mouse models of RA, to reset the immune response so that the antigen-specific autoimmune responses are suppressed. If initiated early enough, this intervention can prevent the permanent damage to joints and tissue in the patient suffering from RA. For this reason, early treatments have been encouraged for the current therapy of RA [70]. It should be further noted that while our focus was on RA in this review, LEAPS vaccines could be applied to other autoimmune conditions driven by Th1 or Th17 cytokines. Likewise, other vaccine technologies discussed in the section ‘Composition of vaccines’ may also show promise as long as they elicit an appropriate immunomodulatory response. As we look for vaccine therapies for autoimmune diseases, it is important that the following issues be addressed:

- Appropriate and multiple animal models should be used that closely mimic the human disease to allow choice of appropriate candidates for human testing as well as determination of the parameters that predict success, problems or failure in future clinical trials.

- The immunogen, the adjuvant or other vaccine components should not exacerbate the ongoing disease or initiate immune responses (e.g., autoantibodies) that may lead to adverse effects (see Box 1).
- The therapy should be shown to be safe for the intended population. The safety parameters and benefit to risk ratio for older individuals and those already experiencing chronic disease are likely to be different from younger and non-diseased individuals or patients with early disease.
- Since the effects of vaccines are first analyzed in animals, the appropriate model for the patient population may not exist and appropriate dosing may be difficult to establish without performing clinical (human) trials [124].
- The mode of action of the treatment should be understood to allow prediction of appropriate and inappropriate outcomes.
- The cost to benefit ratio should be better than for current therapies.

Box 1

Cautions regarding severe adverse events following vaccination

SAE include inflammation or anaphylaxis, but are often specific to the animal model and often occur in random individuals within a group. Concerns for humans may not be relevant. SAE were seen in vaccine trials for Alzheimer's disease [152] and for multiple sclerosis [153] as well as peptide treatments [154,155], and were also observed in autoimmune models such as EAE [156], Type I diabetes [157] and perhaps in experimental autoimmune myocarditis [121]. For J-LEAPS™ vaccines, spurious cases of SAE were observed in very young inbred mice (A/J or DBA/1J) only after two closely administered doses, but not in older inbred DBA/1J mice with the same vaccine, or with other J-LEAPS vaccines in BALB/c or C57BL/6 or outbred adult mice populations even after five immunizations over a 150-day time span [Zimmerman DH, unpublished data]. Adverse reactions can be minimized by using modified peptides that neutralize the charge or alter solubility, employing bifunctional agents [158–163] or changing the dosing schedule. Slow-release adjuvants, such as incomplete Freund's adjuvant [164], may be better at reducing an anaphylactic reaction. The SAE may not resemble classical responses such as anaphylaxis, but may be due to high levels of IgG1 or a Type II or III hypersensitivity response rather than classical IgE and Type I hypersensitivity. The nature of the pro-inflammatory T-cell response may also be difficult to predict and may actually be due to a combination of cytokine responses. It is important to remember that our understanding of immunology has progressed and older explanations for SAE may not be applicable anymore. For example, in 2004, McDevitt focused on the role of IL-2 in autoimmune conditions as it was much more recognized than the role of IL-1 or IL-17, and thus, some of his comments need to be tempered based on current knowledge [154].

EAE: Experimental autoimmune encephalomyelitis;

LEAPS: Ligand epitope antigen presentation system;

SAE: Severe adverse events.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Hyrich KL, Watson KD, Silman AJ, et al. Predictors of response to anti-TNF-alpha therapy among patients with rheumatoid arthritis: results from the British Society for Rheumatology Biologics Register. Rheumatology (Oxford). 2006; 45:1558–65. [PubMed: 16705046]
2. Tak PP, Kalden JR. Advances in rheumatology: new targeted therapeutics. Arthritis Res Ther. 2011; 13(Suppl 1):S5. [PubMed: 21624184]
3. Rosman Z, Shoenfeld Y, Zandman-Goddard G. Biologic therapy for autoimmune diseases: an update. BMC Med. 2013; 11:88. [PubMed: 23557513]
4. Kurkó J, Besenyei T, Laki J, et al. Genetics of rheumatoid arthritis - a comprehensive review. Clin Rev Allergy Immunol. 2013; 45:170–9. [PubMed: 23288628]
5•. Van Vollenhoven RF. Switching between anti-tumour necrosis factors: trying to get a handle on a complex issue. Ann Rheum Dis. 2007; 66:849–51. Review and discusion of success and issues for TNF-α associated therapies for arthritis and there was a significant number of non-responsive patents. [PubMed: 17576784]
6. Stratton, KR.; Durch, JS.; Lawrence, RS. Vaccines for the 21st century: a tool for decisionmaking. The National Academies Press; Washington, DC USA: 1999.
7. Akdis M, Burgler S, Crameri R, et al. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. J Allergy Clin Immunol. 2011; 127:701–21. [PubMed: 21377040]
8. Banchereau J, Pascual V, O'Garra A. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. Nat Immunol. 2012; 13:925–31. [PubMed: 22990890]
9. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011; 365:2205–19. [PubMed: 22150039]
10. Sakaguchi S, Powrie F, Ransohoff RM. Re-establishing immunological self-tolerance in autoimmune disease. Nat Med. 2012; 18:54–8. [PubMed: 22227673]
11. Xing Y, Hogquist KA. T-Cell tolerance: central and peripheral. Cold Spring Harb Perspect Biol. 2012; 4:1–15.
12. Pelanda R, Torres RM. Central B-cell tolerance: where selection begins. Cold Spring Harb Perspect Biol. 2012; 4:a007146. [PubMed: 22378602]
13. Sabouri Z, Schofield P, Horikawa K, et al. Redemption of autoantibodies on anergic B cells by variable-region glycosylation and mutation away from self-reactivity. Proc Natl Acad Sci USA. 2014; 111:E2567–75. [PubMed: 24821781]
14. Sarkar S, Cooney LA, Fox DA. The role of T helper type 17 cells in inflammatory arthritis. Clin Exp Immunol. 2010; 159:225–37. [PubMed: 19758374]
15. Notley CA, Ehrenstein MR. The yin and yang of regulatory T cells and inflammation in RA. Nat Rev Rheumatol. 2010; 6:572–7. [PubMed: 20808295]
16. Wehrens EJ, Prakken BJ, van Wijk F. T cells out of control – impaired immune regulation in the inflamed joint. Nat Rev Rheumatol. 2013; 9:34–42. [PubMed: 23390638]
17. Miyara M, Ito Y, Sakaguchi S. TREG-cell therapies for autoimmune rheumatic diseases. Nat Rev Rheumatol. 2014; 4:1–9.
18. Chu Y, Wang F, Zhou M, et al. A preliminary study on the characterization of follicular helper T (Tfh) cells in rheumatoid arthritis synovium. Acta Histochem. 2014; 116:539–43. [PubMed: 24287433]
19. Wright HL, Moots RJ, Edwards SW. The multifactorial role of neutrophils in rheumatoid arthritis. Nat Rev Rheumatol. 2014; 10:593–601. [PubMed: 24914698]

