Autoreactive B cells in rheumatoid arthritis

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Chapter I

General introduction

ACPA-positive rheumatoid arthritis: the role of autoreactive B cells and targeting strategies

1 Rheumatoid arthritis and its burden

Rheumatoid arthritis (RA) is a chronic autoimmune disorder which is characterized by persistent inflammation of the joints leading to progressive joint destruction, deformity, and immobility. The American College of Rheumatology and the European League Against Rheumatism joint working group has set out criteria for the classification of patients with at least 1 joint with clinical synovitis which cannot be better explained by another disease. These patients are classified as having 'definite RA' when fulfilling at least 6 criteria out of 10 which include the number and kind of joints involved (maximal score of 5); the presence and levels of RA-related autoantibodies, i.e. Rheumatoid Factor (RF) and Anti-Citrullinated Proteins Antibodies (ACPA) (maximal score of 2); the evidence of systemic inflammation as shown by increased acute-phase reactants (score 1); and persistence of symptoms (for at least 6 weeks, score 1).[1] The signs and symptoms of RA include painful, warm and/or swollen joints, joint stiffness particularly after inactivity, fatigue, and occasionally fever and loss of appetite. Moreover, RA is associated with increased risk of lung involvement and cardiovascular morbidity as well as premature death.[2,3,4,5]

There are six histologic characteristics of rheumatic joints: (1) increased vascularization due to vasodilation and neo-angiogenesis; (2) aggregation of fibrin in the joint cavity, forming rice bodies; (3) accumulation of neutrophils in the synovial fluid; (4) infiltration of synovial stroma by dense perivascular inflammatory cells, including B cells, T cells, plasma cells and macrophages; (5) osteoclast activity in underlying bone; and (6) formation of pannus tissue, a mass of synovium and synovial stroma which grows over the articular cartilage. Osteoclast activity and pannus formation lead to bone and cartilage erosion in severe RA, respectively.[6]

RA affects between 0.3% and 1% of the population world-wide, predominantly women, and is more frequent in developed countries.[7] As RA tends to develop during productive years of adulthood, the societal impact and economic burden are particularly high. It is estimated that RA costs the National Health Service of the UK approximately £560 million a year in health care costs with additional £1.8 billion a year for work-related absence and disability.[8]

2 RA subsets: ACPA positive and negative

RA originates from a heterogenous immune-pathogenesis that initiates, promotes and maintains the disease. One of the serological markers of a distinct subset of RA

is ACPA. ACPA discriminate two subsets of RA which differ in genetic background and clinical course.[9-11,12,13] ACPA are present in 46% to 80% of RA patients and serve as highly specific biomarkers for RA.[14,15] These autoantibodies are detectable years before the onset of arthritis, are associated with early, rapidly progressive disease, and prognosticate erosive joint destruction in established RA.[16,17] In patients who achieve drug-free remission or treatment reduction, relapses occur more frequently in ACPA-positive individuals, suggesting persistent immunological dysregulation in spite of clinical improvement.[18] Due to rapid disease progression and the lower chance of reaching sustained drug-free remission, ACPA-positive RA patients have higher annual medical expenditures compared to their ACPA-negative counterparts.[19] The focus of this thesis will be on ACPA-positive RA.

Next to clinical, epidemiological and genetic differences, there are also distinctive features in the synovium and circulating chemokines that differentiate these two subsets of RA. The synovium from ACPA-positive RA shows higher numbers of B and T cell infiltrates compared to ACPA negative RA, with the formation of germinal centre-like follicular structures in some but not all patients with ACPA-positive disease. Moreover, ACPA positive RA patients have higher serum levels of CXCL13, a B cell chemotactic factor.[20] ACPA-positive RA patients also respond better to B cell depletion therapy and T cell co-stimulation inhibitor compared to ACPA-negative patients.[21,22] These differences suggest a pivotal role for B and T cells in the pathogenesis of ACPA-positive RA.

3 Antibodies

Antibodies or immunoglobulins (Ig) are Y-shaped proteins that collectively form the humoral part of the adaptive immune response. In physiological terms, antibody production aims to neutralize pathogens. Antibodies are composed of a pair of heavy and light chains, which form two fragment antigen-binding (Fab) variable regions and one fragment crystallizable (Fc) region. While the Fab region is remarkably diverse to allow for specific binding towards divergent pathogens, the Fc region has a constant structure with limited isotypes and subclasses. Antibody isotypes include early produced IgM, scarcely circulating IgD, most prevalent IgG, mucosa-associated IgA, and parasite-fighting IgE.

The Fc region determines the effector functions of an antibody by its distinct capacity to activate complement and by binding specific Fc receptors (FcR) expressed by effector cells such as macrophages, natural killer cells, neutrophils and mast cells. Antibody binding towards different FcR is regulated by the presence of

different glycan groups in the Fc regions.[23] Upon binding to their cognate antigens, antibodies form immune complexes (ICs) that enhance complement and FcR activation. Consequently, pro-inflammatory cytokines and other mediators that contribute to inflammation are produced. In addition to this Fc tail-dependent effector cell activation, antibodies can also function directly by neutralizing the infective capacity of pathogens, by agonistically activating receptors or by disturbing intermolecular interaction.

IgM and IgA antibodies have a half-life of five to six days while IgG has a 21-day half-life due to recycling by the neonatal FcR (FcRn). Antibody effector functions are also regulated by the presence of soluble FcR which binds to the antibody without subsequent FcR signaling and by binding to the inhibitory FcR, i.e., Fc-gamma-receptor 2b or CD32b.[24-26]

Antibodies are produced by two distinct late-phase differentiated types of B cells, namely plasmablasts and plasma cells which have different characteristics. Plasmablasts are rapidly produced, short-lived and proliferating cells while plasma cells can either be short-lived or long-lived and can reside as quiescent producers of antibodies in tissues and bone marrow.[27] Both cell types are the product of B cell differentiation in the germinal centre at secondary lymphoid organs where B cells undergo substantial changes in B cell receptor (BCR) variable domain genes due to somatic hypermutation. In addition, an alternative pathway of B cell extrafollicular development supports rapid production of antibodies at the site of inflammation. During the course of adaptive humoral immune responses, the antibody repertoire evolves to increase its binding capacity towards the cognate antigens, to broaden the binding targets for efficient clearance of pathogens, and to diversify the isotype usage in order to expand the effector functions of the antibodies. These processes, which are respectively termed as avidity maturation, epitope spreading and class switch recombination, occur primarily in the germinal center.

4 ACPA

ACPA are a group of antibodies that recognize a variety of self-proteins including fibrinogen, filaggrin, vimentin, myelin basic protein, histone and alpha-enolase in their citrullinated forms. The commonality of the majority of these autoantibodies is their binding to a single synthetic peptide, i.e. CCP2. When tested for their fine specificity, most ACPA bind with varying affinity to various citrullinated proteins, indicating that they cross-react. In addition, ACPA can also recognize and thus frequently cross-react with proteins or peptides that undergo other post-translational

modifications.[28,29]

Citrullination or deimination is a post-translational modification of a positively charged arginine residue to the neutrally charged citrulline residue in a peptide or protein backbone, catalysed by a family of enzymes called peptidylarginine deiminases (PADs). The alteration of amino acid charge leads to changes in protein folding and molecular interactions. PADs are expressed in five isoforms where each one has tissue-specific distribution and distinct cellular substrates.[30,31] PAD function is tightly regulated by calcium homeostasis. Under physiological conditions, PADs play roles in the regulation of gene transcription, skin keratinization, brain plasticity, the formation of neutrophil extracellular traps and the regulation of chemokines function such as CXCL8 and CXCL12.[32,33] Aberrant expression and activation of PADs are observed in many pathologic conditions such as cancer; neurodegenerative diseases including Alzheimer's and prion diseases; and autoimmune diseases such as multiple sclerosis, psoriasis and RA.[34]

Despite ubiquitous citrullination in many physiologic and pathologic conditions, the presence of ACPA is strongly associated with RA. Moreover, there is so far only a limited number of citrullinated proteins that have been identified as binding targets of ACPA. It is still unclear whether ACPA targets *in vivo* are the physiologic substrates of PADs.[35] It has been hypothesized that aberrant citrullination by PADs may lead to new citrullinated epitopes that are not tolerized by the immune system. Subsequently these new epitopes could evoke an autoimmune response in susceptible individuals. As rheumatic joints contain numerous citrullinated proteins, it is likely that ACPA can bind to antigens in the joint *in vivo* and evoke inflammatory immune responses.[36,37]

5 Effector functions of ACPA

Autoantibodies can exert their activity by directly binding to organ-specific targets which induces agonistic or inhibitory effects on a particular receptor, thereby activating alternative signalling pathways or disturbing essential molecular interactions.[38] These organ-specific autoantibodies play central roles in autoimmune diseases such as neuromyelitis optica, Grave's disease, bullous pemphigoid, myasthenia gravis, alopecia areata, and a subset of Sjögren's syndrome.[39-44] Other autoantibodies do not have organ-specific targets but bind to ubiquitous autoantigens which lead to systemic disease manifestations. An example of these autoantibodies are anti-double stranded DNA antibodies which contribute to systemic lupus erythematous through IC deposition in the kidney, complement

activation and subsequent tissue injury. Unlike these autoantibodies, however, the disease-causing roles of ACPA are still under debate.

The study of ACPA pathogenicity in human RA is challenging, partly due to the strict definition of ACPA which are defined as antibodies that bind to citrullinated antigens but not to the unmodified "parent" peptide or protein. This means that the isolation of ACPA for functional studies requires strict controls that ensure binding of purified ACPA to citrullinated antigens but not arginine control antigens. Moreover, ACPA isolation could generate antibody aggregation, immune complex formation, co-purification of rheumatoid factor and introduction of pyrogens which can lead to various false positive immunological and non-immunological read-outs.[45] Consequently, there has been considerable debate as to the reproducibility of data with regard to ACPA effector functions derived from isolated polyclonal ACPA preparations.

To circumvent some of the issues regarding the isolation of patient-derived polyclonal ACPA, monoclonal ACPA have been produced and tested for their pathogenicity. Infusion of monoclonal ACPA which bind citrullinated fibrinogen into an RA mouse model induced worsening of arthritis and increased interleukin-6 levels in the joints and serum.[46] In addition, Fab fragment of two monoclonal ACPA, namely D10 and B02, have been shown to induce osteoclast activation and bone resorption through binding with a PAD-mediated citrullinated target on the osteoclast precursors which leads to autocrine IL-8 production.[47] This study suggests a direct activating role of ACPA by binding to a citrullinated target on osteoclast precursors. Moreover IL-8 produced by ACPA monoclonal-activated osteoclast induces pain behaviour in mice through activation of sensory neurons.[48] One fundamental caveat of these studies is the uncertainty of whether these D10 and B02 monoclonals are true ACPA since previous work describing the generation of these monoclonals have been retracted due to the inability of these monoclonals to bind citrullinated peptides.[49] Furthermore, two cohorts of arthralgia and early RA did not show independent association between ACPA and bone erosion or pain.[17,50] In the arthralgia cohort, it is suggested that ACPA indirectly increases bone erosion through the induction of local inflammation in RF-dependent manner.[50] These findings indicate the putative role of ACPA as a part of inflammatory network in the development of RA.

The evidence is clear that ACPA positivity is associated with rapidly progressing, severely destructive RA. Moreover, in individuals without RA, ACPA positivity is already associated with joint complaints.[51] ACPA effector functions have been

researched extensively *in vitro*. It has been reported that ACPA IC bind to Fc-gamma-receptor IIa on macrophages and induce tumor necrosis factor alpha (TNFα) production.[52] As the ACPA response matures prior to disease onset, ACPA may bind to a growing number of diverse citrullinated proteins. These characteristics enable ACPA to form large and numerous ICs which could potently stimulate TNFα production by macrophages.[53]

Moreover, ACPA often co-exist with another autoantibody group, namely the anti-IgG antibodies rheumatoid factor (RF). RA patients who have both autoantibodies exhibit higher disease activity and systemic inflammation compared to patients with either autoantibodies.[54] *In vitro* studies show that IgM RF is able to augment the pro-inflammatory cytokine response of ACPA-IC-activated macrophages.[54,55] Besides binding to and activating macrophages, ACPA IC can also bind to activated neutrophils which are abundant in RA synovial fluid.[56] This binding can intensify joint inflammation. Moreover, IC in the joint can activate FcR on the neurons which induces pain sensation.[57] Apart from activating effector cells, ACPA are also able to activate complement systems *in vitro*.[58] Accordingly, the number of individual synovial fluid ACPA reactivities in the IC, but not monomeric ACPA in the free form, associate with joint inflammation and destruction.[59] These findings support inflammation inducing and/or enhancing roles of ACPA primarily via the formation of IC, in particular in the synovial fluid.

Despite these findings, the clinical outcomes of RA are not mirrored by the levels of these autoantibodies. For instance, clinical benefits of medication are generally not coupled with a decrease in ACPA levels.[60] Moreover, plasmapheresis was shown not to benefit chronic RA.[61] In addition, the presence of ACPA alone is not associated with bone erosions in the patients with clinically suspect arthralgia.[50] Therefore, it is possible that ACPA might propagate joint inflammation once it is started and that the underlying immune dysregulations play a more dominant role in the establishment of synovitis, which is the main clinical correlate of RA.