20. Niedbala W, Wei X-Q, Cai B, et al. IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. Eur J Immunol. 2007; 37:3021–9. [PubMed: 17874423]
21. Zheng SG. Regulatory T cells vs Th17: differentiation of Th17 versus Treg, are the mutually exclusive? Am J Clin Exp Immunol. 2013; 2:94–106. [PubMed: 23885327]
22. Holmdahl R, Malmström V, Burkhardt H. Autoimmune priming, tissue attack and chronic inflammation - the three stages of rheumatoid arthritis. Eur J Immunol. 2014; 44:1593–9. [PubMed: 24737176]
23. Burmester GR, Feist E, Dörner T. Emerging cell and cytokine targets in rheumatoid arthritis. Nat Rev Rheumatol. 2014; 10:77–88. [PubMed: 24217582]
24. Van Herwijnen MJC, Wieten L, van der Zee R, et al. Regulatory T cells that recognize a ubiquitous stress-inducible self-antigen are long-lived suppressors of autoimmune arthritis. Proc Natl Acad Sci USA. 2012; 109:14134–9. [PubMed: 22891339]
25. Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol. 2010; 10:849–59. [PubMed: 21107346]
26. Hanyecz A, Olasz K, Tarjanyi O, et al. Proteoglycan aggrecan conducting T cell activation and apoptosis in a murine model of rheumatoid arthritis. Biomed Res Int. 2014; 2014:942148. [PubMed: 24605340]
27. Van de Stadt LA, de Koning MHMT, van de Stadt RJ, et al. Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. Arthritis Rheum. 2011; 63:3226–33. [PubMed: 21792832]
28. Jardetzky TS, Lane WS, Robinson RA, et al. Identification of self peptides bound to purified HLA-B27. Nature. 1991; 353:326–9. [PubMed: 1922338]
29. Van Boekel MAM, van Venrooij WJ. Modifications of arginines and their role in autoimmunity. Autoimmun Rev. 2003; 2:57–62. [PubMed: 12848959]
30. 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. [PubMed: 16585962]
31. Bäcklund J, Treschow A, Bockermann R, et al. Glycosylation of type II collagen is of major importance for T cell tolerance and pathology in collagen-induced arthritis. Eur J Immunol. 2002; 32:3776–84. [PubMed: 12516572]
32. Maresz KJ, Hellvard A, Sroka A, et al. Porphyromonas gingivalis facilitates the development and progression of destructive arthritis through its unique bacterial peptidylarginine deiminase (PAD). PLoS Pathog. 2013; 9:e1003627. [PubMed: 24068934]
33. Shoda H, Fujio K, Shibuya M, et al. Detection of autoantibodies to citrullinated BiP in rheumatoid arthritis patients and pro-inflammatory role of citrullinated BiP in collagen-induced arthritis. Arthritis Res Ther. 2011; 13:R191. [PubMed: 22108001]
34. Mydel P, Wang Z, Brisslert M, et al. Carbamylation-dependent activation of T cells: a novel mechanism in the pathogenesis of autoimmune arthritis. J Immunol. 2010; 184:6882–90. [PubMed: 20488785]
35. Law SC, Street S, Yu C-HA, et al. T cell autoreactivity to citrullinated autoantigenic peptides in rheumatoid arthritis patients carrying HLA-DRB1 shared epitope alleles. Arthritis Res Ther. 2012; 14:R118. [PubMed: 22594821]
36. Thiele GM, Duryee MJ, Dusad A, et al. Citrullinated mouse collagen administered to DBA/1J mice in the absence of adjuvant initiates arthritis. Int Immunopharmacol. 2012; 13:424–31. [PubMed: 22626832]
37. Vassallo R, Luckey D, Behrens M, et al. Cellular and humoral immunity in arthritis are profoundly influenced by the interaction between cigarette smoke effects and host HLA-DR and DQ genes. Clin Immunol. 2014; 152:25–35. [PubMed: 24631425]
38. Von Delwig A, Locke J, Robinson JH, et al. Response of Th17 cells to a citrullinated arthritogenic aggrecan peptide in patients with rheumatoid arthritis. Arthritis Rheum. 2010; 62:143–9. [PubMed: 20039419]

39. Wegner N, Lundberg K, Kinloch A, et al. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. Immunol Rev. 2010; 233:34–54. [PubMed: 20192991]
40. Burkhardt H, Holmdahl R, Deutzmann R, et al. Identification of a major antigenic epitope on CNBr-fragment 11 of type II collagen recognized by murine autoreactive B cells. Eur J Immunol. 1991; 21:49–54. [PubMed: 1703964]
41•. Terato K, Hasty KA, Cremer MA, et al. Collagen-induced arthritis in mice. Localization of an arthritogenic determinant to a fragment of the type II collagen molecule. J Exp Med. 1985; 162:637–46. Review and discusion of success and issues for TNF-α associated therapies for arthritis and there was a significant number of non-responsive patents. [PubMed: 2410532]
42•. Glant T, Csongor J, Szücs T. Immunopathologic role of proteoglycan antigens in rheumatoid joint disease. Scand J Immunol. 1980; 11:247–52. Demonstration of immunopathologic role of proteoglycan in joint disease (arthritis). [PubMed: 9537052]
43. Von Delwig A, Altmann DM, Charlton FG, et al. T cell responses to a non-glycosylated epitope predominate in type II collagen-immunised HLA-DRB1*0101 transgenic mice. Ann Rheum Dis. 2007; 66:599–604. [PubMed: 17114189]
44. Glant TT, Buzás EI, Finnegan A, et al. Critical roles of glycosaminoglycan side chains of cartilage proteoglycan (aggrecan) in antigen recognition and presentation. J Immunol. 1998; 160:3812–19. [PubMed: 9558085]
45. Misják P, Bösze S, Horváti K, et al. The role of citrullination of an immunodominant proteoglycan (PG) aggrecan T cell epitope in BALB/c mice with PG-induced arthritis. Immunol Lett. 2013; 152:25–31. [PubMed: 23578666]
46. Kinne RW. (Auto)immunity to cartilage matrix proteins - a time bomb? Arthritis Res Ther. 2013; 15:101. [PubMed: 23336107]
47. Kobezda T, Ghassemi-Nejad S, Mikecz K, et al. Of mice and men: how animal models advance our understanding of T-cell function in RA. Nat Rev Rheumatol. 2014; 10:160–70. [PubMed: 24394350]
48. Strzępa A, Szczepanik M. IL-17-expressing cells as a potential therapeutic target for treatment of immunological disorders. Pharmacol Rep. 2011; 63:30–44. [PubMed: 21441609]
49. Van den Berg WB. Lessons from animal models of arthritis over the past decade. Arthritis Res Ther. 2009; 11:250. [PubMed: 19849822]
50. Schellekens GA, de Jong BA, van den Hoogen FH, et al. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest. 1998; 101:273–81. [PubMed: 9421490]
51. Hu Y, Cheng W, Cai W, et al. Advances in research on animal models of rheumatoid arthritis. Clin Rheumatol. 2013; 32:161–5. [PubMed: 22885986]
52. Andersson M, Holmdahl R. Analysis of type II collagen-reactive T cells in the mouse. I. Different regulation of autoreactive vs. non-autoreactive anti-type II collagen T cells in the DBA/1 mouse. Eur J Immunol. 1990; 20:1061–6. [PubMed: 1694131]
53. Backlund J, Li C, Jansson E, et al. C57BL/6 mice need MHC class II Aq to develop collagen-induced arthritis dependent on autoreactive T cells. Ann Rheum Dis. 2013; 72(7):1225–32. [PubMed: 23041839]
54. Campbell IK, Hamilton JA, Wicks IP. Collagen-induced arthritis in C57BL/6 (H-2b) mice: new insights into an important disease model of rheumatoid arthritis. Eur J Immunol. 2000; 30:1568–75. [PubMed: 10898492]
55. Lindh I, Snir O, Lönnblom E, et al. Type II collagen antibody response is enriched in the synovial fluid of rheumatoid joints and directed to the same major epitopes as in collagen induced arthritis in primates and mice. Arthritis Res Ther. 2014; 16:R143. [PubMed: 25005029]
56. Glant TT, Mikecz K, Arzoumanian A, et al. Proteoglycan-induced arthritis in BALB/c mice. Clinical features and histopathology. Arthritis Rheum. 1987; 30:201–12. [PubMed: 3827960]
57. Mikecz K, Glant TT, Poole AR. Immunity to cartilage proteoglycans in BALB/c mice with progressive polyarthritis and ankylosing spondylitis induced by injection of human cartilage proteoglycan. Arthritis Rheum. 1987; 30:306–18. [PubMed: 3566822]

58. Glant TT, Radacs M, Nagyeri G, et al. Proteoglycan-induced arthritis and recombinant human proteoglycan aggrecan G1 domain-induced arthritis in BALB/c mice resembling two subtypes of rheumatoid arthritis. Arthritis Rheum. 2011; 63:1312–21. [PubMed: 21305522]

59. Glant TT, Finnegan A, Mikecz K. Proteoglycan-induced arthritis: immune regulation, cellular mechanisms, and genetics. Crit Rev Immunol. 2003; 23:199–250. [PubMed: 14584879]

60. Deane KD. Can rheumatoid arthritis be prevented? Best Pract Res Clin Rheumatol. 2013; 27:467–85. [PubMed: 24315049]

61. Farkas B, Boldizsar F, Tarjanyi O, et al. BALB/c mice genetically susceptible to proteoglycan-induced arthritis and spondylitis show colony-dependent differences in disease penetrance. Arthritis Res Ther. 2009; 11:R21. [PubMed: 19220900]

62. Maggi L, Santarlasci V, Capone M, et al. Distinctive features of classic and nonclassic (Th17 derived) human Th1 cells. Eur J Immunol. 2012; 42:3180–8. [PubMed: 22965818]

63••. Rodeghero R, Cao Y, Olalekan SA, et al. Location of CD4+ T cell priming regulates the differentiation of Th1 and Th17 cells and their contribution to arthritis. J Immunol. 2013; 190:5423–35. Key demonstration that using exactly the same reagents and mice for induction of proteoglycan-induced arthritis (PGIA) disease could have either a Th1 or Th17 signature cytokine phenotype based solely on the route of inductive immunization. [PubMed: 23630349]

64. Domingues HS, Mues M, Lassmann H, et al. Functional and pathogenic differences of Th1 and Th17 cells in experimental autoimmune encephalomyelitis. PLoS One. 2010; 5:e15531. [PubMed: 21209700]

65. Luger D, Silver PB, Tang J, et al. Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. J Exp Med. 2008; 205:799–810. [PubMed: 18391061]

66. Smolen JS, van der Heijde D, Machold KP, et al. Proposal for a new nomenclature of disease-modifying antirheumatic drugs. Ann Rheum Dis. 2014; 73:3–5. [PubMed: 24072562]

67. Mócsai A, Kovács L, Gergely P. What is the future of targeted therapy in rheumatology: biologics or small molecules? BMC Med. 2014; 12:43. [PubMed: 24620738]

68. Crofford LJ. Use of NSAIDs in treating patients with arthritis. Arthritis Res Ther. 2013; 15(Suppl 3):S2. [PubMed: 24267197]

69. Colmegna I, Ohata BR, Menard HA. Current understanding of rheumatoid arthritis therapy. Clin Pharmacol Ther. 2012; 91:607–20. [PubMed: 22357455]

70•. Smolen JS, Landewé R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann Rheum Dis. 2014; 73:492–509. Addresses current therapies for rheumatoid arthritis. [PubMed: 24161836]

71. Lee EB, Fleischmann R, Hall S, et al. Tofacitinib versus Methotrexate in Rheumatoid Arthritis. N Engl J Med. 2014; 370:2377–86. [PubMed: 24941177]

72. Ferraro B, Morrow MP, Hutnick NA, et al. Clinical applications of DNA vaccines: current progress. Clin Infect Dis. 2011; 53:296–302. [PubMed: 21765081]

73. Kallen K-J, Theß A. A development that may evolve into a revolution in medicine: mRNA as the basis for novel, nucleotide-based vaccines and drugs. Ther Adv Vaccines. 2014; 2:10–31. [PubMed: 24757523]

74. Wu T-C, Xu K, Banchereau R, et al. Reprogramming tumor-infiltrating dendritic cells for CD103+CD8+ mucosal T-cell differentiation and breast cancer rejection. Cancer Immunol Res. 2014; 2:487–500. [PubMed: 24795361]

75. Snook AE, Magee MS, Schulz S, et al. Selective antigen-specific CD4(+) T-cell, but not CD8(+) T- or B-cell, tolerance corrupts cancer immunotherapy. Eur J Immunol. 2014; 44:1956–66. [PubMed: 24771148]

76. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 2007; 7:429–42. [PubMed: 17525752]

77. Marabelle A, Kohrt H, Sagiv-Barfi I, et al. Depleting tumor-specific Tregs at a single site eradicates disseminated tumors. J Clin Invest. 2013; 123:2447–63. [PubMed: 23728179]

78. Bäckström NF, Dahlgren UIH. Bystander suppression of collagen-induced arthritis in mice fed ovalbumin. Arthritis Res Ther. 2004; 6:R151–60. [PubMed: 15059279]

79. Oki S, Tomi C, Yamamura T, et al. Preferential T(h)2 polarization by OCH is supported by incompetent NKT cell induction of CD40L and following production of inflammatory cytokines by bystander cells in vivo. Int Immunol. 2005; 17:1619–29. [PubMed: 16291659]

80. Lu S, Holmdahl R. Different therapeutic and bystander effects by intranasal administration of homologous type II and type IX collagens on the collagen-induced arthritis and pristane-induced arthritis in rats. Clin Immunol. 1999; 90:119–27. [PubMed: 9884360]

81. Perez SA, von Hofe E, Kallinteris NL, et al. A new era in anticancer peptide vaccines. Cancer. 2010; 116:2071–80. [PubMed: 20187092]

82. Tovey MG, Lallemand C. Adjuvant activity of cytokines. Methods Mol Biol. 2010; 626:287–309. [PubMed: 20099135]

83. Assier E, Semerano L, Duvallet E, et al. Modulation of anti-tumor necrosis factor alpha (TNF-α) antibody secretion in mice immunized with TNF-α kinoid. Clin Vaccine Immunol. 2012; 19:699–703. [PubMed: 22441388]

84. Hasselberg A, Schön K, Tarkowski A, et al. Role of CTA1R7K-COL-DD as a novel therapeutic mucosal tolerance-inducing vector for treatment of collagen-induced arthritis. Arthritis Rheum. 2009; 60:1672–82. [PubMed: 19479868]

85•. Dzhambazov B, Nandakumar KS, Kihlberg J, et al. Therapeutic vaccination of active arthritis with a glycosylated collagen type II peptide in complex with MHC class II molecules. J Immunol. 2006; 176:1525–33. Demonstration of therapeutic vaccination of glycosylated collagen peptide complex after disease induction to downregulate disease in collagen-induced arthritis model. [PubMed: 16424181]

86••. Boonnak K, Vogel L, Orandle M, et al. Antigen-activated dendritic cells ameliorate influenza A infections. J Clin Invest. 2013; 123:2850–61. Demonstration that J-ligand epitope antigen presentation system activated dendritic cells in the infleunza model hone to the site of antigen (infection), recruit antigen-specific CD8T cells to the site of infection, upmodulate select cytokines (including IFN-γ and IL-12p70) and downmodulate other cytokines (TNF-α, IL-1α, IL-4 and IL-6), and these result in an isotype shift of antibodies toward an IgG2a (Th1) direction, resulting in protection as measured by morbidity, death and viral isolation. [PubMed: 23934125]