6 The evolution of the ACPA response during RA development

ACPA can be detected years before the onset of RA. During this period, the ACPA response is not stable and can also revert to a negative state.[62] In some individuals, however, it undergoes substantial changes. Most of the knowledge about the evolution of the ACPA response comes from the study of unaffected first degree relatives (FDR) of RA patients and from blood donors who later developed RA. FDR have a higher prevalence of ACPA positivity than the general population and share

genetic as well as environmental risk factors for RA, including a high prevalence of HLA-SE alleles and, in some populations, smoking.[63] FDRs are more likely to have joint symptoms than healthy controls, without fulfilling the criteria of definite RA.[64] In this "pre-RA" stage, ACPA titres are generally low with more limited epitope recognition and isotype usage compared to established RA.[64-66] Notably, the most prominent ACPA class at this stage is IgA, indicating that a mucosal trigger could breach the tolerance towards citrullinated antigens. ACPA-positive FDR gradually expand their ACPA binding targets until the onset of disease, indicating a maturation of the ACPA response through epitope spreading.[67] Accordingly, unaffected FDRs who harbour ACPA with a broad epitope recognition profile are more likely to develop RA in the future than those with limited recognition profile.[63,66]

ACPA avidity also increases over time until disease onset. Thereafter, no further avidity changes take place.[68] Compared to antibodies against vaccination antigens such as tetanus toxoid, however, ACPA from patients with established RA have substantially lower avidity despite undergoing extensive somatic hypermutation.[69,70] Another major difference between ACPA and protective antibodies is that the majority of ACPA IgG contain glycans in their variable domain (VD).[71] This unique feature is thought to result from a selective advantage inferred by the introduction of N-glycosylation sites during the development of ACPA-expressing B cells.[70,72] This modification is an acquired feature of the ACPA response which occurs before the onset of disease. Generally, unaffected FDR exhibit low ACPA IgG VD glycosylation. However, those with extensive VD glycosylation are six times more likely to develop RA in the future.[73] ACPA IgG also undergo changes in the glycosylation pattern at the Fc tails at the onset of RA. Based on murine studies, it is assumed that the newly acquired Fc tail glycosylation pattern renders these autoantibodies more inflammatory.[74]

The evolving nature of the ACPA response before the onset of disease can be explained by a two-hit theory (Figure 1a and 1b). In this model, the first hit occurs when tolerance towards citrullinated antigens is breached, resulting in the appearance of a limited ACPA response without clinical manifestations. This event is likely to occur in the mucosal organs. The following, second hit triggers maturation of the ACPA response, resulting in increased titers, avidity maturation, epitope spreading, isotypes diversification and the acquisition of VD glycosylation prior to disease onset. These events require close interaction between ACPA-expressing B cells and T cells in at least two discrete episodes that are reminiscent of a prime-boost strategy during vaccination. Eventually, this conceptual model might be too

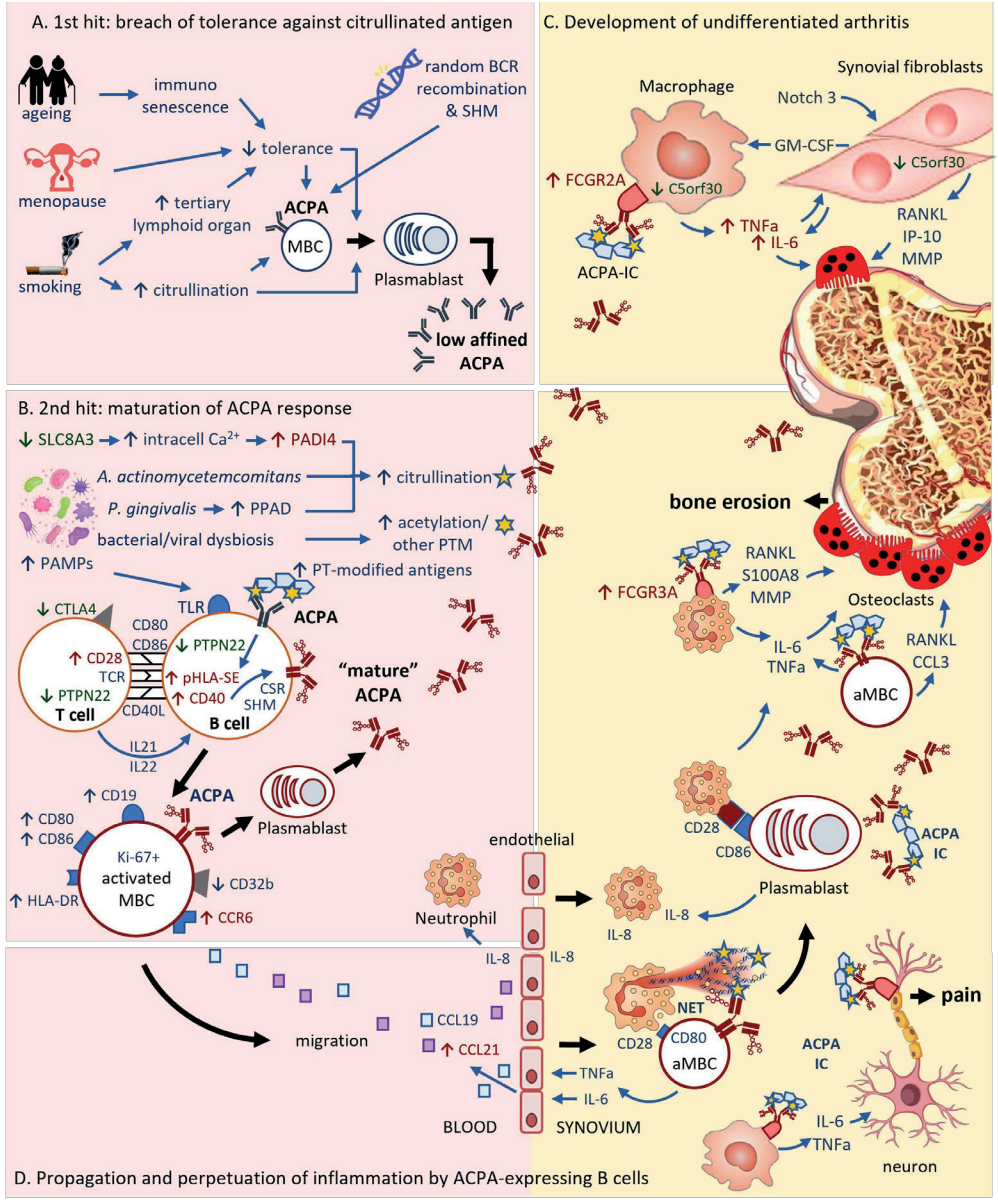

Figure 1. The development and roles of ACPA-expressing B cells in the aetiopathogenesis of RA (**A**). ACPA-expressing B cells develop due to the breach of tolerance towards citrullinated self-antigen, resulting in the production of low affinity ACPA. (**B**) ACPA-expressing B cells are reactivated due to several environmental and genetic factors with the help of T cell, resulting in activated memory phenotype and maturation of pro-inflammatory ACPA. (**C**) Mature ACPA, together with activated macrophages and synovial fibroblasts, elicit the development of joint inflammation resulting in undifferentiated arthritis. (**D**) ACPA-expressing activated B cells migrate to the synovium, propagate and sustain inflammation, resulting in RA.

simplistic, however, as the dynamics of changes in ACPA characteristics suggest that multiple events/hits are required that induce a step-wise maturation of the ACPA-response ('multiple hit model'). Nonetheless, upon formation of a "mature" ACPA response, inflammation and other clinical manifestations may follow.

7 The Aetiology of ACPA-positive RA

Numerous pre-clinical, epidemiological, genetic, epigenetic and therapeutic studies have provided evidence and clues on the distinct causes of the development of ACPA and ACPA-positive RA. Intricate interactions between environmental and genetic factors are presumably responsible for the breach of tolerance towards citrullinated autoantigens, which gives rise to ACPA positivity and the development of RA which is preceded by an enhanced ACPA response (Figure 1b).

7.1 The breach of tolerance towards citrullinated autoantigens

In a large population-based cohort study from the Netherlands, ACPA were detectable in around 1% of the general, mostly Caucasian population. ACPA positivity is more prevalent in women, the elderly, smokers, people with joint complaints, RA patients and first-degree relatives of patients with rheumatic diseases. When RA patients are excluded, ACPA positivity still associates with older age, smoking and joint complaints.[51] In a Swiss cohort, the presence of ACPA peaks in women around perimenopausal age which indicates that pronounced hormonal decline may support the breach of tolerance towards self-antigens, including citrullinated proteins.[75] Accordingly, hormone replacement therapy associates with a reduced risk of developing ACPA-positive RA but not with ACPA-negative RA in post-menopausal women.[76]

In a Swedish twin study, heavy cigarette smoking is significantly associated with ACPA positivity without RA.[77] Similarly, exposure to air pollutants such as sulphur dioxide is weakly associated with the presence of ACPA in a large Canadian cohort.[78] Indeed, smoking has been shown to increase protein citrullination in the bronchial mucosal and alveolar compartments as well as to alter cytokine balance.[79,80] Based on these epidemiological studies, ageing, the decline of ovarian function and mucosal exposure to air pollutants are linked to the breach of tolerance towards citrullinated self-antigens. These associations could be explained by the accumulation of citrullinated self-proteins related to ageing and smoking as well as by the dysregulation of immunological tolerance related to endocrine changes and immunosenescence.[81-85]

The association of autoimmunity with smoking, ageing, female gender as well as endocrinological transition is, however, not exclusive for the development of ACPA. Data from multinational cohorts showed that smoking is associated with concurrent multiple RA-associated autoantibodies and not with a specific autoantibody.[86] Smoking is also associated with the presence of other disease-specific autoantibodies including anti-myeloperoxidase and anti-topoisomerase I antibodies.[87,88] Moreover, many autoimmune diseases are more prevalent in women while the course of the disease is tightly linked to major female endocrinological changes, namely puberty, pregnancy and menopause. These life events are known to have considerable influence on both the innate and adaptive immune systems.[84] Ageing, on the other hand, is associated with the involution of the thymus, the major T cell central tolerance organ; a decrease in B cell lymphopoiesis and quality control; and increased levels of autoantibodies.[89-93]

As the B cell receptor (BCR) repertoire originates from random recombination of gene segments in the bone marrow, mature naïve B cells that recognize citrullinated antigens may arise stochastically. Moreover, new evidence shows that some germline encoded B cell receptors already have propensity to weakly bind citrullinated and other post-translationally modified proteins.[46,94] These ACPA-expressing B cells may subsequently get selected to become plasmablasts that eventually produce low levels of ACPA as a result of increased citrullination and dysregulation of immunological tolerance. Autoimmunity against citrullinated antigens is, then, born. At this stage, however, the ACPA response is still 'immature'. The emergence of ACPA is necessary but not sufficient for the development of ACPA-positive RA. Accordingly, some ACPA-positive individuals may never develop RA in their lifetime, and without additional triggers and/or genetic predisposition the ACPA response is likely to disappear again at this stage.[62] The development of autoimmune disease from autoimmunity can take several years and requires interaction between additional genetic and environmental factors and, in particular, the presence of specific helper T cells, as indicated by HLA association which is restricted to ACPA-positive RA but not ACPA only.

7.2 The development of ACPA-positive RA

7.2.1 Environmental factors

Bacterial and viral dysbiosis has been hypothesized to induce ACPA-positive RA in genetically susceptible individuals.[95-97] Some studies link ACPA-positive RA with severe chronic periodontitis, an inflammation of the gums and supporting structures

of the teeth caused by bacterial dysbiosis. Both RA and periodontitis share similar risk factors, namely the HLA-DRB1 shared epitope, smoking, and ageing.[98]

Two periodontal pathogens have been suggested to increase citrullination of self- as well as bacterial proteins which, in theory, can subsequently activate ACPA-expressing B cell by means of both the citrullinated antigens and pathogen-associated molecular patterns (PAMPs). These bacteria are *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. *P. gingivalis* contains a bacterial citrullinating enzyme (termed *Prophyromonas* PAD or PPAD) and bacterial enolase. Anti-*P.gingivalis* antibodies are increased in RA patients and are detectable years before the onset of RA.[95] Moreover, a positive correlation between the serum levels of anti-PPAD IgG and ACPA IgG has been reported in Japanese subjects.[99] This correlation is, however, could not be shown for subjects in the US.[100]

It has been hypothesized that humans develop antibodies against PPAD-mediated citrullinated bacterial proteins which eventually cross-react with human citrullinated self-proteins due to molecular mimicry.[101,102] To test this hypothesis, several groups have assessed the cross-reactivity of ACPA from RA patients with citrullinated *P. gingivalis*-derived proteins and peptides. Using patient-derived anti-citrullinated alpha-enolase peptide antibodies, Lundberg *et al* showed that these autoantibodies bind a citrullinated bacterial enolase peptide which shares sequence similarity with human enolase.[103] At the protein level, however, Muñoz-Atienza *et al*, could not identify ACPA binding specificity towards auto-citrullinated *P. gingivalis* proteins.[104] In addition, PPAD has been shown to citrullinate human fibrinogen and enolase which are two binding targets of ACPA, in a manner different from human PAD.[105,106] These findings suggest possible roles of *P.gingivalis* in the development of RA, particularly via PPAD-mediated bacterial and human protein citrullination, although the mechanistic basis for this hypothesis is still under debate.