87•. Pleguezuelos O, Robinson S, Stoloff GA, et al. Synthetic Influenza vaccine (FLU-v) stimulates cell mediated immunity in a double-blind, randomised, placebo-controlled Phase I trial. Vaccine. 2012; 30:4655–60. Demonstration that synthetic influenza peptide vaccine stimulates cell-mediated immunity in a Phase I clinical trial. [PubMed: 22575166]

88. Melief CJM. Treatment of established lesions caused by high-risk human papilloma virus using a synthetic vaccine. J Immunother. 2012; 35:215–16. [PubMed: 22421938]

89•. Quakkelaar ED, Melief CJM. Experience with synthetic vaccines for cancer and persistent virus infections in nonhuman primates and patients. Adv Immunol. 2012; 114:77–106. Review of work on synthetic long peptide for therapy in man and non-human primates for cancer and infectious diseases. [PubMed: 22449779]

90. Zeestraten ECM, Speetjens FM, Welters MJP, et al. Addition of interferon-α to the p53-SLP® vaccine results in increased production of interferon-γ in vaccinated colorectal cancer patients: a phase I/II clinical trial. Int J Cancer. 2013; 132:1581–91. [PubMed: 22948952]

91. Feng GD, Xue XC, Gao ML, et al. Therapeutic effects of PADRE-BAFF autovaccine on rat adjuvant arthritis. Biomed Res Int. 2014; 2014:854954. [PubMed: 24791002]

92. Baleeiro RB, Wiesmüller K-H, Dähne L, et al. Direct activation of human dendritic cells by particle-bound but not soluble MHC class II ligand. PLoS One. 2013; 8:e63039. [PubMed: 23658796]

93. Xu M, Capraro GA, Daibata M, et al. Cathepsin B cleavage and release of invariant chain from MHC class II molecules follow a staged pattern. Mol Immunol. 1994; 31:723–31. [PubMed: 8035834]

94. Mizel SB, Bates JT. Flagellin as an adjuvant: cellular mechanisms and potential. J Immunol. 2010; 185:5677–82. [PubMed: 21048152]

95. Mitchell D, Yong M, Raju J, et al. Toll-like receptor-mediated adjuvanticity and immunomodulation in dendritic cells: implications for peptide vaccines. Hum Vaccin. 2011; 7(Suppl):85–93. [PubMed: 21245658]

96. Kim S-J, Chen Z, Chamberlain ND, et al. Ligation of TLR5 promotes myeloid cell infiltration and differentiation into mature osteoclasts in rheumatoid arthritis and experimental arthritis. J Immunol. 2014; 193:3902–13. [PubMed: 25200955]

97••. Rosenthal KS, Taylor P, Zimmerman DH. J-LEAPS peptide and LEAPS dendritic cell vaccines. Microb Biotechnol. 2012; 5:203–13. Key review on ligand epitope antigen presentation system technology, applications in various models for infectious and autoimmune diseases, for cells, cytokines and antibodies involved and mode of action. [PubMed: 21895992]

98. Zimmerman DH. LEAPS therapeutic vaccines as antigen specific suppressors of inflammation in infectious and autoimmune diseases. J Vaccines Vaccin. 2012; 03:1000149.

99. Ohnishi Y, Tsutsumi A, Matsumoto I, et al. Altered peptide ligands control type II collagen-reactive T cells from rheumatoid arthritis patients. Mod Rheumatol. 2006; 16:226–8. [PubMed: 16906372]

100. Wakamatsu E, Matsumoto I, Yoshiga Y, et al. Altered peptide ligands regulate type II collagen-induced arthritis in mice. Mod Rheumatol. 2009; 19:366–71. [PubMed: 19444549]

101•. Doodes PD, Cao Y, Hamel KM, et al. IFN-gamma regulates the requirement for IL-17 in proteoglycan-induced arthritis. J Immunol. 2010; 184:1552–9. IFN-γ and IL-17 have the potential to induce PGIA, but it is the strength of the IFN-γ response that regulates the contribution of each of these to disease. [PubMed: 20028652]

102. Alzabin S, Abraham SM, Taher TE, et al. Incomplete response of inflammatory arthritis to TNFα blockade is associated with the Th17 pathway. Ann Rheum Dis. 2012; 71:1741–8. [PubMed: 22550316]

103. Chackerian B, Lowy DR, Schiller JT. Conjugation of a self-antigen to papillomavirus-like particles allows for efficient induction of protective autoantibodies. J Clin Invest. 2001; 108:415–23. [PubMed: 11489935]

104. Kochetkova I, Trunkle T, Callis G, et al. Vaccination without autoantigen protects against collagen II-induced arthritis via immune deviation and regulatory T cells. J Immunol. 2008; 181:2741–52. [PubMed: 18684965]

105. Luross JA, Heaton T, Hirst TR, et al. Escherichia coli heat-labile enterotoxin B subunit prevents autoimmune arthritis through induction of regulatory CD4+ T cells. Arthritis Rheum. 2002; 46:1671–82. [PubMed: 12115200]

106. Zimmerman DH, Taylor P, Bendele A, et al. CEL-2000: a therapeutic vaccine for rheumatoid arthritis arrests disease development and alters serum cytokine/chemokine patterns in the bovine collagen type II induced arthritis in the DBA mouse model. Int Immunopharmacol. 2010; 10:412–21. [PubMed: 20074669]

107. Sidhu M, Griffiths MM, Bradley DS. Vaccination with collagen-pulsed dendritic cells prevents the onset and reduces the disease severity in the mouse model of spontaneous polychondritis. Clin Exp Immunol. 2009; 157:350–8. [PubMed: 19664142]

108. Zimmerman D, Mikecz K, Kurko J, et al. LEAPS peptide vaccination alters T cell phenotype and suppresses joint inflammation in a murine model of rheumatoid arthitis (P5223). J Immunol. 2013; 190:67.6.

109. Prakken BJ, Roord S, Ronaghy A, et al. Heat shock protein 60 and adjuvant arthritis: a model for T cell regulation in human arthritis. Springer Semin Immunopathol. 2003; 25:47–63. [PubMed: 12904891]

110. Koffeman EC, Genovese M, Amox D, et al. Epitope-specific immunotherapy of rheumatoid arthritis: clinical responsiveness occurs with immune deviation and relies on the expression of a cluster of molecules associated with T cell tolerance in a double-blind, placebo-controlled, pilot phase II tria. Arthritis Rheum. 2009; 60:3207–16. [PubMed: 19877047]

111. Cammarota G, Scheirle A, Takacs B, et al. Identification of a CD4 binding site on the beta 2 domain of HLA-DR molecules. Nature. 1992; 356:799–801. [PubMed: 1574119]

112. König R, Huang LY, Germain RNN. MHC class II interaction with CD4 mediated by a region analogous to the MHC class I binding site for CD8. Nature. 1992; 356:796–8. [PubMed: 1574118]

113. Taylor PR, Paustian CC, Koski GK, et al. Maturation of dendritic cell precursors into IL12-producing DCs by J-LEAPS immunogens. Cell Immunol. 2010; 262:1–5. [PubMed: 20163792]

114. Taylor PR, Koski GK, Paustian CC, et al. J-LEAPS vaccines initiate murine Th1 responses by activating dendritic cells. Vaccine. 2010; 28:5533–42. [PubMed: 20600501]

115. Zimmerman DH, Morris S, Rouse D, et al. Immunization with peptide heterconjugates primes a t helper cell type 1-associated antibody (IgG2a) response that recognizes the native epitope on the 38-kDA protein of Mycobacterium tuberculosis. Vaccine Res Liebert. 1996; 5:103–18.

116. Goel N, Zimmerman DH, Rosenthal KS. Ligand epitope antigen presentation system vaccines against herpes simplex virus. Front Biosci. 2005; 10:966–74. [PubMed: 15569635]

117. König R, Shen X, Germain RN. Involvement of both major histocompatibility complex class II alpha and beta chains in CD4 function indicates a role for ordered oligomerization in T cell activation. J Exp Med. 1995; 182:779–87. [PubMed: 7650484]

118. Rosenthal KS, Mao H, Horne WI, et al. Immunization with a LEAPS heteroconjugate containing a CTL epitope and a peptide from beta-2-microglobulin elicits a protective and DTH response to herpes simplex virus type 1. Vaccine. 1999; 17:535–42. [PubMed: 10075159]

119. Goel N, Rong Q, Zimmerman D, et al. A L.E.A.P.S heteroconjugate vaccine containing a T cell epitope from HSV-1 glycoprotein D elicits Th1 responses and protection. Vaccine. 2003; 21:4410–20. [PubMed: 14505924]

120. Zimmerman D, Bergmann K, Rosenthal K, et al. A new approach to T-cell activation: natural and synthetic conjugates capable of activating T cells. Vaccine Res. 1996; 5:91–102.

121. Cihakova D, Barin JG, Baldeviano GC, et al. L.E.A.P.S heteroconjugate is able to prevent and treat experimental autoimmune myocarditis by altering trafficking of autoaggressive cells to the heart. Int Immunopharmacol. 2008; 8:624–33. [PubMed: 18387504]

122••. Finnegan A, Mikecz K, Tao P, et al. Proteoglycan (aggrecan)-induced arthritis in BALB/c mice is a Th1-type disease regulated by Th2 cytokines. J Immunol. 1999; 163:5383–90. Role of Th1 cytokines in upregulating and Th2 cytokines in downregulating disease in PGIA model. [PubMed: 10553063]

123•. Cao Y, Brombacher F, Tunyogi-Csapo M, et al. Interleukin-4 regulates proteoglycan-induced arthritis by specifically suppressing the innate immune response. Arthritis Rheum. 2007; 56:861–70. Demonstration that IL-4 can downregulate disease in PGIA model. [PubMed: 17330244]

124. Cook D, Brown D, Alexander R, et al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. Nat Rev Drug Discov. 2014; 13:419–31. [PubMed: 24833294]

125. Bendele A. Animal models of rheumatoid arthritis. J Musculoskelet Neuronal Interact. 2001; 1:377–85. [PubMed: 15758488]

126•. Bevaart L, Vervoordeldonk MJ, Tak PP. Evaluation of therapeutic targets in animal models of arthritis: how does it relate to rheumatoid arthritis? Arthritis Rheum. 2010; 62:2192–205. Excellent review and very good collection of various features of arthritis models. [PubMed: 20506322]

127. Billiau A, Matthys P. Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. J Leukoc Biol. 2001; 70:849–60. [PubMed: 11739546]

128. Schurgers E, Billiau A, Matthys P. Collagen-induced arthritis as an animal model for rheumatoid arthritis: focus on interferon-γ. J Interferon Cytokine Res. 2011; 31:917–26. [PubMed: 21905879]

129. Boissier MC, Chiocchia G, Bessis N, et al. Biphasic effect of interferon-gamma in murine collagen-induced arthritis. Eur J Immunol. 1995; 25:1184–90. [PubMed: 7774621]

130. Bolon B, Stolina M, King C, et al. Rodent preclinical models for developing novel antiarthritic molecules: comparative biology and preferred methods for evaluating efficacy. J Biomed Biotechnol. 2011; 2011:569068. [PubMed: 21253435]

131. Brand DD, Latham KA, Rosloniec EF. Collagen-induced arthritis. Nat Protoc. 2007; 2:1269–75. [PubMed: 17546023]

132. Brand DD. Rodent models of rheumatoid arthritis. Comp Med. 2005; 55:114–22. [PubMed: 15884771]

133••. Chu C-Q, Swart D, Alcorn D, et al. Interferon-gamma regulates susceptibility to collagen-induced arthritis through suppression of interleukin-17. Arthritis Rheum. 2007; 56:1145–51. Demonstration that collagen-induced arthritis is a Th17-driven arthritic disease and that

modulation can occur by conversion to IFN-γ producing Th1 responses and that IFN-γ ablation increases disease severity. [PubMed: 17393396]

134. Kannan K, Ortmann RA, Kimpel D. Animal models of rheumatoid arthritis and their relevance to human disease. Pathophysiology. 2005; 12:167–81. [PubMed: 16171986]
135. Lindqvist A-KB, Bockermann R, Johansson ÅC, et al. Mouse models for rheumatoid arthritis. Trends Genet. 2002; 18:S7–S13.
136. Malfait AM, Butler DM, Presky DH, et al. Blockade of IL-12 during the induction of collagen-induced arthritis (CIA) markedly attenuates the severity of the arthritis. Clin Exp Immunol. 1998; 111:377–83. [PubMed: 9486407]
137. Glant TT, Mikecz K. Proteoglycan aggrecan-induced arthritis: a murine autoimmune model of rheumatoid arthritis. Methods Mol Med. 2004; 102:313–38. [PubMed: 15286393]
138. Nandakumar KS, Holmdahl R. Antibody-induced arthritis: disease mechanisms and genes involved at the effector phase of arthritis. Arthritis Res Ther. 2006; 8:223. [PubMed: 17254316]
139. Nandakumar KS, Holmdahl R. Efficient promotion of collagen antibody induced arthritis (CAIA) using four monoclonal antibodies specific for the major epitopes recognized in both collagen induced arthritis and rheumatoid arthritis. J Immunol Methods. 2005; 304:126–36. [PubMed: 16125192]
140. Patten C, Bush K, Rioja I, et al. Characterization of pristane-induced arthritis, a murine model of chronic disease: response to antirheumatic agents, expression of joint cytokines, and immunopathology. Arthritis Rheum. 2004; 50:3334–45. [PubMed: 15476226]
141. Vingsbo C, Sahlstrand P, Brun JG, et al. Pristane-induced arthritis in rats: a new model for rheumatoid arthritis with a chronic disease course influenced by both major histocompatibility complex and non-major histocompatibility complex genes. Am J Pathol. 1996; 149:1675–83. [PubMed: 8909256]
142. Cromartie WJJ. Arthritis in rats after systemic injection of streptococcal cells or cell walls. J Exp Med. 1977; 146:1585–602. [PubMed: 336836]
143. Joosten LA, Helsen MM, van Den Berg WB. Blockade of endogenous interleukin 12 results in suppression of murine streptococcal cell wall arthritis by enhancement of interleukin 10 and interleukin 1Ra. Ann Rheum Dis. 2000; 59:196–205. [PubMed: 10700428]
144. Plater-Zyberk C, Joosten LAB, Helsen MMA, et al. GM-CSF neutralisation suppresses inflammation and protects cartilage in acute streptococcal cell wall arthritis of mice. Ann Rheum Dis. 2007; 66:452–7. [PubMed: 17020908]
145. Holmdahl R, Lorentzen JCC, Lu S, et al. Arthritis induced in rats with nonimmunogenic adjuvants as models for rheumatoid arthritis. Immunol Rev. 2001; 184:184–202. [PubMed: 12086312]
146. Curtis JR, Singh JA. Use of biologics in rheumatoid arthritis: current and emerging paradigms of care. Clin Ther. 2011; 33:679–707. [PubMed: 21704234]
147. Bajtner E, Nandakumar KS, Engström A, et al. Chronic development of collagen-induced arthritis is associated with arthritogenic antibodies against specific epitopes on type II collagen. Arthritis Res Ther. 2005; 7:R1148–57. [PubMed: 16207332]
148. Mould AW, Scotney P, Greco SA, et al. Prophylactic but not therapeutic activity of a monoclonal antibody that neutralizes the binding of VEGF-B to VEGFR-1 in a murine collagen-induced arthritis model. Rheumatology (Oxford). 2008; 47:263–6. [PubMed: 18204088]
149. Hamel K, Doodes P, Cao Y, et al. Suppression of proteoglycan-induced arthritis by anti-CD20 B Cell depletion therapy is mediated by reduction in autoantibodies and CD4+ T cell reactivity. J Immunol. 2008; 180:4994–5003. [PubMed: 18354225]
150•. Prakken BJ, Samodal R, Le TD, et al. Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. Proc Natl Acad Sci USA. 2004; 101:4228–33. Evaluation of peptide delivered orally in arthritis and demonstration of both safety and potential immunotherapy application. [PubMed: 15024101]
151. Iizuka M, Wakasa Y, Tsuboi H, et al. Suppression of collagen-induced arthritis by oral administration of transgenic rice seeds expressing altered peptide ligands of type II collagen. Plant Biotechnol J. 2014; 12:1143–52. [PubMed: 24989432]