Aggregatibacter actinomycetemcomitans (*Aa*), on the other hand, does not contain bacterial PAD. Instead, it produces leukotoxin A (LtxA), a pore-forming toxin, which increases calcium influx in neutrophils, triggers hypercitrullination of intracellular neutrophils proteins, and releases the hypercitrullinated cargo in a similar fashion as neutrophil extracellular trap (NET) formation.[96] These hypercitrullinated neutrophil proteins are thought to trigger immune responses towards citrullinated proteins in susceptible individuals. In a study from the US, exposure to the bacterial LtxA was found, indeed, associated with ACPA, suggesting a putative role of *Aa* as a trigger for the breach of tolerance towards citrullinated proteins in RA.[96] This association, however, was absent in a large cohort of Dutch RA patients.[107]

To further complexify the possible mechanisms triggering ACPA immune responses, it was found that ACPA can also cross-react with antigens bearing other post-translational modifications (PTM) such as acetyl-lysine and homocitrulline.[94] This cross-reactivity is likely to be more relevant as it can directly induce a breach of tolerance. Apart from ACPA, other anti-modified proteins antibodies (AMPA) such as anti-carbamylated proteins antibodies (anti-CarP) and anti-acetylated proteins antibodies (AAPA) are also prevalent in RA.[29,108] Due to this cross-reactivity, AMPA are thought to originate from a common B-cell response which differentiates into a cross-reactive AMPA immune response with varying avidity for different PTM antigens.[29] Importantly, it has been shown in a murine model that such diversification can occur *in-vivo*. Immunization with either acetylated or carbamylated murine proteins induced both AAPA and anti-CarP antibodies.[29] These findings have opened many possible originating antigens which can commence and activate the ACPA immune response, particularly because acetylation in bacterial proteins is commonplace.[109]

The mucosal bacterial origin of ACPA-positive RA theory is further supported by the fact that some antibiotic therapies are also effective in treating ACPA-positive RA. Double-blind trials have shown that minocycline, a tetracycline antibiotic, is associated with improved clinical outcome in both early and established seropositive RA.[110-113] Furthermore, the patients who benefit most from minocycline are those possessing HLA-SE which is a major susceptibility gene in ACPA-positive RA.[114] Other anti-bacterial therapies such as levofloxacin and clarithromycin have also been associated with clinical improvements in RA.[115] These drugs, however, have also anti-inflammatory properties which are independent of their antibacterial effects.[115-118] It is therefore not clear whether the clinical response of these antibiotics occurs primarily due to the anti-bacterial or anti-inflammatory properties of these drugs. Nonetheless, these findings indicate putative roles of mucosal bacteria in the development of ACPA-positive RA which may be different in each population or individuals.

7.2.2 Genetic and epigenetic factors

Apart from environmental factors, genetic elements also play roles in the origin of ACPA-positive RA. It is estimated that the heritability of ACPA-positive RA is approximately 68%.[119] Although the variation in the susceptibility genes may vary in different populations and occur in only a fraction of RA patients, they can provide insights in the development of ACPA-positive RA. Single nucleotide polymorphisms (SNPs) that are known to render people susceptible for developing

ACPA-positive RA include those at *HLA*, *PTPN22*, *PAD4*, *SLC8A3*, *CD28*, *CTLA-4*, *CD40*, *FCGR2*, *FCRL3*, *IL6R*, *TRAF1/C5* and *CCR6* genes.[120] Using this information, a chain of events can be made to decipher the mechanism by which ACPA-positive RA develops (Figure 1b and 1c.

Excessive and/or ectopic protein citrullination may induce the development and activation of ACPA-expressing B cells. SNPs in the genes involved in protein citrullination, including the citrullinating enzyme peptidylarginine deiminase 4 (*PADI4* and sodium/calcium exchanger membrane protein *SLC8A3*, associate with ACPA-positive RA.[121,122] A SNP at *PADI4* also associates with progressive joint destruction in ACPA-positive RA in Japanese patients.[123] The excessive citrullinated peptides can then be presented by antigen presenting cells (APC, which include ACPA-expressing B cells and dendritic cells, to $CD4^+$ T cells with the help of human leukocyte antigen (HLA class II.[124]

HLA polymorphisms have been estimated to contribute 11-37% to RA heritability which can either increase or decrease the susceptibility of getting ACPA-positive RA.[125,126] A group of HLA-DRB1 alleles that bears similar sequence motifs comprising of five amino acids in residues 70-74 of the DR chain, termed the shared epitope (SE, has a major influence on ACPA-positive RA susceptibility. ACPA-positive individuals with HLA-SE are 4-6 times more likely to develop RA than those without these alleles.[13] Moreover, HLA-SE alleles are linked to the levels of ACPA, indicating a direct role of these genes in the production of ACPA.[127-129] HLA-SE alleles also associate with ACPA-IgG VD glycosylation, a hallmark of mature ACPA response in RA.[130] Remarkably, there is no significant positive association between HLA-SE with ACPA positivity in individuals without RA.[77] These findings suggest that HLA-SE alleles have central roles in the activation and maturation of the ACPA response that precedes the onset of RA, and hence with the transition of ACPA positivity to ACPA-positive disease.

Activation of T cells via antigen presentation requires not only peptide-HLA molecule complexes but also co-stimulatory molecules including CD80 and CD86. These co-stimulatory signals are then received by two competing receptors, the stimulatory CD28 and the inhibitory CTLA-4 on T cells. Gain-of-function SNPs of *CD28* and loss-of-function SNPs of *CTLA-4* are known to render people susceptible to develop RA.[131-133]

Once activated, $CD4^+$ T cells produce CD40 ligand which activates ACPA-expressing B cells and induces BCR somatic hypermutation. Subsequently, epitope

spreading, affinity maturation and VD glycosylation of ACPA may occur. These events mark the maturation of the ACPA immune response. Accordingly, SNPs at *CD40* genes as well as the genes related to CD40 signalling such as *TRAF1* and *TNFAIP3*, have been associated with the development of joint destruction in ACPA-positive RA.[134-136] The activation of both T and B cells requires intracellular signalling regulations which calibrate the threshold of activation. RA susceptibility genes which are involved in the modulation of lymphocyte activation are *PTPN22* and *FCRL3*.[137] Individuals who have a combination of a *PTPN22* variant and ACPA positivity confer a much higher relative risk to develop RA than those who have either risk factor, suggesting pathogenic roles of hyperactive lymphocytes, especially ACPA-expressing B cells, in the development of ACPA-positive RA.[138] A genetic variance on *PRDM1*, a transcription factor essential for B cell differentiation into plasmablasts/cells (PB/C), also associates with seropositive RA, indicating putative pathological roles of autoreactive PB/C in the development of RA.[131] Activated B cells are indeed found in the RA synovial tissue. One of the chemokine receptors which regulates migration towards inflamed organs is CCR6. SNP of this receptor as well as chemokine CCL21 are associated with ACPA-positive RA.[136,139-141]

Once mature ACPA are produced at a certain level and bind to citrullinated antigens in the joints, they form immune complexes that activate effector cells through FcR binding as well as complement activation, resulting in joint inflammation. Accordingly, polymorphisms in the FcR and complement factor-expressing genes, *FCGR2A*, *FCGR3A* and *TRAF1-C5*, have been shown to associate with ACPA-positive RA in Caucasian subjects.[131,142-144]

Other than polymorphisms at the susceptibility genes, epigenetic factors, such as DNA methylation at *PCDHB14* is associated with ACPA positivity, while DNA methylations at *PCDHB5* and *EXOSC1* are associated with ACPA-positive RA.[145,146] This supports the distinct mechanisms of the breach of tolerance towards citrullinated antigens and development of ACPA-positive RA also at epigenetic levels.

There are some overlaps between the genetic basis of ACPA-positive and -negative RA.[13,120,147] This indicates that B cell-independent factors also contribute to the pathogenesis of RA while the ACPA response enhances the progression and the chronicity of the disease (Figure 1c). These overlaps include a genetic variant at *C5orf30* which encodes a 206-aa protein that regulates tissue damage response and activation of synovial fibroblasts and macrophages.[13,148,149]

A *C5orf30* polymorphism increases the invasiveness and the production of gamma interferon-inducible protein-10 (IP-10) by synovial fibroblasts.[150] Synovial fibroblasts physiologically constitute the inner layer of synovium which is essential for the production of the components of the synovial fluid. These cells also produce optineurin to inhibit osteoclast differentiation in inflammatory conditions.[151] In RA, however, these cells display an altered phenotype, grow uncontrollably and produce IL-6, RANKL as well as matrix metalloproteases which mediate joint inflammation and bone erosion.[152,153] The altered phenotype may stem from the upregulation of endothelium-derived Notch3 signalling.[154] Moreover, synovial fibroblast subsets may have specific pathological functions in RA. Adoptive transfer of different subtypes of synovial fibroblasts in mouse model demonstrate specialized function of fibroblast subtypes in mediating inflammation or joint damage.[155] Targeting synovial fibroblasts is possible using anti-cadherin-11 mAb which leads to reduction of joint inflammation in mouse models of arthritis.[152]

Furthermore, polymorphisms of genes encoding cytokine receptor and signal transducer, TRAF1/C5 and STAT4, are also associated with RA irrespective of the ACPA status.[13,120] The roles of pro-inflammatory cytokines in the development of RA are further evidenced by the association of *IL6ST* and *TRAF1* gene polymorphisms with RA as well as the success of IL-6 and TNF-alpha inhibitors for the treatment of RA regardless of the ACPA status.[120,139,156] These data suggest that dysregulation of synovial fibroblasts, macrophages and the cytokine network in the joints may be the determinants of arthritis development which are further aggravated and sustained by the ACPA immune response.

Altogether, these susceptibility genes highlight the plethora of immune dysregulations that play roles in the development of ACPA-positive RA. The mere presence of any of these susceptibility genes, however, does not cause RA as shown in large twin studies which prove low concordance rates for ACPA-positive RA in monozygous twin. [157,158] This observation suggests a complex interplay between susceptibility genes and environmental factors in the development of ACPA-positive RA.[159,160]

7.2.3 The perpetrators in established ACPA-positive RA: lessons from clinical trials

The importance of specific immune cells and cytokines in the pathogenesis of ACPA-positive RA has been substantiated or disapproved by RA clinical trials testing targeted therapies in the form of biological and targeted synthetic disease-modifying anti-rheumatic drugs (bDMARDs and tsDMARDSs). Inhibitors of the

IL1 receptor (anakinra), TNF-alpha (infliximab), IL-6 (tocilizumab, sirukumab), the IL-6 receptor (sarilumab), GM-CSF (otilimab) and the GM-CSF receptor (mavrilimumab) are efficacious in treating RA regardless of ACPA status.[156,161-164] Moreover, inhibitors of JAK-STAT signalling (tofacitinib, baricitinib, filgotinib), which relay cytokine signals, also show good clinical response in both types of RA.[165-169] Therefore, these clinical benefits highlight the central roles of pro-inflammatory cytokines in the aetiopathogenesis of RA regardless of ACPA status.

Under physiological condition, these mediators of inflammation are produced locally at the site of tissue injury which activate both local and systemic inflammatory responses. Such responses aim to enhance the clearance of pathogens and injured tissue as well as to stimulate the healing process. To this end, extra resources from the body are required and therefore compensatory mechanisms take place; for instance, the suppression of red blood cell formation and demineralization of the bone.[170,171] To regulate this process, pro-inflammatory cytokines are normally modulated by negative feedback signalling and the activation of anti-inflammatory cytokines as well as other pro-resolving mediators. In RA, however, the disbalance of the cytokine network occurs chronically due to persistent tissue injury, hyperactive immune cells and dysfunctional resolution of inflammation.[172]

Pro-inflammatory cytokines such as IL-1, IL-6, TNF alpha, and GM-CSF have pleiotropic effects on immune cells, synovial fibroblasts, neuron excitation, osteoclast maturation, and activation of endothelial cells all of which play roles in RA pathogenesis. These cytokines are produced mainly by activated synovial fibroblasts, monocytes and innate lymphoid cells which in turn enhance the activation, differentiation and survival of the immune cells as well as osteoclasts that play direct roles in joint inflammation and damage.[173-176] Indeed, the levels of these cytokines are elevated in the blood and/or synovial tissue of RA patients.[177-179] As a result, they promote the up-regulation of body temperature, fatigue and joint pain which characterize the symptoms of RA.[180-183]

The association of genetic variants of HLA-DR, CD28, CTLA4, PTPN22 and CD40 signalling with ACPA-positive RA as well as the abundance of T cells in RA synovium strongly suggest the involvement of T cell activation in the aetiopathogenesis of the disease.[184,185] $CD4^+$ helper T cells develop from common lymphoid progenitors in the bone marrow and migrate to the cortex of the thymus to undergo positive and negative selection in which T cells expressing T cell receptors (TCR) that bind self-proteins are largely eliminated. This central T cell tolerance is essential in ensuring that competent T cells are not autoreactive.

Accordingly, mutations of *AIRE*, a gene central in T cell tolerance, cause multiorgan autoimmunity.[186] Once emigrating from the thymus and getting activated by antigen-presenting cells, naïve T helper cells further develop into intracellular pathogens-counteracting TH1, extracellular parasites-counteracting TH2, B cell-stimulating follicular helper T cells (TFH) and peripheral helper T cells (TPH), IL-17-producing TH17, and immune regulatory T cells (Treg).[187]

Clinical trials targeting T cells, their subsets and cytokines to treat RA, however, have shown less promising results. The phase 1 clinical trials of a pan-T cell depletion therapy using anti-CD3 monoclonal antibody (mAb) otelixizumab to treat RA have been terminated due to a "changed risk-benefit ratio for the patients". Moreover, trials of anti-CD4 mAbs for RA have shown mixed results, with the most positive ones only resulting in modest improvement in clinical parameters.[188-194] By depleting $CD4^+$ T cells, it is hypothesized that not only pro-inflammatory TH17 cells but also anti-inflammatory Treg cells are affected. Therefore, a few trials have attempted to specifically target TH17-associated cytokines and to enhance Treg cell functions. Monoclonal antibodies against TH17-associated cytokines, such as anti-IL-12/23 (ustekinumab), anti-IL-23 (guselkumab) and anti-IL17A (secukinumab) failed to induce clinical improvement in RA.[195,196] Efforts to activate Treg by targeting unique CD4 epitopes using tregalizumab have also failed to improve clinical outcome of RA patients.[197,198]

The failure of T-cell targeting trials may stem from the fact that T cells in established RA are hypo-responsive to stimulation.[199] This anergic phenotype may be linked to T cell exhaustion due to persistent antigen and inflammatory signals.[200-203] T cells seem to have passive roles in perpetuating joint inflammation in established RA and therefore targeting T cells at this stage of RA does not render significant clinical response. T cells are more likely to have major roles in the maturation of the ACPA response, the second hit of RA development. This hypothesis is supported by the fact that TH17-produced IL-21 and IL-22 can change the Fc-glycosylation profile of IgG.[204] Glycosylation changes hallmark the maturation of ACPA and are strongly associated with the progression toward RA. These findings indicate that T cells may play more pivotal roles during the maturation of the ACPA response before the onset of disease rather than in the established and/or chronic phase of RA.