152. Orgogozo J-M, Gilman S, Dartigues J-F, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. Neurology. 2003; 61:46–54. [PubMed: 12847155]

153. Kappos L, Comi G, Panitch H, et al. Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The Altered Peptide Ligand in Relapsing MS Study Group. Nat Med. 2000; 6:1176–82. [PubMed: 11017151]

154. McDevitt H. Specific antigen vaccination to treat autoimmune disease. Proc Natl Acad Sci USA. 2004; 101(Suppl 2):14627–30. [PubMed: 15466699]

155. Finkelman FD. Anaphylaxis: lessons from mouse models. J Allergy Clin Immunol. 2007; 120:506–15. quiz 516–7. [PubMed: 17765751]

156. Pedotti R, DeVoss JJ, Youssef S, et al. Multiple elements of the allergic arm of the immune response modulate autoimmune demyelination. Proc Natl Acad Sci USA. 2003; 100:1867–72. [PubMed: 12576552]

157. Pedotti R, Sanna M, Tsai M, et al. Severe anaphylactic reactions to glutamic acid decarboxylase (GAD) self peptides in NOD mice that spontaneously develop autoimmune type 1 diabetes mellitus. BMC Immunol. 2003; 4:2. [PubMed: 12597780]

158. Kobayashi M, Abiru N, Arakawa T, et al. Altered B:9-23 insulin, when administered intranasally with cholera toxin adjuvant, suppresses the expression of insulin autoantibodies and prevents diabetes. J Immunol. 2007; 179:2082–8. [PubMed: 17675466]

159. Mitamura M, Nakano N, Yonekawa T, et al. T cells are involved in the development of arthritis induced by anti-type II collagen antibody. Int Immunopharmacol. 2007; 7:1360–8. [PubMed: 17673151]

160. Kobayashi N, Kiptoo P, Kobayashi H, et al. Prophylactic and therapeutic suppression of experimental autoimmune encephalomyelitis by a novel bifunctional peptide inhibitor. Clin Immunol. 2008; 129:69–79. [PubMed: 18676182]

161. Liu E, Moriyama H, Abiru N, et al. Anti-peptide autoantibodies and fatal anaphylaxis in NOD mice in response to insulin self-peptides B:9-23 and B:13-23. J Clin Invest. 2002; 110:1021–7. [PubMed: 12370280]

162. Liu E, Moriyama H, Paronen J, et al. Nondepleting anti-CD4 monoclonal antibody prevents diabetes and blocks induction of insulin autoantibodies following insulin peptide B:9-23 immunization in the NOD mouse. J Autoimmun. 2003; 21:213–19. [PubMed: 14599846]

163. Liu E, Moriyama H, Abiru N, et al. Preventing peptide-induced anaphylaxis: addition of C-terminal amino acids to produce a neutral isoelectric point. J Allergy Clin Immunol. 2004; 114:607–13. [PubMed: 15356565]

164. Quakkelaar ED, Fransen MF, van Maren WWC, et al. IgG-mediated anaphylaxis to a synthetic long peptide vaccine containing a B cell epitope can be avoided by slow-release formulation. J Immunol. 2014; 192:5813–20. [PubMed: 24813207]

Key issues

- When investigating autoimmune diseases such as rheumatoid arthritis, it is important to identify the antigens and antigenic epitopes that initiate and maintain the disease, as well as the signature cytokine(s) driving the disease pathogenesis in humans.
- Appropriate animal models should be identified and their relevance to human disease established with regard to the autoantigens and pro-inflammatory immune responses (T-cell response and cytokines) driving the disease.
- Passive administration of antibodies against inflammatory mediators (e.g., cytokines, receptors or other cell surface molecules) may offer some practical relief from arthritic symptoms, but will not address the initiators or the drivers of the autoimmune response unlike antigen-specific vaccination.
- Current treatments are not effective in a substantial number of cases, ablate responses that are important for immune protection and are contraindicated in many cases.
- Upon identification of an appropriate antigen(s), the vaccine formulation (including adjuvant) and route of administration must be determined so that the vaccine will activate the proper T-cell response to modulate the ongoing inflammatory response. Vaccines inducing antibody to autoantigens will likely exacerbate the condition.
- Practical considerations for stability, safety and dosing may involve changes to the structure, design, formulation or the route of immunization. As examples, we present two ligand epitope antigen presentation system vaccines for rheumatoid arthritis that modulate either Th1- or Th17-driven disease in different animal models, as indicated by cessation of disease progression and change in the production of signature cytokines.

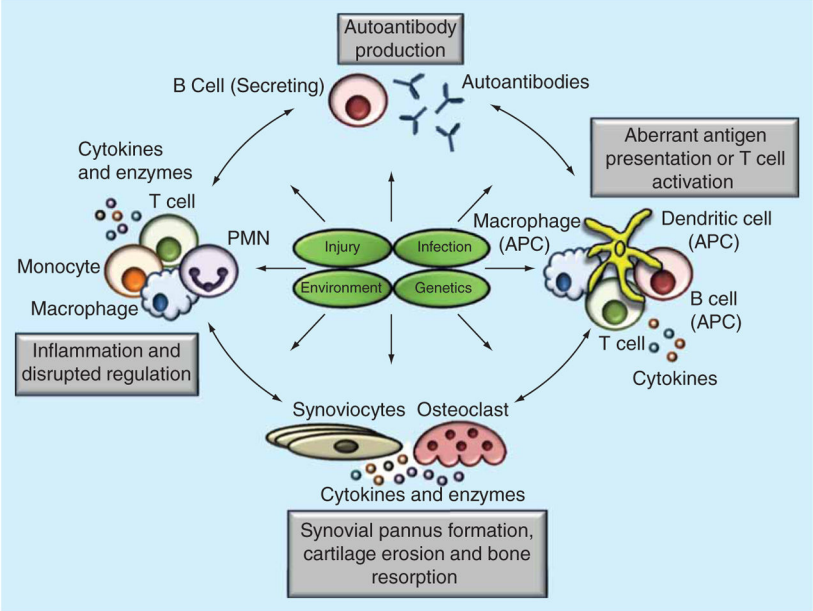

Figure 1. A generic view of the multifocal nature of autoimmune diseases with rheumatoid arthritis as an example

(Center) For many of these diseases, there is no singular, identified cause, but many of the underlying mechanisms can create a synergistic feedback that result in progression. (Right) By various mechanisms, an APC may present self-antigen to autoreactive T cells. These cells may become resistant to Tregs and other regulatory mechanisms due to the persistence of activation signals. (Left) Autoreactive T cells, generally Th1 or Th17, become actively involved in an inflammatory cascade, featuring strongly in the dysregulation of the production of inflammatory (IL-1, IL-17, IL-23, TNF-α and IFN-γ) and regulatory (IL-4, IL-10, TGF-β) cytokines. (Top) Antibodies targeting self-antigens can initiate and exacerbate the inflammatory process. Autoanti-bodies facilitate recruitment of PMNs and monocytes, and augment local inflammatory reactions. (Bottom) In rheumatoid arthritis, cytokines produced by Th1 and Th2 cells, macrophages and other inflammatory cells stimulate the proliferation of synoviocytes. These synovial cells then form a granulation tissue (pannus) that invades and destroys articular cartilage and bone. Th1 and Th17 cells also induce the differentiation of macrophage-like precursor cells into osteoclasts that mediate bone resorption.

APC: Antigen presenting cell; PMN: Polymorphonuclear leukocyte.

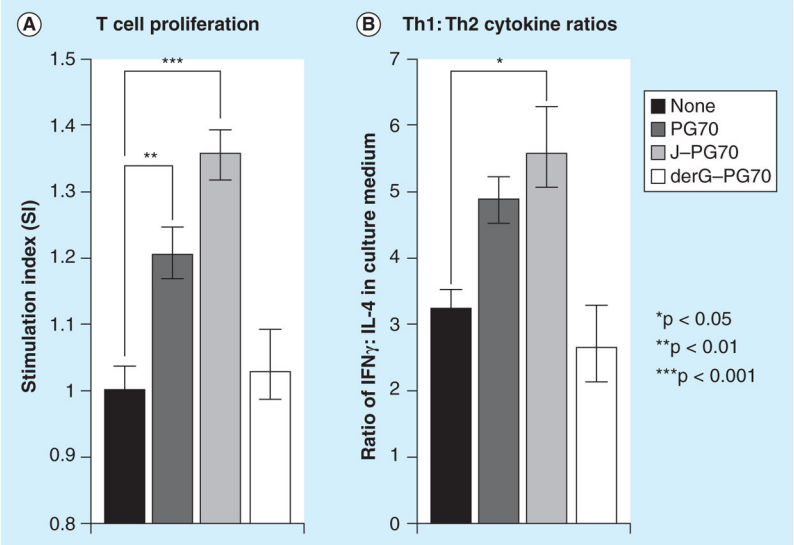

Figure 2. Effects of PG70 peptide and LEAPS–PG70 conjugates on *in vitro* immune cell responses of mice with PGIA

Spleen cells from BALB/c mice with PGIA were cultured without (none) or with the peptides (5 M). **(A)** T-cell proliferation, expressed as an SI, was significantly increased in the presence of PG70 and J–PG70 peptides, but not in the presence of derG–PG70 as compared to untreated cells. **(B)** As determined by the ratio of Th1 and Th2 cytokines (IF-γ:IL-4, measured by ELISA of supernatants), T cells showed a shift toward Th1 polarization in the presence of PG70 and J–PG70, but not in response to derG–PG70 as compared to untreated cells. This finding suggests that the J, but not the derG, LEAPS conjugate of PG70 is capable of steering PGIA spleen T cells toward the Th1 phenotype. LEAPS: Ligand epitope antigen presentation system; PGIA: Proteoglycan-induced arthritis; SI: Stimulation index.

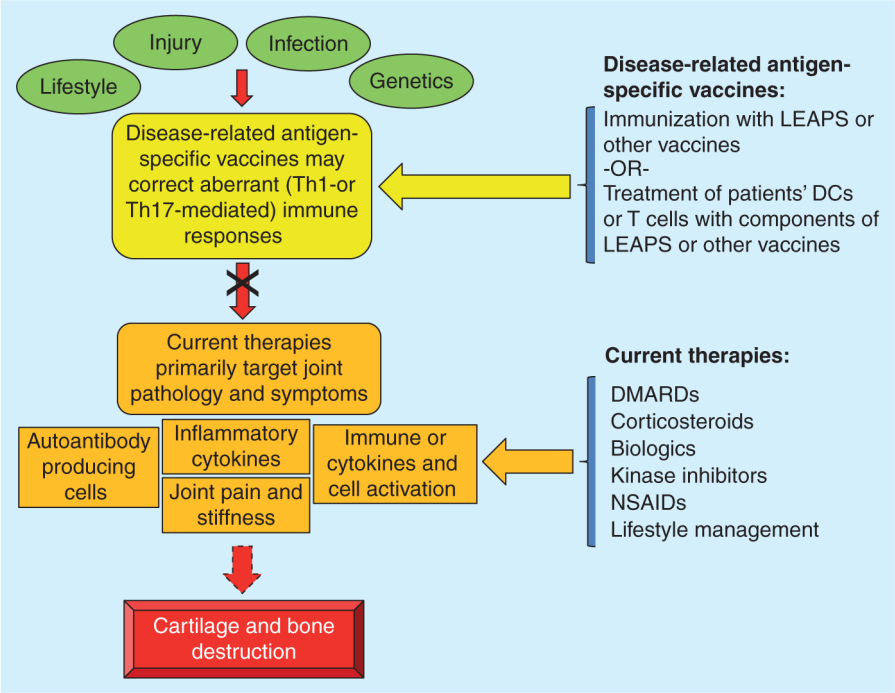

Figure 3. Schematic view of disease progression and potential therapeutic interventions in RA

Therapeutic agents and strategies are listed, indicating the mechanism or targeted stage of disease. Antigen-specific immunomodulatory vaccines (LEAPS and others) act at an earlier point in the progression of RA, and with more specificity than current treatments and have the potential to halt arthritis progression. Therapies targeting the autoimmune pathology and joint symptoms (especially those used for early aggressive treatment) may delay disease progression, but are not curative for RA.

DCs: Dendritic cells; DMARDs: Disease modifying anti-rheumatic drugs; LEAPS: Ligand epitope antigen presentation system; RA: Rheumatoid arthritis.

Table 1

Characteristics of induced animal models of rheumatoid arthritis.

Model	**Collagen-induced arthritis**		**Proteoglycan-induced arthritis**		**Collagen antibody–induced arthritis**	**Pristane-induced arthritis**		**SCWA**	**AIA**
Full name	Collagen-induced arthritis		Proteoglycan-induced arthritis		Collagen antibody–induced arthritis	Pristane-induced arthritis		Streptococcal cell wall–induced arthritis	Adjuvant arthritis
Disease-inducing agent	Type II collagen		Human cartilage proteoglycan aggrecan		Anti-collagen antibodies	Pristane		Bacterial cell wall peptidoglycan	CFA
Primary species	Mouse	Rat	Mouse		Mouse	Mouse	Rat	Rat	Rat
Route	id., sc.	id., sc.	ip.	id.	iv.	ip.	id.	ip.	id.
Required injections	1–2	1–2	2–4	3	1	1–2	1	2–3	2–3
Peak days of onset†	30–40	14–21	50–70	55–80	~8	ND	~25	32 (3 after reactivation)	~17
Range of incidence	60–90%	80–100%	90–100%	80–100%	>95%	40–80%	100%	90–100%	100%
Genetic background	DBA/1J	DA	BALB/c	BALB/c	BALB/c, DBA/1	CBA, BALB/c	DA	LEW	LEW
Induction	2–3 weeks	10–13 days	7–8 weeks	8–9 weeks	1–3 days	60–180 days	~10 days	1–2 days	10 days–6 weeks
Duration	1–2 months	NA	Progressive	Progressive	3 weeks	200+ days	4+ weeks	4–6 weeks	~1 month
Preferred age/sex	5–8 weeks old males	Adult females	>12 weeks old females	>12 weeks old females	6–8 weeks old males	6–8 weeks old males	Adult females	7–8 weeks old females	7–8 weeks old males
Spondylitis	No	No	Yes	yes	no	NS/ND	NS/ND	NS/ND	NS/ND
T-cell involvement	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Exacerbating cytokines	TNF-α, IL-1, IL-17, IFN-γ‡	TNF-α, IL-1, IL-17	TNF-α, IFN-γ, IL-12, IL-17§	IL-6, IL-17, IL-23	TNF-α, IL-1, IL-6	TNF-α, IL-1, IL-6	TNF-α, IL-1, IL-6, IFN-γ	IL-1, IL-17, IL-18, TNF-α, IFN-γ, IL-12¶, GM-CSF	TNF-α, IL-1, IL-17
Ameliorating cytokines#	IL-4, IL-10, IL-12¶, IFN-γ‡	IL-4, IL-10	IL-4, IL-10	IFN-γ	N/A	NA	NA	IL-10	IL-4, IL-10, IFN-γ
References	[49,53,102,125–136]		[49,58,63,126,132,135,137]		[134,138,139]	[49,135,140,141]		[49,126,130,132,134,142–144]	[49,125–127,130,145]

†Peak days of onset are the day(s) at which disease severity reaches a maximum.