In contrast to T cell depletion, B cell depletion therapy using anti-CD20 mAb rituximab (RTX) is significantly more efficacious than methotrexate in reducing RA symptoms and progression.[21,205,206] Besides for RA, RTX is authorised in Europe for

the treatment of B cell malignancies such as follicular lymphoma, diffuse large B cell non-Hodgkin's lymphoma and chronic lymphocytic leukaemia; and B cell-related autoimmune diseases such as granulomatosis with polyangiitis, microscopic polyangiitis and pemphigus vulgaris. The depletion of B cell subpopulations in peripheral blood using RTX is significant. In solid tissues, however, the depletion is incomplete.[207]

B cell development starts in the bone marrow where common lymphoid progenitors sequentially differentiate into pro-B cells, pre-B cells and immature B cells. These B cell precursors are selected based on productive rearrangement of immunoglobulin heavy (IgH) and light (IgL) chains as well as the expression of CD79a/b which together form the functional BCR complex. The expression of the transcription factor PAX5, which associates with the expression of CD19, is essential for B cell identity. Furthermore, the expression of CD20 starts from the pre-B cell stage.[207] Transitional B cells egress from the bone marrow into the peripheral blood and mature further to become antigen-inexperienced IgD^+ IgM^+ naïve B cells.[208] Upon binding to antigen, naïve B cells migrate into secondary lymphoid organs where B cells undergo class-switching recombination and somatic hypermutation (SHM) in their BCR genes with the help of TFH. B cells with high affinity to the antigen receive T cell stimulation and undergo clonal expansion as well as further development into memory B cells and antibody-secreting cells (ASCs). ASCs cease to express CD20 as well as PAX5 and some migrate back to the bone marrow or inflamed tissues as long-lived plasma cells. Therefore, RTX only selectively depletes pre-B cells up until the memory B cell subsets, but not ASC.

There are several biomarkers which can predict good and poor response to RTX. The serological biomarkers that predict good clinical response are ACPA, RF, IL-33 and increased erythrocyte sedimentation rate (ESR).[209-211] A good response to RTX in RA is not only predicted by ACPA positivity but also by high levels of ACPA.[210,212,213] Another good predictive biomarker is IL-33 which is a cytokine released from necrotic cells to alert the immune system for tissue damage or stress. In RA, IL-33 circulates at higher levels compared to healthy individuals and is decreased after anti-TNF treatment.[214,215] The fact that IL-33 synergistically predicts positive RTX responses with the autoantibodies suggests that tissue damage may support the activation and survival of ACPA-expressing B cells.[211]

Poor predictors of RTX responses in RA include elevated CD20-negative ASC markers and an increased type I interferon (IFN) signature.[216-219] In active RA, general type I IFN signature does not correlate with the presence and titres of ACPA

and RF.[220] Moreover, people with a high type I IFN signature are more likely to develop RA, independent of the autoantibody status.[221] On the other hand, specific type I IFN signature genes which regulate helper T cells and B cell proliferation correlate significantly with the levels of ACPA and anti-CarP antibodies.[222] These findings suggest that type I IFN activation plays diverse roles in the development of RA, one of which is through the activation of autoreactive B cells.

All in all, clinical trials provide clear evidence for the involvement of specific cytokines and immune cells in established RA. Pro-inflammatory cytokines are central mediators of RA symptoms regardless of serological status. Moreover, CD20-positive B cells, particularly ACPA-expressing B cells, are essential for the progression of ACPA-positive RA. T cells, on the other hand, seem to be dispensable in sustaining and progressing joint inflammation in established RA.

8 ACPA-expressing B cells

8.1 The origin of ACPA-expressing B cells

Serological, genetic and clinical studies discussed above demonstrate the putative central roles of ACPA-expressing B cells in the aetiopathogenesis of ACPA-positive RA. The two hit theory of the ACPA response in RA development suggests that prior to disease onset ACPA-expressing B cells develop and mature at different time points which depend on distinctive factors (Figure 1a and 1b). In the bone marrow, B cells undergo random V(D)J recombination of BCR genes to enable the binding to infinitely diverse pathogenic antigens. The by-product of this process is the occurrence of autoreactive B cells. In fact, it has been suggested that 75% of pre-B cell clones are autoreactive, as defined by the ability to bind HEp-2 cell extract.[223] The percentage of autoreactive BCR is decreasing during B cell development from an estimated 43% in the immature B cell stage, 40% in the transitional B cell stage and around 20% in the mature naïve B cell stage.[223] Self-reactive B cells are, therefore, largely removed in the bone marrow at the immature B cell stage and in the blood during the transition to become mature naïve B cells.

Immunological tolerance is essential to prevent the immune system from attacking its own body. Besides autoantigen-specific B cell removal, immunological tolerance is safeguarded by receptor editing, autoantigen-specific anergy and ignorance induction.[224] Switching the BCR light chain from Ig-kappa to Ig-lambda, for example, is typically effective in modifying BCR reactivity away from autoreactivity.[225] Under physiologic conditions, surviving autoreactive naïve B

cells that are not deleted during B cell development adopt an anergic phenotype, such as lower IgM expression, and are prevented to become ASC by tolerance checkpoints.[226] These cells are preserved in the periphery as they increase the diversity of the BCR pool which may benefit the protective immune response. Upon infection, these autoreactive B cells may undergo somatic hypermutation (SHM) and selection processes in the germinal centre which results in decreased affinity towards self-antigens and increased binding to infectious antigens.[226,227] In fact, broadly neutralizing antibodies against HIV-1 retain some degree of poly- and autoreactivity.[228] It is hypothesized that these viral responses may arise from poly/autoreactive B cell clones which progressively lose their autoreactivity and become protective.[229,230]

TFH cell-dependent BCR selection procedures and multiple rounds of BCR gene editing are necessary for dampening autoimmunity. BCR with high affinity to an antigen are able to bind and capture the antigen and present the processed antigen to antigen-specific T helper cells. In turn, TFH cells provide survival and activation signals to B cells. Antigen-stimulated germinal centre B cells which do not receive stimulation from TFH will undergo apoptosis.[231-233] On the other hand, strong T cell stimulation with weak BCR signalling may also induce FAS-dependent B cell death.[234] Therefore, the requirement of balanced BCR and T cell-derived signals in the survival and activation of germinal centre B cells provides immunological checkpoints to prevent the activation of autoreactive B cells in the periphery.

This multi-step process, however, is not error-free and can be hijacked. For instance, up-regulation of anti-apoptotic signals can inhibit the elimination of autoreactive B cell clones in the germinal centre.[232] The random nature of BCR SHM, just like V(D)J recombination, can give rise to autoreactive BCR clones. In fact, autoreactive IgG^+ MBC can be detected in the blood of healthy donors. These cells express BCR which become autoreactive only after undergoing SHM in the germinal centre.[235]

The germinal centre reaction normally occurs in secondary lymphoid organs (SLO) such as lymph nodes and spleen. In inflamed tissues, germinal centre reactions may also develop ectopically in inducible tertiary lymphoid organs (TLO).[236] Physiologically, TLOs are able to develop local and quick humoral immune responses to foreign antigens. The immunological tolerance check-points at TLOs, however, may be less strict compared to SLOs which favours the development of autoreactive B cells.[237,238] Persistent TLOs can be found in mice exposed to chronic cigarette smoke exposure, a risk factor of ACPA development.[239] Therefore, dysregulation of immunological tolerance, which associates with cigarette smoking,

may support the development of low-affine ACPA-expressing B cells. Such cells mark the first hit in the development of the ACPA response.

8.2 The activation of ACPA-expressing B cells

The intricate genetic and environmental factors discussed previously may lead to the activation of ACPA-expressing B cells. The hallmark of this process is an enhanced T cell-dependent germinal centre activity including SHM and class-switch recombination. The BCR of ACPA-expressing MBC in RA patients is mutated extensively, contains predominantly immunoglobulin lambda light chains, and is mostly class-switched. These characteristics indicate several rounds of *IgH* and *IgL* gene editing in the germinal centre which lead to the expansion of ACPA repertoire.[70,240] Moreover, approximately 90% of ACPA-IgG expressing MBCs in RA have BCR variable domain (VD) glycosylation that is introduced during SHM.[70] In mouse models of autoimmunity, VD glycans on the BCR occupy the majority of antigen binding sites and decrease the avidity of residual unglycosylated sites.[227] On ACPA, however, VD glycosylation does not generally decrease the avidity towards citrullinated antigens but may affect the accessibility of antigen binding sites through steric hindrance. The high proportion of ACPA-expressing B cells with VD glycosylation indicates that this modification may provide survival advantage during maturation of ACPA response.

Remarkably, the VD glycans on ACPA contain sialic acids which can have immunomodulatory properties through binding to siglecs.[241,242] Theoretically, the highly sialylated BCR of ACPA-expressing B cells may dampen negative selection mechanisms allowing for their survival in the periphery. Moreover, the bulky VD glycan of ACPA may interact with the BCR and other proteins which increase tonic BCR signalling.[243] It has been shown that BCR signalling dysregulation can disturb B cell tolerance mechanisms. For instance, genetic variance of *PTPN22* and *PIK3CD* disturb BCR signalling and, consequently, reduce the removal of self-reactive B cells.[244,245] Therefore it is possible that highly sialylated VD glycans provide survival advantages to ACPA-expressing B cells through dysregulation of BCR signalling and/or inhibition of immunological tolerance check-points.

The activation of MBC is also regulated by negative feedback from antibodies. Binding of antigen-antibody immune complex to both BCR and inhibitory Fc gamma receptor 2b (FcGR2b or CD32b induces apoptosis of antigen-specific MBC which limits antigen-specific humoral immune response and leaves room for other plasma cells in bone marrow.[246,247] Down-regulation of CD32b

expression and function on MBC impairs this negative feedback mechanism and associates with increased autoreactive IgG^+ MBC.[248] Therefore it is possible that ACPA-expressing MBCs modulate their CD32b signalling to remain active despite high levels of circulating ACPA and the presence of citrullinated antigens.

8.3 The migration and pathogenic roles of ACPA-expressing B cells

Activated B cells have effector functions locally by migrating towards inflamed or injured tissue, attracting and activating other immune cells *in situ* as well as systemically by production of antibody and cytokines. Peripheral MBCs express chemokine receptors which allow them to migrate to inflammatory and secondary lymphoid tissues following a gradient of chemokines.[249,250] The serum levels of the chemokine CCL19 are increased in ACPA-positive RA patients, positively correlate with B cell activation markers and predict RTX response at 24 week.[251] CCL19 is primarily produced by stimulated RA macrophages and synovial fibroblasts and attracts CCR7-expressing cells to the inflamed synovial tissue.[252] Through the putative expression of chemokine receptors, activated ACPA-expressing MBC may migrate to inflamed and/or injured synovial tissues.

In the inflamed synovial tissue, ACPA-expressing MBC may get activated by citrullinated proteins and the inflammatory milieu to become ACPA-producing plasmablasts/cells (PB/C).[253,254] The source of citrullinated proteins includes neutrophil extracellular traps which are present in RA synovium.[255] ACPA-expressing MBC may also interact with specialized PD-1 high, CXCR5 negative peripheral helper T cells in RA synovium to differentiate into ACPA-producing PB/C in IL-21-dependent manner.[187] ACPA-producing PB/Cs are indeed present and active in RA synovial fluid.[253,256,257] Furthermore, their survival is supported by the presence of other mononuclear cells in RA synovial fluid.[256]

In the joint, activated B cells can propagate local inflammation and induce bone erosion through the expression of co-stimulatory molecules and the production of cytokines and antibodies. The importance of the co-stimulatory molecules CD80/86 in ACPA-positive RA pathogenesis is demonstrated by the fact that ACPA positivity correlates with better treatment response to the CD80/86 blocking agent abatacept.[22] CD80/86 is expressed by various antigen presenting cells and co-stimulates immune responses through an interaction with CD28 which is mainly expressed by T cells. Given the lack of clinical evidence for a role of T cell role in sustaining arthritis, the expression of CD80/86 by ACPA-expressing B cells can, theoretically, stimulate innate immune responses. Clinical study of the superagonist

anti-CD28 mAb TGN1412 led to an abrupt fatal cytokine storm which may not stem from T cell activation.[258] Human immune cells that express functional CD28 and secrete pro-inflammatory cytokines immediately include neutrophils and eosinophils. In fact, neutrophils are the most abundant immune cells in the RA synovial fluid.[259] Crosslinking of CD28 on these cells results in IFN gamma, IL-13 and IL-2 production.[260,261] Moreover, Immune complex-activated neutrophils produce osteoclast-activating S100A8 and MMP-9 which play roles in bone erosion.[262,263] These cells also release their nuclear components to form neutrophils extracellular traps (NETs) which contain citrullinated histones.[255] Hypothetically, ACPA-expressing MBC can be activated by these citrullinated histones and differentiate into ACPA-producing PB/Cs which in turn activate neutrophils in CD86, IL-8 and ACPA-IC-dependent manners. This hypothesis argues for the bidirectional interactions between ACPA-expressing B cells and neutrophils that propagate and perpetuate inflammation as well as bone erosion.

Furthermore, BCR-stimulated B cells can produce pro-inflammatory cytokines and chemokines which are involved in bone erosion.[264-267] Synovial B cells from RA patients spontaneously produce CCL3 and TNF-alpha which suppress osteoblast differentiation.[265] These cells also produce RANKL which induces bone-resorbing osteoclast development.[266,267] Taken together, ACPA-expressing B cells may migrate to the inflamed synovium, get activated and remain in the synovial tissue to contribute to the progression of RA by not only producing ACPA but also co-stimulatory molecules, cytokines and chemokines which perpetuate joint inflammation and enhance bone erosion (Figure 1d).

8.4 The nature of ACPA-expressing B cells: lessons from rituximab and abatacept

The nature of ACPA-expressing B cells in RA can be deduced by studying the kinetics of clinical responses, ACPA levels and fine specificity, as well as B cell counts after treatment using RTX and abatacept. ACPA are strong positive predictors of the response to both medicines.[268] Two infusions of 1000 mg rituximab on days 1 and 15 deplete circulating B cells at week four post first treatment. At this timepoint, synovial B cells are significantly decreased though not completely absent. Moreover, there is also a significant decrease of both synovial T cells and macrophages. The 28-joint Disease Activity Score (DAS28) as well as ACPA levels are not yet significantly reduced at this timepoint, while at weeks 16 and 24 post first treatment, the DAS28 is significantly reduced. The clinical improvement can be predicted by the reduction of synovial PB/C. At around 24 weeks, peripheral B

cells start to return. ACPA levels, however, only start to decrease significantly at 36 weeks.[269] These observations provide evidence for the role of B cells in triggering local immune infiltration and the incapacity of ACPA in inducing arthritis. This notion is also supported by the fact that ACPA levels are frequently unaltered during disease remission.[270]

Moreover, the delay of the clinical response after CD20 depletion suggests that $CD20^+$ B cells may mediate joints inflammation indirectly through activation of other immune cells and differentiation into plasma cells.[271] In fact, unaltered plasma cell counts in the joints associates with poorer response to RTX.[271]

Relapse occurs after discontinuation of RTX treatment and is preceded by the re-occurrence of IgM^+ naïve B cells from the bone marrow. Remarkably, the circulating ACPA at relapse consist of new and old antigen binding specificities. These findings suggest that the mechanism underlying ACPA responses is self-sustaining in RA and that interactions between new and residual ACPA-expressing B cells may be essential in resurgence of symptoms.[272,273]

In contrast to RTX therapy, a decline in ACPA levels at 3 months predicts sustained improvement of RA symptoms at 12 months after treatment with abatacept, a chimeric CTLA4-Fc construct.[274] Given that ACPA-producing plasma cells can live at least for several months, the early reduction of ACPA indicates that abatacept may not only inhibit the development of ACPA-expressing MBC into plasma cells but also directly inhibit ACPA-producing PB/C. CD80/86 proteins contain binding domains for signalling proteins in their cytoplasmic tails, suggesting the ability to transduce signals upon activation. In dendritic cells, cross-linking of CD80/86 by CD28 results in IL-6 production while crosslinking by CTLA-4 results in immune suppressive enzyme IDO production.[275] Thus, the engagement of abatacept with CD80/86 on ACPA-producing PB/C may reduce their pro-inflammatory capacity which lead to a decrease in ACPA levels and, possibly, clinical improvement.

ACPA B cell responses seem to differ from responses against vaccine antigens or virus infection. Years after sustained MBC depletion, rhesus macaques still produce anti-virus antibodies which are attributed to long-lived plasma cells.[276] MBCs, on the other hand, have the capacity to mutate their BCR to neutralize virus mutants which escape the binding to pre-existing antibodies.[277] This division of tasks safeguards the body not only from the same pathogen but also from their evolving mutants. Accordingly, a two-year period of $CD20^+$ B cell depletion in RA patients does not change the levels of protective antibodies against measles, mumps and

rubella.[278] The levels of ACPA, however, decrease significantly in this period; even though in general no seroconversion was observed. Moreover, the positive correlation between circulating IgG levels and antigen-specific MBC is found in ACPA but not in anti-tetanus responses.[279] These findings strongly indicate that ACPA-producing PB/C may frequently be regenerated by circulating ACPA-expressing MBC.

9 Strategies to target ACPA-expressing B cells for RA therapy

The pathogenic potential of ACPA-expressing MBC and ACPA-producing PB/C make them promising targets to treat ACPA-positive RA in a targeted manner. Depletion of $CD20^+$ B cells increases the probability for developing *de novo* infections due to the reduction of protective MBCs. Specific targeting of ACPA-expressing B cells and other autoreactive B cells, on the other hand, provide a venue of next generation curative as well as preventative medicines for B cell-mediated autoimmune diseases in the era of targeted therapy. The aim of such therapies is the restoration of immunological tolerance by either inducing autoantigen-specific cellular anergy or by depletion of autoantigen-specific B cells. To this end, several strategies can be outlined: 1) blocking prominent signalling pathways on autoreactive B cells, 2) inducing specific tolerance by autoantigen-based immunotherapy, 3) targeting autoreactive B cells with autoantigen-drug conjugates, 4) employing a sequential pro-drug strategy to avoid circulating autoantibodies, 5) inhibiting autoreactive response by anti-idiotype immunotherapy, and 6) using cell-based therapies. The holy grail of immune tolerizing therapies is to reset tolerance towards self-antigens and to induce life-long protection against B cell-mediated autoimmune diseases. Passive tolerizing therapies such as the depletion of autoreactive B cells, however, may not result in sustained tolerance. Active tolerizing therapies, on the other hand, may provide sustained tolerance as a preventive and curative measure in autoimmune disease.

9.1 Signaling inhibitors

Autoreactive B cells breach immunological tolerance through dysregulation of signalling pathways which promote their survival and activation. This is well demonstrated by the association of a genetic variant of the Protein Tyrosine Phosphatase Non-Receptor Type 22 (*PTPN22*) gene with many autoimmune diseases, including RA, SLE, Grave's disease, anti-neutrophil cytoplasmic antibody-associated vasculitis, generalised vitiligo, myasthenia gravis and type 1 diabetes.[280] The *PTPN22* R620W variant increases its degradation and enhances

BCR, BAFF receptor and CD40 signalling which leads to increased positive selection of autoreactive B cells.[281-284] Therefore, autoreactive B cells may be more sensitive to the blockage of these hyperactive signalling pathways than protective B cells. B cell signalling targets which may have clinical relevance in RA include Bruton's tyrosine kinase (BTK) and phosphatidyl-inositol-3 kinase (PI3K) (Figure 2a).

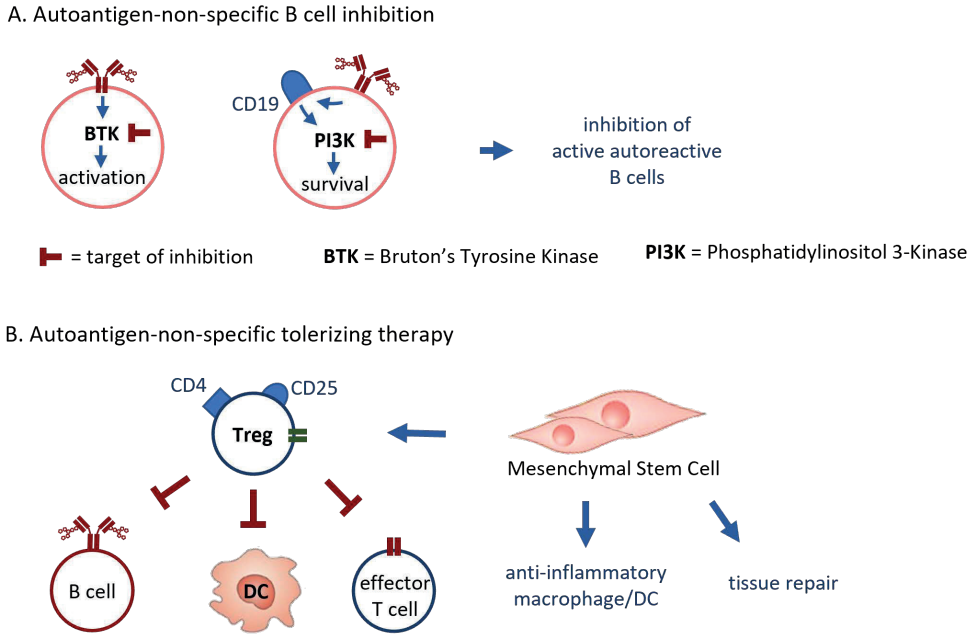

Figure 2. Autoantigen-non-specific B cell inhibition and tolerizing therapy. (**A**) Inhibitors of signalling pathways which play roles in the activation and survival of activated autoreactive B cells such as Bruton's Tyrosine Kinase (BTK) and Phosphatidylinositol 3-Kinase (PI3K) are promising therapy for autoimmune diseases. (**B**) Adoptive transfer of activated regulatory t cells (Treg) or mesenchymal stem cells can induce tolerance in autoantigen-non-specific manner.

BTK plays a role in conveying BCR and Fc-gamma-R III signalling.[285] Loss-of-function mutations at the kinase domain of BTK result in X-linked agammaglobulinemia, which is characterized by defective B cell development.[286] In ACPA-positive RA patients, B cells upregulate the expression and function of BTK.[287,288] In B cells, BTK inhibition suppresses not only BCR signalling but also signals from CD40 ligand (CD154, IL-21 and BAFF.[288] Inhibiting BTK in mouse models of arthritis reduces B cell proliferation and autoantibody levels. As activated autoreactive B cells are proliferative, BTK inhibitors may have specific targeting effects on these cells. Moreover, BTK inhibitors also reduce Fc-gamma-R III-induced production of pro-inflammatory cytokines by macrophages.[285] Therefore, in RA, BTK inhibition may selectively inhibit hyperactive autoreactive B cells while

reducing the pro-inflammatory capacity of ACPA-IC-activated macrophages.

B cell survival, differentiation and activation require metabolic reprogramming to meet the changing energy and building block requirements. The PI3K signalling pathway is crucial for this function.[289] PI3K signalling is also essential for the expression of PAX5 and CD19 which control B cell commitment during the development from common lymphoid progenitors.[290] Moreover, PI3K signalling has an indispensable role in BCR signalling.[291] Constitutively active PI3K-P110alpha expressed by autoreactive B cells abrogates negative selection in the bone marrow.[292] Furthermore, active PI3K-p110delta induces the production of autoantibodies through the suppression of pre-germinal centre peripheral tolerance.[245] Specific inhibition of PI3k-p110gamma suppresses joint inflammation and damage in RA mouse models, reduces glomerulonephritis in SLE mouse models, and reverses autoimmune diabetes in mouse models of type 1 diabetes.[293-295] Moreover, broad PI3K signalling inhibitors such as artesunate and metformin are able to suppress proliferation of chondrocytes and RA synovial fibroblasts in RA models.[296,297] Therefore, targeting PI3K may benefit patients with ACPA-positive RA by enhancing autoreactive B cell negative selection and inhibiting synovial fibroblasts. Future studies that delineate which signalling pathways support the survival and activation of ACPA-expressing B cells will open more options on how to target these cells specifically using respective pathway inhibitors.

9.2 Autoantigen-based immunotherapy

Antigen immunotherapy has been used for more than a hundred years to induce allergen-specific desensitization in patients with allergy.[298] By frequent administration of the allergen in increasing amounts for a long period of time, allergen-specific immune responses can be reduced. Despite differences in the immunopathological basis, autoimmune diseases may also benefit from autoantigen-based therapy.

A four double-stranded oligo-DNA abetimus is an immunomodulating agent which aims to induce tolerance for double stranded DNA and to reduce nephritic flares in SLE.[299] Weekly infusion of abetimus was safe and reduced the levels of circulating anti-dsDNA-antibodies in a dose-dependent manner. In phase 3 clinical trials, patients with high-affinity anti-dsDNA antibodies experienced less renal flares; furthermore, abetimus reduced the need to use high-dose corticosteroids and cyclophosphamide.[300,301] These findings show that antigen-based immunotherapy can serve as a decoy for autoantibody binding leading to reduced tissue-specific

effector functions (Figure 3a). For this purpose, autoantibody affinity to the therapeutic antigen may be essential for improving clinical parameters.

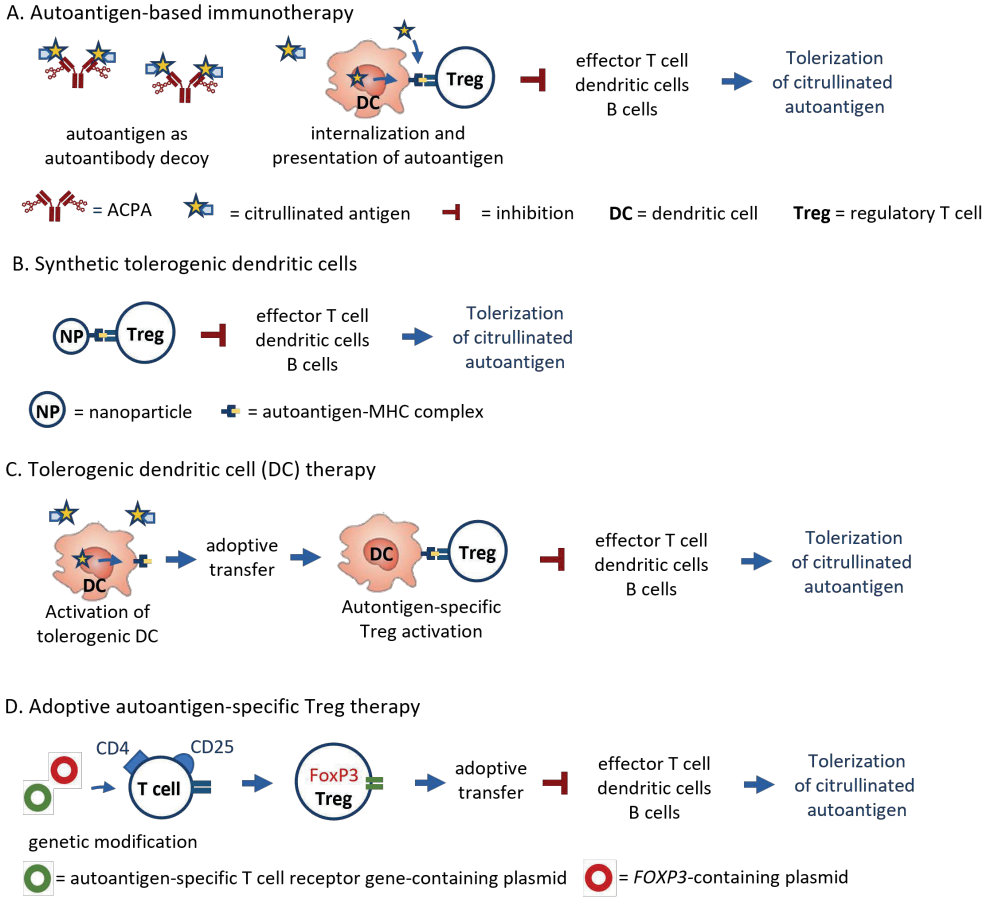

Figure 3. Autoantigen-specific tolerizing therapy. (**A**) autoantigen-based immunotherapy can be used as decoy to prevent autoantibodies from binding to their targets and to induce antigen-specific immunological tolerance through binding with tolerogenic dendritic cells (DC) and activation of antigen-specific Treg. (B) Synthetic tolerogenic DCs can be made by conjugating autoantigen-MHC complex on nanoparticle. (C) Adoptive transfer of modified DC treated with autoantigen can induce autoantigen-specific immunological tolerance. (D) CD4+CD25bright T cells can be modified to express autoantigen-specific T cell receptor and FoxP3 to become autoantigen-specific Tregs.