‡IFN-γ has a biphasic effect, exacerbating the disease during induction but ameliorating during the chronic phase.

§IL-17 knockout does not affect disease unless IFN-γ and IL-17 are absent.

¶Some reports based on measurement/ablation of IL-12p40, a component of both IL-12p70 and IL-23, which may affect Th1 and Th17 pathways.

#TGF-β was not reported or studied in this context.

AIA: Adjuvant-induced arthritis; CFA: Complete Freund's adjuvant; id.: Intradermal; ip.: Intraperitoneal; iv.: Intravenous; NA: Data not available; NS/ND: Not studied or not detected; sc.: Subcutaneous; SCWA: Streptococcal wall arthritis.

Table 2

Experimental vaccines for arthritis in animal models and in rheumatoid arthritis.

Name	Active or passive	Immunogen	Nature of immunogen	Conjugate type	Status	Comments from or shown in paper	Ref.
Denosumab	Passive	RANKL	Cytokine	huMab; NA	Phase II		[146]
Anti-collagen	Passive	Collagen	Collagen-II	mMab; NA	Preclinical	Used to induce arthritis in mice	[139,147]
Mab VEGF-B	Passive	VEGF-B	Cytokine	mMab; NA	Preclinical	CIA ↓AI in DBA/1J or C57BL/6, prophylactic not therapeutic	[148]
Anti-CD20	Passive	CD20	CD20	mMab; NA	Human form approved	In proteoglycan-induced arthritis model, ↓AI, RF, ACPA, $CD4^{+}IFN\text{-}\gamma^{+}$, $CD4^{+}IL\text{-}17^{+}$	[149]
rPADRE-BAFF	Active	rBAFF	Cytokine	PADRE-BAFF	Preclinical	Evaluated in rats sc. with CFA; induces anti-BAFF which neutralizes BAFF and ameliorates disease	[91]
dnzJP1 peptide	Active	dnazJP1	Disease Ag	None	Phase I/Ia	Oral delivery, no adjuvant ↓TNF-α, IFN-γ, IL-2, ↑IL-4, IL-10	[150]
$C\text{-}II_{256–271}$	Active	CII peptide	Disease Ag	$APL\text{-}262_{G\ to\ A}$	Preclinical	Used PBMC from RA patients only IVS exhibited antagonistic on three cell lines	[99]
$C\text{-}II_{256–271}$	Active	CII peptide	Disease Ag	$APL\text{-}262_{G\ to\ A}$	Preclinical	ip. no adjuvant, oral no adjuvant; CIA ↓AI in DBA/1J therapeutic	[100,151]
CTA1R7K-COL-DD	active	Collagen II_{259}	Collagen	Fusion protein of peptide and CT-A1	Preclinical	CIA in DBA/1J, with Ribi as adjuvant ↓AI, IL-6, IL-17, IFN-γ ↑IL-10	[84]
GalOK264/Aq IN or IV delivery	Active	Collagen II I_{259}	Collagen	Complex of peptide and MHC	Preclinical	CIA in hybrid mice and GalOK264 peptide complexed with MHC, iv. or in.; ↓AI, involves T cells as shown by adoptive transfer	[85]
CEL-2000	Active	Collagen II_{254}		J-LEAPS of collagen II peptide	Preclinical	CIA in DBA/1J; sc. with IFA ↓AI, IL-6, IL-17, TNF-α ↑IFN-γ, IL-12p70, IL-10	[106]
Salmonella vector with colonization factor antigen I	Active	Unrelated	Colonization factor	NA	Preclinical	DBA/1J CIA, oral, Ag specificity unknown, bystander effect? ↓AI, IL-17, IL-27 ↑IL-4, IL-10, IL-13, TGF-β by immune deviation or bystander effect, Treg	[104]
Escherichia coli enterotoxin B heat labile	Active	Unrelated	NA	NA; oral/gastric delivery	Preclinical	DBA/1J CIA, in.; Ag specificity unknown, acts on Treg	[105]

Name	Active or passive	Immunogen	Nature of immunogen	Conjugate type	Status	Comments from or shown in paper	Ref.
						↓AI, IFN-γ, IgG2a isotype anti- C-II antibodies , IgG2a isotype anti- C-II antibodies Involves T cells as shown by adoptive cell transfer	

For comments column: type of adjuvant (IFA, CFA, Ribi is MPL or lipid A) and route of administration (sc., id., in., iv.). Upward pointing arrows depict increases in the identified cytokines or cell type. Downward pointing arrows indicate decreases in the identified item(s).

ACPA: Anti-citrulline protein antibodies; AI: Arthritic index; BAFF: B-cell activating factor; CFA: Complete Freund's adjuvant; CIA: Collagen-induced arthritis; huMab/mMab: human/mouse monoclonal antibody; IFA: Incomplete Freund's adjuvant; in.: Intranasal; iv.: Intravenous; ip.: Intraperitoneal; IVS: *In vitro* stimulation; LEAPS: Ligand epitope antigen presentation system; NA: Not applicable; PBMC: Peripheral blood mononuclear cells; PADRE: pan DR epitope; RA: Rheumatoid arthritis; RF: Rheumatoid factor; sc.: Subcutaneous.

Table 3

End point mean changes in arthritic index score in mice with proteoglycan-induced arthritis treated with adjuvant only (control) or with J– or derG–PG70 conjugates in the adjuvant.

Treatment	Increase in AI score over 36 days
Adjuvant only	10.0 ± 0.9
J–PG70	9.9 ± 0.9
derG–PG70	2.6 ± 0.7

In a pilot study, LEAPS therapy was evaluated in the cartilage PGIA model of rheumatoid arthritis. PGIA was induced in retired breeder female BALB/c mice by intraperitoneal immunizations with cartilage PG in DDA adjuvant [58,137]. After arthritis onset, the mice were sorted into three groups and vaccinated subcutaneously with ISA51vg adjuvant emulsified with PBS (adjuvant only) (control), J–PG70 in adjuvant or derG-PG70 in adjuvant on therapy day 0, and once again on therapy day 14. After the first vaccination (therapy day 0), AI was determined by visual scoring. The animals were euthanized 36 days after the first LEAPS vaccination. Shown are the changes in AI scores at the end of the study. Disease severity (AI) was dramatically suppressed in mice vaccinated with the LEAPS conjugate derG–PG70 as compared with controls or animals receiving J–PG70 vaccine.

AI: Arthritic index; DDA: Dimethyldioctacecyl ammonium bromide; LEAPS: Ligand epitope antigen presentation system; PBS: Phospate buffered saline; PG: Proteoglycan; PGIA: Proteoglycan-induced arthritis.