Another autoantigen-based immunotherapy, ATX-MS-1467, is a peptide mixture of four soluble epitopes from myelin basic protein for the treatment of multiple sclerosis (MS). In a mouse model of MS, ATX-MS-1467 induced the activation of antigen-specific Treg. In phase 2a trials, ATX-MS-1467 was safe and able to reduce MS-related brain lesions.[302] Similarly, a phase one trial showed that a peptide mixture of two soluble epitopes from thyroid stimulating hormone receptor (TSHR), ATX-GD-59, was able to suppress T cell and antibody response towards TSHR in patients with Grave's disease.[303] These autoantigen-derived peptides

bind to the HLA on tolerogenic DC, supress the pro-inflammatory capacity of antigen-specific T cells, and induce the development of Treg (Figure 3a).[304]

The route of delivery of autoantigen-based immunotherapy may be crucial for the development of tolerance. Notably, oral or intra nasal immunization can induce mucosal tolerance which mimics tolerization against non-pathogenic foreign antigens such as food and airborne proteins such as pollens.[305,306] Moreover, a combination of autoantigens with immunomodulating drugs can enhance antigen-specific tolerance. Intranasal immunization with proinsulin peptides, in combination with an anti-CD3 epsilon-specific antibody, can induce insulin-specific Tregs and reverse recent-onset type 1 diabetes in mouse models.[307] This combinatorial therapy was more efficacious than monotherapy using the proinsulin peptide or anti-CD3 alone. Anti-CD3 treatment has been shown to expand T cells with regulatory capacities. Besides anti-CD3 antibodies, histone deacetylases inhibitors such as trichostatin A, microbial metabolites such as butyrate and proprionate, all trans-retinoic acid, low dose of IL-2 and rapamycin are known to induce Treg differentiation and enhance their immune regulatory functions.[308-316] Therefore, a mucosal route of autoantigen delivery as well as combinatorial therapy with Treg-enhancing drugs may increase the success of autoantigen-based immunotherapy.

Hitherto, robust data suggest that ACPA pathogenicity may stem from their ability to form immune complexes (IC). Due to the pro-inflammatory glycosylation profile of the Fc tail of "mature" ACPA, it is conceivable that ACPA-IC can induce FcR-dependent inflammation.[74] Therefore, administration of citrullinated antigens as a putative immunotherapy for RA must be done cautiously. Co-administration of FcR inhibitors may help preventing ACPA-IC-induced inflammation.

Another approach of antigen-based tolerizing immunotherapy mimics tolerogenic dendritic cells. Nanoparticles (NP) coated with autoantigen-derived peptides which are linked to major histocompatibility complexes (MHC or human leukocyte antigen, HLA, in human), resulting in pMHC-NP, induce the expansion of antigen-specific Tregs, suppress natural autoantigen presentation and prevent type 1 diabetes in mouse model (Figure 3b).[317] In humanized mouse models, systemic delivery of pMHC-NP induces autoantigen-specific regulatory $CD4^+$ T cell type 1 (TR1)-like cells and drives the differentiation of regulatory B cells without affecting protective immunity.[318] This approach prevents the formation of IC that may worsen the disease and directly induces antigen-specific tolerance. Its efficacy in humans remains to be tested.

HLA molecules have complex associations with the development of ACPA-positive RA. While HLA shared epitope alleles increase the risk for RA development, other HLA-DR variants containing the amino acid sequence of "DERAA" are associated with protection from RA.[319] HLA-DERAA reduces the risk for RA development possibly by inducing central negative selection of DERAA-binding T cells, which bind not only HLA-DERAA-derived peptides on dendritic cells but also DERAA-containing foreign and autoantigens such as vinculin. Accordingly, HLA-DERAA-negative women who are pregnant with a HLA-DERAA-positive foetus have increased risk for developing RA.[320] This may be due to foetal HLA-DERAA-containing cell micro-chimerism in the mother's body which activates DERAA-binding T cells and subsequently stimulates ACPA-expressing B cells. The selection of HLA molecules as well as citrullinated peptides to form pHLA-NP for RA therapy can therefore be challenging. Citrullinated-vinculin-derived DERAA-containing peptides with HLA-SE nanoparticles may be an option to induce tolerance towards citrullinated autoantigens in ACPA-positive healthy individuals as a preventative vaccine against ACPA-positive RA.

9.3 Autoantigen-drug conjugate

Along with being able to decoy autoantibodies from their anatomical targets and to induce tolerance, autoantigens can also serve to deliver drugs specifically to autoreactive B cells. The conjugated autoantigen can deliver drugs both on the surface and the inside of autoantigen-specific B cells. BCR-mediated internalization of autoantigen-drug conjugate can promote delivery of the drug intracellularly. Depending on the mode of action of the drugs, a suitable linker between the autoantigen and drug can be added. While cleavable linkers can benefit the release of intracellular drug upon internalization by antigen-specific B cells, non-cleavable linkers may sustain the inhibitory capacity of drugs targeting surface protein.

The risk of this approach is that autoantigen-drug conjugates bind to circulating autoantibodies which may induce toxicity against FcR-expressing cells. To avoid adverse toxicity, drugs which target B cell receptor signalling pathways such as BTK inhibitors may be favourable choices. Citrullinated peptide-BTK inhibitor conjugates may not only inhibit ACPA-expressing B cell activation but also macrophages which have active BTK signalling and are able to bind to the drug-immune complex.[321] For that reason, citrullinated peptide-BTK inhibitor conjugates may be able to target two pathogenic cells in RA, namely ACPA-expressing B cells and activated macrophages.

Besides BTK, B cell-specific inhibitory receptors may also be targeted using the autoantigen-drug conjugate strategy described. Some inhibitory receptors, such as CD22 and CD32b, are indispensable for the induction of peripheral B cell tolerance. Knocking-out of the genes expressing these receptors leads to autoimmunity in mice.[322,323] CD22 binds to alpha2,6-linked sialic acids in cis to regulate B cell receptor-induced proliferation.[324] On the other hand, CD32b binds with low affinity to the Fc tail of antibodies in the form of immune complexes to inhibit antigen-specific B cell activation.[325]

Inhibitory receptors generally require co-localization with the BCR to enact their inhibitory capacity. Co-ligation of the BCR with CD22 or CD32b ligands inhibits the development arthritis in mouse models.[326,327] Therefore, co-ligation of ACPA BCRs with an inhibitory receptor through citrullinated antigen-inhibitory ligand conjugates may be effective in inhibiting ACPA-expressing B cells (Figure 4a). It is hypothesized that ACPA-expressing B cells may survive from immunological tolerance checkpoints by downregulating their inhibitory receptors. Therefore, the usefulness of this strategy depends on the expression and function of the inhibitory receptors on these cells.

9.4 Sequential pro-drug strategy: targeting B cells and plasma cells

The autoantigen-drug conjugate has two possible shortcomings. First, circulating autoantibodies may have neutralizing effects on the autoantigen-drug conjugates which limits the availability of the conjugates for autoreactive B cells. Second, there are limited options of drugs that can be used to avoid toxicity. To circumvent these issues, a sequential pro-drug strategy can be an option. First, a cytotoxic drug is conjugated with an inactive form of an autoantigen using a cleavable linker. Subsequently, an activating drug which binds to the surface of B cells is given to activate the autoantigen-drug conjugate in the vicinity of BCR. Only autoreactive BCRs will bind to the active form of the autoantigen-drug conjugate which is then internalized through antigen-induced BCR endocytosis. Upon linker cleavage in the endolysosome, the cytotoxic drug is released inside the cell (Figure 4b). We have shown that this "Trojan horse" strategy can be feasible in targeting ACPA-expressing B cells *in vitro*.[328]

Some autoreactive B cells exert their pathogenicity through the production of pathogenic autoantibodies that activate a receptor aberrantly or disturb essential molecular interactions. These autoantibodies are produced by plasma cells which cease to express BCR on their surface. To target these cells in an antigen-specific

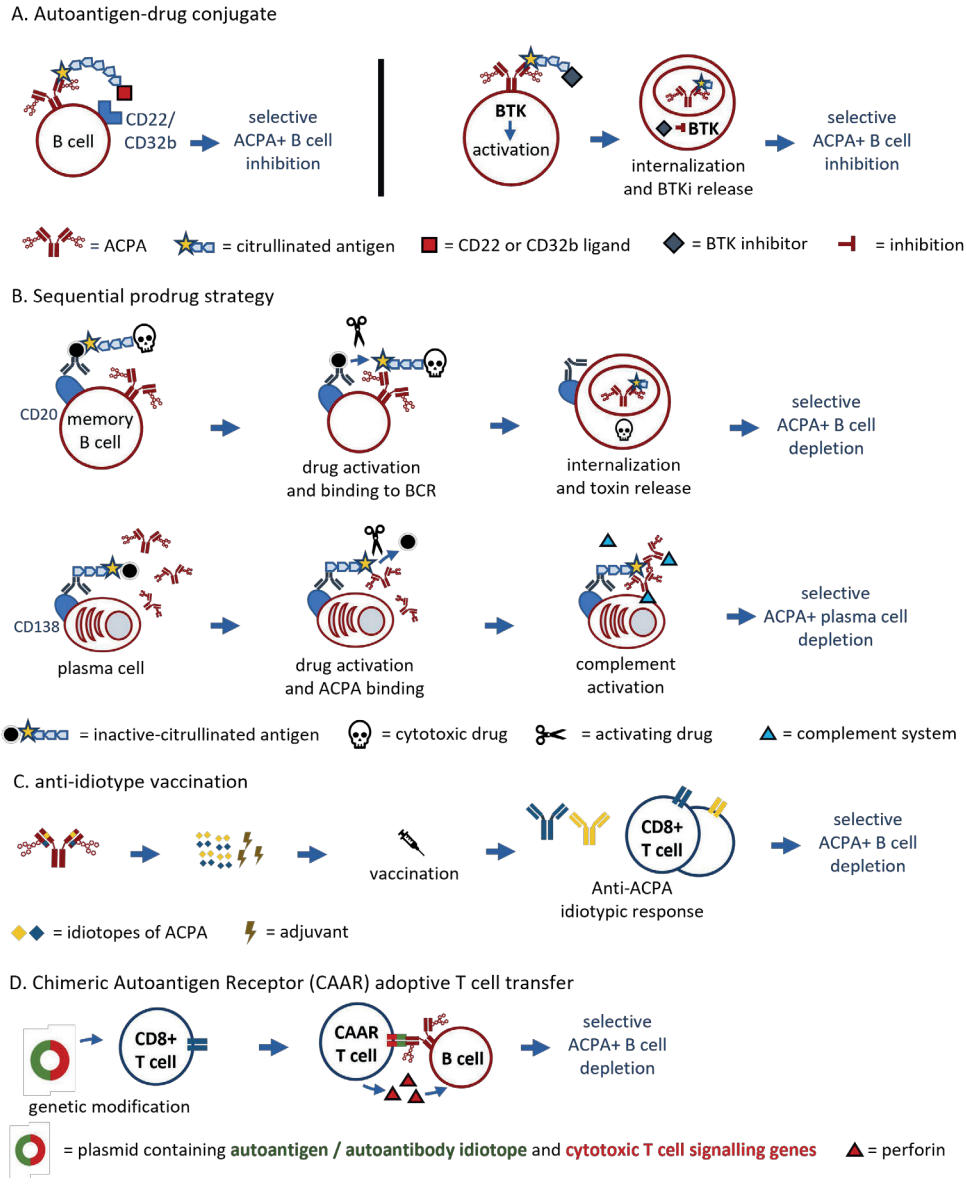

Figure 4. Autoantigen-specific B cell inhibition or depletion. (A) Autoantigen-drug conjugate can deliver immunomodulating drugs only on autoantigen-specific B cells. (B) Sequential prodrug strategy can circumvent neutralization by circulating autoantibodies and deliver cytotoxic drug to autoantigen-specific memory B cells. Moreover, this strategy can also specifically target autoantigen-specific plasma cells in complement system-dependent manner. (C) The variable domain of autoantibodies contain idiotopes which can induce immune response. Idiotype of ACPA, alongside with adjuvants, can be used as vaccine against ACPA-positive RA through development of anti-ACPA idiotypic immune response. (D) Adoptive therapy using geneticall modified $CD8^+$ T cells which express chimeric autoantigen or autoantibody idiotype receptor are effective in depleting pathogenic autoreactive B cells.

manner, an "affinity matrix" technology was develop by conjugating an antigen to an antibody fragment against CD138, a marker of plasma cells.[329] The specific antibodies will then bind to the antigen and induce complement-dependent cytotoxicity.

The possible caveat of this strategy is that autoantibodies may bind to the autoantigen on the affinity matrix before binding to plasma cells. This can lead to depletion of plasma cells regardless of antigen specificity. To circumvent this potential issue, a sequential pro-drug approach could be employed. In this approach, an inactive form of autoantigen is conjugated to an antibody fragment against CD138. A few hours after the injection of the pro-drug, an activating drug can be administered to activate the autoantigen. The period between the injection of the pro-drug and the activating drug would need to reflect the time needed by the pro-drug to bind to plasma cells. The activated autoantigen then bind autoantibodies and induce complement-dependent cytotoxicity (Figure 4b). This approach could increase the prospect of success in targeting antigen-specific plasma cells.

9.5 Anti-idiotype immunotherapy

During B and T cell development, BCR and TCR undergo extensive recombination and hypermutation leading to highly diverse variable domains (VD). The novel VD BCR and TCR sequences and structures may not be tolerated by the immune system and therefore can serve as immune target determinants or epitopes. The parts of VD BCR and TCR which serve as epitopes are referred to as idiotype. In this concept, an antibody idiotype represents a new antigen to which immune responses can develop. Anti-idiotypic immune responses are hypothesized to regulate antigen-specific immune responses through the formation of idiotype-reactive cytotoxic T cells and anti-idiotypic antibodies.[330,331] In fact, anti-idiotypic antibodies binding to autoantibodies are detectable in healthy individuals.[332] These anti-idiotypic antibodies may induce autoantibody clearance and inhibit autoantibody binding to their anatomical targets.[333]

Moreover, anti-idiotype responses can also be induced. Mice immunized with murine autoreactive antibodies against *N*-glycolylated gangliosides develop anti-idiotype B cell responses which can stimulate cytotoxic T cells in an antigen-specific manner.[334] Furthermore, serum transfer from autoantibody-immunized rabbits protects guinea pigs from developing autoimmune nephritis.[335] These findings suggest that it may be possible to induce anti-idiotype responses also against ACPA. ACPA are diverse antibodies which may have various idiotypes. It is therefore

interesting to gain knowledge on dominant idiotypes of ACPA which then can be used to make either active or passive immunization which targets ACPA-expressing B cells (Figure 4c).

9.6 Cell-based therapy

Cell-based therapy can ameliorate RA by direct killing of autoreactive B cells or by inducing autoantigen-specific tolerance. Depletion of autoreactive B cells can be achieved by adoptive cell therapy using CAAR T cells. Engineered human cytotoxic T cells which express chimeric autoantibody receptors (CAAR) consisting of an autoantigen fused with T cell signalling domains selectively eliminate desmoglein 3-specific B cells in a mouse model of pemphigus vulgaris (Figure 4d).[336] The use of CAAR technology circumvents the possible restriction mediated by the HLA genotype of the patients. As citrullination is a posttranslational modification, methods to conjugate a citrullinated peptide to the CAAR signalling domain construct will be needed to enable this technology for RA treatment. Alternatively, the antigen binding domain of anti-ACPA idiotype antibody can replace the need of a citrullinated peptide. However, as discussed previously, the ACPA idiotypes and the antibodies that recognize these are still unknown.

Other cell-based RA therapies aim to induce tolerance in either antigen-specific or non-specific manners. Antigen-specific tolerance can be induced by autologous transfer of modified, tolerogenic dendritic cells (DCs) which are exposed to citrullinated peptides *ex vivo* (Figure 3c). In a phase 1 trial of this DC-based tolerogenic vaccine, the ratio of Treg to effector T cells was increased, T cell responses against a citrullinated peptide were inhibited and the disease activity score within one month was decreased.[337] This trial provides a rationale to develop DC-based vaccine against ACPA-positive RA.

Besides autologous DC, regulatory T cell (Treg) and mesenchymal stem cell (MSC) transplantation are also promising strategies to induce immunological tolerance in autoimmune diseases (Figure 2b). Tregs promote peripheral immunological tolerance through competition with effector T cells for activating signals, production of immunosuppressive molecules and cytolysis of DC.[338] In RA synovial fluid, Tregs are enriched.[339-341] Their immunoregulatory capacity is, however, impaired in the inflammatory milieu.[172,342] Moreover, the activated phenotype of T and B cells in the synovial tissue confers relative resistance towards immunoregulation by Tregs.[341] To enhance the number and functionality in the inflammatory arthritic milieu, Tregs can be treated with a combination of anti-CD3 anti-CD28 and IL-

2, as well as all-trans retinoic acids *ex vivo*.[343,344] Moreover, inhibition of IL-6 and TNF alpha also recovers Treg functions.[172,342]

Tregs require TCR activation by antigen-MHC complexes to induce their immunoregulatory capacity. Once activated, they can exert their suppressive properties in an antigen-independent manner.[345] In experimental autoimmune encephalitis, a model of MS, adoptive transfer of myelin basic protein-reactive Treg improves symptom recovery and prevents relapse after disease onset.[346] Polyclonal Tregs are, however, unable to exert similar protection. To gain sufficient numbers of autoantigen-specific Treg, autoantigen-specific TCR gene transfer can be employed before transferring the cells into the patient. Indeed, adoptive therapy using antigen-specific *TCR* and *FOXP3* gene-transferred $CD4^+CD25^+$ T cells to a mouse model of arthritis suppresses arthritic bone destruction (Figure 3d).[347]

Antigen-nonspecific cell therapy using polyclonal Tregs can also be beneficial to induce immunological tolerance without suppressing systemic immunity. Adoptive transfer of $CD4^+CD25^+$ T cells reduces disease progression of arthritis and reverses type 1 diabetes in mouse models of these respective diseases.[343,348] Another antigen-nonspecific cell therapy using mesenchymal stem cell (MSCs) transplantation has also been trialled for therapy against autoimmune diseases. MSCs are non-hematopoietic progenitor cells which reside in many tissues to replace injured cells. These cells possess immunosuppressive and anti-inflammatory properties, which contribute to tissue homeostasis.[349]

Autologous MSCs transplantation is efficacious in treating refractory autoimmune diseases by restoring the frequency of Tregs and inducing anti-inflammatory phenotypes of macrophages as well as autoreactive T cells (Figure 2b).[350] Moreover, MSCs inhibit pro-inflammatory responses by RA synovial cells.[351] Umbilical cord-derived MSC transplantation in RA patients decreases serum levels of IL-6 and TNF-alpha, increases the frequency of circulating Tregs and reduces disease activity.[352] In a phase 1b/2a clinical trial, adipose-derived MSC transplantations showed a trend for clinical efficacy in patients with active refractory RA.[353] In addition, allogeneic MSC transplantation ameliorated disease activity in refractory lupus patients in a pilot clinical study.[354] These data suggest that despite being antigen-nonspecific, polyclonal Tregs and MSCs may be able to induce immunological tolerance in autoimmune diseases.

In conclusion, the breach of tolerance towards citrullinated autoantigens and activated ACPA-expressing B cells have central pathogenic roles in the

development and chronicity of ACPA-positive RA. There are several strategies to induce immunological tolerance in these patients. Patient's data suggest that immune regulatory cells are present in the RA synovial fluid and tissue. However, these cells fail to exert their immunomodulatory properties, most likely due to the inflammatory arthritic milieu.[172,342,355] Therefore, combinatorial therapeutic strategies to deplete existing inflammation-inducing ACPA-expressing B cells and to tolerize citrullinated antigens may provide both short- and long-term protection against ACPA-positive RA.

10 Scope of this thesis

This thesis is divided in two parts, namely **the characterization of ACPA-expressing B cells** which is described in Chapters 2 and 3; and **the development of strategies to specifically target ACPA-expressing B cells** which is described in Chapters 4, 5 and 6.

10.1 The characterization of ACPA-expressing B cells

Epidemiological studies on ACPA and clinical studies using rituximab for the treatment of RA give strong indications that ACPA-expressing B cells may play major roles in the development of RA. However, the characteristics of these rare cells were largely unknown. Using previously developed staining method to identify these cells for flow cytometry, direct characterization of ACPA-expressing B cells was conducted. **Chapter 2** describes the phenotypic and functional characterization of ACPA-expressing B cells. First, the percentage of ACPA-expressing B cells and their development states in both blood and synovial fluid of RA patients are described. Then the expression of activation markers such as CD19, HLA-DR, CD80, CD86 and Ki-67 on these cells from ACPA-positive patients with arthralgia (pre-disease state), early immunosuppressor-naïve RA and established immunosuppressor-treated RA is investigated. These characteristics are subsequently compared to those of tetanus toxoid (TT)-specific B cells in both quiescent and activated states. Next, the production of proinflammatory cytokines by ACPA-expressing B cells in the blood and synovial fluid is explored. This chapter highlights that in RA, ACPA-expressing memory B cells are proliferative and active which are not observed in the arthralgia state. These cells also highly express co-stimulatory molecules, produce abundant pro-inflammatory cytokines, and differentiate into plasma cells at the site of inflammation. These findings argue for direct roles of ACPA-expressing B cells in the pathogenesis of RA. To understand how these cells sustain their activated state, their expression of an essential immune checkpoint

receptor CD32B is also studied. This chapter describes that the expression of CD32 on ACPA-expressing memory B cells (MBC) from RA patients is downregulated. **Chapter 3** further investigates the expression of immune checkpoint receptors on ACPA-expressing MBC including CD5, PECAM-1, CD200R, LAIR-1, FcRL4, CD22 and Fas. Dysregulation of any of these immune checkpoint receptors leads to aberrant B cell function and/or the formation of autoantibodies in murine models. This chapter highlights the fact that the expression of immune checkpoint receptors on ACPA-expressing MBC was generally similar to that on quiescent TT-specific MBC with an exception of Fas which is highly expressed by ACPA-expressing MBC. The high expression of Fas and the downregulation of CD32 on these cells are mimicked by recently boosted TT-specific MBC. Importantly, more than 90% of ACPA-expressing MBC express CD22 which will be targeted in **Chapter 5**.

10.2 Development of strategies to specifically target ACPA-expressing B cells

Due to their pathologic characteristics, ACPA-expressing MBCs are good targets for RA treatment. Therefore, three strategies are described to inhibit or eliminate these pathologic cells specifically while conserving protective MBCs. **Chapter 4** investigates the feasibility of a sequential prodrug strategy to selectively eliminate these pathologic cells using autoantigen-drug conjugate. In this chapter, we test a non-B cell specific cytotoxic drug saporin which is a ribosome inactivating protein. It is hypothesized that a cyclic citrullinated peptide (CCP)-saporin conjugate will be captured by the BCR of ACPA-expressing B cell, get internalized into endolysosome compartment and saporin will be released into the cytosol of the cells to exert its cytotoxicity. A potential problem of this strategy is that the CCP can bind to circulating ACPA. Subsequently, the resulting immune complex-drug conjugate will eventually bind and eliminate Fc receptor-expressing cells. To prevent the circulating ACPA from binding the CCP-saporin conjugate, we aim to block the citrullinated part of the peptide so that it cannot bind to circulating ACPA. ACPA recognition should then be restored upon introduction of an activating drug only when the prodrug is in the vicinity of B cells. This chapter not only elaborates this concept but also shows *in vitro* that the blocking of citrulline residue using carboxy-p-nitrobenzyl (CNBz) group and its activation using nitroreductase occur with 100% efficiency. Moreover, this chapter shows that both CCP-saporin and nitroreductase-treated CNBz-containing CCP-saporin conjugates selectively eliminate ACPA-expressing B cell line *in vitro*. **Chapter 5** explores the second strategy which aims to activate the immune checkpoint receptor CD22 specifically on ACPA-expressing B cells using polyisocyanopeptides (PIC) multivalent scaffold.

As discussed in chapter 3, ACPA-expressing MBC highly express CD22 which can be used as a target. A PIC construct containing both CD22 ligand and CCP can inhibit IL-8 production by ACPA-expressing B cell line as compared to those treated with separate PIC-CCP and PIC-CD22 ligand at the same concentrations. **Chapter 6** investigates the feasibility of another autoantigen-drug conjugate. Using CCP covalently conjugated to a specific inhibitor of Bruton's tyrosine kinase, acalabrutinib, we aim to selectively inhibit the activation and survival of ACPA-expressing MBC by blocking the BCR signalling in antigen-specific manner. CCP-acalabrutinib conjugate induce partial cytotoxicity towards ACPA-expressing B cell line but not to tetanus-specific B cell line. This selective cytotoxicity, however, is not mediated by Btk inhibition. Therefore an off-target effect may play role in the selective toxicity of CCP-acalabrutinib. Finally, **chapter 7** provides a summary and general discussion of the findings in the thesis.

References

1. Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; **69**(9): 1580-8.

2. Hyldgaard C, Ellingsen T, Hilberg O, Bendstrup E. Rheumatoid Arthritis-Associated Interstitial Lung Disease: Clinical Characteristics and Predictors of Mortality. *Respiration* 2019; **98**(5): 455-60.

3. Crowson CS, Liao KP, Davis JM, 3rd, *et al*. Rheumatoid arthritis and cardiovascular disease. *Am Heart J* 2013; **166**(4): 622-8.e1.

4. Khalid U, Egeberg A, Ahlehoff O, *et al*. Incident Heart Failure in Patients With Rheumatoid Arthritis: A Nationwide Cohort Study. *J Am Heart Assoc* 2018; **7**(2).

5. Ometto F, Fedeli U, Schievano E, Botsios C, Punzi L, Corti MC. Cause-specific mortality in a large population-based cohort of patients with rheumatoid arthritis in Italy. *Clinical and experimental rheumatology* 2018; **36**(4): 636-42.

6. Rosenberg AE. Bones, Joints, and Soft Tissue Tumors. In: Kumar V, Abbas AK, Fausto N, eds. Robbins and Cotran Pathologic Basis of Disease. 7th edition ed. Pennsylvania: Elsevier Saunders; 2005.

7. World Health Organization. Chronic rheumatic conditions. 2019. https://www.who.int/chp/topics/rheumatic/en/ 2019).

8. Zhang W, Anis AH. The economic burden of rheumatoid arthritis: beyond health care costs. *Clinical rheumatology* 2011; **30** Suppl 1: S25-32.

9. Burgers LE, van Steenbergen HW, Ten Brinck RM, Huizinga TW, van der Helm-van Mil AH. Differences in the symptomatic phase preceding ACPA-positive and ACPA-negative RA: a longitudinal study in arthralgia during progression to clinical arthritis. *Annals of the rheumatic diseases* 2017; **76**(10): 1751-4.

10. Padyukov L, Seielstad M, Ong RT, *et al*. A genome-wide association study suggests contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis. *Annals of the rheumatic diseases* 2011; **70**(2): 259-65.

11. Ohmura K, Terao C, Maruya E, *et al*. Anti-citrullinated peptide antibody-negative RA is a genetically distinct subset: a definitive study using only bone-erosive ACPA-negative rheumatoid arthritis. *Rheumatology (Oxford, England)* 2010; **49**(12): 2298-304.

12. Han B, Diogo D, Eyre S, *et al*. Fine mapping seronegative and seropositive rheumatoid arthritis to shared and distinct HLA alleles by adjusting for the effects of heterogeneity. *American journal of human genetics* 2014; **94**(4): 522-32.

13. Viatte S, Plant D, Bowes J, *et al*. Genetic markers of rheumatoid arthritis susceptibility in anti-citrullinated peptide antibody negative patients. *Annals of the rheumatic diseases*

2012; **71**(12): 1984-90.

14. Boeters DM, Mangnus L, Ajeganova S, *et al*. The prevalence of ACPA is lower in rheumatoid arthritis patients with an older age of onset but the composition of the ACPA response appears identical. *Arthritis research & therapy* 2017; **19**(1): 115.

15. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *The Journal of clinical investigation* 1998; **101**(1): 273-81.

16. Willemze A, Trouw LA, Toes RE, Huizinga TW. The influence of ACPA status and characteristics on the course of RA. *Nature reviews Rheumatology* 2012; **8**(3): 144-52.

17. Hafström I, Ajeganova S, Forslind K, Svensson B. Anti-citrullinated protein antibodies are associated with osteopenia but not with pain at diagnosis of rheumatoid arthritis: data from the BARFOT cohort. *Arthritis research & therapy* 2019; **21**(1): 45.

18. Haschka J, Englbrecht M, Hueber AJ, *et al*. Relapse rates in patients with rheumatoid arthritis in stable remission tapering or stopping antirheumatic therapy: interim results from the prospective randomised controlled RETRO study. *Annals of the rheumatic diseases* 2016; **75**(1): 45-51.

19. Shafrin J, Tebeka MG, Price K, Patel C, Michaud K. The Economic Burden of ACPA-Positive Status Among Patients with Rheumatoid Arthritis. *Journal of managed care & specialty pharmacy* 2018; **24**(1): 4-11.

20. Orr C, Najm A, Biniecka M, *et al*. Synovial Immunophenotype and Anti-Citrullinated Peptide Antibodies in Rheumatoid Arthritis Patients: Relationship to Treatment Response and Radiologic Prognosis. *Arthritis & rheumatology (Hoboken, NJ)* 2017; **69**(11): 2114-23.

21. Lopez-Olivo MA, Amezaga Urruela M, McGahan L, Pollono EN, Suarez-Almazor ME. Rituximab for rheumatoid arthritis. *The Cochrane database of systematic reviews* 2015; **1**: Cd007356.

22. Genovese MC, Becker JC, Schiff M, *et al*. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *The New England journal of medicine* 2005; **353**(11): 1114-23.

23. Lofano G, Gorman MJ, Yousif AS, *et al*. Antigen-specific antibody Fc glycosylation enhances humoral immunity via the recruitment of complement. *Science immunology* 2018; **3**(26).

24. Fridman WH, Teillaud JL, Bouchard C, *et al*. Soluble Fc gamma receptors. *Journal of leukocyte biology* 1993; **54**(5): 504-12.

25. Galon J, Paulet P, Galinha A, *et al*. Soluble Fc gamma receptors: interaction with ligands and biological consequences. *Int Rev Immunol* 1997; **16**(1-2): 87-111.

26. Roghanian A, Stopforth RJ, Dahal LN, Cragg MS. New revelations from an old receptor: Immunoregulatory functions of the inhibitory Fc gamma receptor, FcgammaRIIB

(CD32B). Journal of leukocyte biology 2018.

27. Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. *Nature reviews Immunology* 2015; **15**(3): 160-71.

28. Scherer HU, van der Woude D, Willemze A, *et al*. Distinct ACPA fine specificities, formed under the influence of HLA shared epitope alleles, have no effect on radiographic joint damage in rheumatoid arthritis. *Annals of the rheumatic diseases* 2011; **70**(8): 1461-4.

29. Kampstra ASB, Dekkers JS, Volkov M, *et al*. Different classes of anti-modified protein antibodies are induced on exposure to antigens expressing only one type of modification. *Annals of the rheumatic diseases* 2019; **78**(7): 908-16.

30. Darrah E, Rosen A, Giles JT, Andrade F. Peptidylarginine deiminase 2, 3 and 4 have distinct specificities against cellular substrates: novel insights into autoantigen selection in rheumatoid arthritis. *Annals of the rheumatic diseases* 2012; **71**(1): 92-8.

31. Wang S, Wang Y. Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. *Biochimica et biophysica acta* 2013; 1829(10): 1126-35.

32. Zhai Q, Wang L, Zhao P, Li T. Role of citrullination modification catalyzed by peptidylarginine deiminase 4 in gene transcriptional regulation. *Acta biochimica et biophysica Sinica* 2017; **49**(7): 567-72.

33. Baka Z, Gyorgy B, Geher P, Buzas EI, Falus A, Nagy G. Citrullination under physiological and pathological conditions. *Joint, bone, spine : revue du rhumatisme* 2012; **79**(5): 431-6.

34. Jang B, Ishigami A, Maruyama N, Carp RI, Kim YS, Choi EK. Peptidylarginine deiminase and protein citrullination in prion diseases: strong evidence of neurodegeneration. *Prion* 2013; **7**(1): 42-6.

35. Darrah E, Andrade F. Rheumatoid arthritis and citrullination. *Current opinion in rheumatology* 2018; **30**(1): 72-8.

36. Smeets TJ, Vossenaar ER, Kraan MC, *et al*. Expression of citrullin-containing antigens in RA synovium. *Arthritis research & therapy* 2001; **3**(2): P004.

37. van Beers JJ, Schwarte CM, Stammen-Vogelzangs J, Oosterink E, Božič B, Pruijn GJ. The rheumatoid arthritis synovial fluid citrullinome reveals novel citrullinated epitopes in apolipoprotein E, myeloid nuclear differentiation antigen, and β-actin. *Arthritis and rheumatism* 2013; **65**(1): 69-80.

38. Ludwig RJ, Vanhoorelbeke K, Leypoldt F, *et al*. Mechanisms of Autoantibody-Induced Pathology. *Frontiers in immunology* 2017; **8**: 603.

39. Bennett JL, Lam C, Kalluri SR, *et al*. Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. *Ann Neurol* 2009; **66**(5): 617-29.

40. Zhou D, Srivastava R, Nessler S, *et al*. Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis. *Proceedings of*

the National Academy of Sciences of the United States of America 2006*;* **103**(50): 19057-62.

41. Morshed SA, Davies TF. Graves' Disease Mechanisms: The Role of Stimulating, Blocking, and Cleavage Region TSH Receptor Antibodies. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* 2015*;* **47**(10): 727-34.

42. Hammers CM, Stanley JR. Mechanisms of Disease: Pemphigus and Bullous Pemphigoid. *Annual review of pathology* 2016*;* **11**: 175-97.

43. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis - autoantibody characteristics and their implications for therapy. *Nat Rev Neurol* 2016*;* **12**(5): 259-68.

44. Waterman SA, Gordon TP, Rischmueller M. Inhibitory effects of muscarinic receptor autoantibodies on parasympathetic neurotransmission in Sjogren's syndrome. *Arthritis and rheumatism* 2000*;* **43**(7): 1647-54.

45. Toes R, Pisetsky DS. *Pathogenic effector functions of ACPA: Where do we stand? Annals of the rheumatic diseases* 2019*;* **78**(6): 716-21.

46. Ozawa T, Ouhara K, Tsuda R, *et al*. Physiologic Target, Molecular Evolution, and Pathogenic Functions of a Monoclonal Anti-Citrullinated Protein Antibody Obtained From a Patient With Rheumatoid Arthritis. *Arthritis & rheumatology (Hoboken, NJ)* 2020*;* **72**(12): 2040-9.

47. Krishnamurthy A, Joshua V, Haj Hensvold A, *et al*. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. *Annals of the rheumatic diseases* 2016*;* **75**(4): 721-9.

48. Wigerblad G, Bas DB, Fernades-Cerqueira C, *et al*. Autoantibodies to citrullinated proteins induce joint pain independent of inflammation via a chemokine-dependent mechanism. *Annals of the rheumatic diseases* 2016*;* **75**(4): 730-8.

49. Amara K, Steen J, Murray F, *et al*. Retraction: Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition. *The Journal of experimental medicine* 2019*;* **216**(1): 245.

50. Ten Brinck RM, Toes REM, van der Helm-van Mil AHM. Inflammation functions as a key mediator in the link between ACPA and erosion development: an association study in Clinically Suspect Arthralgia. *Arthritis research & therapy* 2018*;* **20**(1): 89.

51. van Zanten A, Arends S, Roozendaal C, *et al*. Presence of anticitrullinated protein antibodies in a large population-based cohort from the Netherlands. *Annals of the rheumatic diseases* 2017*;* **76**(7): 1184-90.

52. Clavel C, Nogueira L, Laurent L, *et al*. Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. *Arthritis and rheumatism* 2008*;* **58**(3): 678-88.

53. Elliott SE, Kongpachith S, Lingampalli N, *et al*. Affinity Maturation Drives Epitope Spreading and Generation of Proinflammatory Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis. *Arthritis & rheumatology (Hoboken, NJ)* 2018; **70**(12): 1946-58.

54. Sokolove J, Johnson DS, Lahey LJ, *et al*. Rheumatoid factor as a potentiator of anti-citrullinated protein antibody-mediated inflammation in rheumatoid arthritis. *Arthritis & rheumatology (Hoboken, NJ)* 2014; **66**(4): 813-21.

55. Laurent L, Anquetil F, Clavel C, *et al*. IgM rheumatoid factor amplifies the inflammatory response of macrophages induced by the rheumatoid arthritis-specific immune complexes containing anticitrullinated protein antibodies. *Annals of the rheumatic diseases* 2015; **74**(7): 1425-31.

56. Kempers AC, Nejadnik MR, Rombouts Y, *et al*. Fc gamma receptor binding profile of anti-citrullinated protein antibodies in immune complexes suggests a role for FcγRI in the pathogenesis of synovial inflammation. *Clinical and experimental rheumatology* 2018; **36**(2): 284-93.

57. Bersellini Farinotti A, Wigerblad G, Nascimento D, *et al*. Cartilage-binding antibodies induce pain through immune complex-mediated activation of neurons. *The Journal of experimental medicine* 2019; **216**(8): 1904-24.

58. Trouw LA, Haisma EM, Levarht EW, *et al*. Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. *Arthritis and rheumatism* 2009; **60**(7): 1923-31.

59. Sohrabian A, Mathsson-Alm L, Hansson M, *et al*. Number of individual ACPA reactivities in synovial fluid immune complexes, but not serum anti-CCP2 levels, associate with inflammation and joint destruction in rheumatoid arthritis. *Annals of the rheumatic diseases* 2018; **77**(9): 1345-53.

60. de Moel EC, Derksen V, Trouw LA, *et al*. In rheumatoid arthritis, changes in autoantibody levels reflect intensity of immunosuppression, not subsequent treatment response. *Arthritis research & therapy* 2019; **21**(1): 28.

61. Dwosh IL, Giles AR, Ford PM, Pater JL, Anastassiades TP. Plasmapheresis therapy in rheumatoid arthritis. A controlled, double-blind, crossover trial. *The New England journal of medicine* 1983; **308**(19): 1124-9.

62. Tanner S, Dufault B, Smolik I, *et al*. A Prospective Study of the Development of Inflammatory Arthritis in the Family Members of Indigenous North American People With Rheumatoid Arthritis. *Arthritis & rheumatology (Hoboken, NJ)* 2019; **71**(9): 1494-503.

63. Barra L, Scinocca M, Saunders S, *et al*. Anti-citrullinated protein antibodies in unaffected first-degree relatives of rheumatoid arthritis patients. *Arthritis and rheumatism* 2013; **65**(6): 1439-47.

64. Smolik I, Robinson DB, Bernstein CN, El-Gabalawy HS. First-degree relatives of

patients with rheumatoid arthritis exhibit high prevalence of joint symptoms. *The Journal of rheumatology* 2013; **40**(6): 818-24.

65. Ioan-Facsinay A, Willemze A, Robinson DB, *et al*. Marked differences in fine specificity and isotype usage of the anti-citrullinated protein antibody in health and disease. *Arthritis and rheumatism* 2008; **58**(10): 3000-8.

66. van der Woude D, Syversen SW, van der Voort EI, *et al*. The ACPA isotype profile reflects long-term radiographic progression in rheumatoid arthritis. *Annals of the rheumatic diseases* 2010; **69**(6): 1110-6.

67. Brink M, Hansson M, Mathsson L, *et al*. Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis. *Arthritis and rheumatism* 2013; **65**(4): 899-910.

68. Suwannalai P, van de Stadt LA, Radner H, *et al*. Avidity maturation of anti-citrullinated protein antibodies in rheumatoid arthritis. *Arthritis and rheumatism* 2012; **64**(5): 1323-8.

69. Suwannalai P, Scherer HU, van der Woude D, *et al*. Anti-citrullinated protein antibodies have a low avidity compared with antibodies against recall antigens. *Annals of the rheumatic diseases* 2011; **70**(2): 373-9.

70. Vergroesen RD, Slot LM, Hafkenscheid L, *et al*. B-cell receptor sequencing of anti-citrullinated protein antibody (ACPA) IgG-expressing B cells indicates a selective advantage for the introduction of N-glycosylation sites during somatic hypermutation. *Annals of the rheumatic diseases* 2018; **77**(6): 956-8.

71. Rombouts Y, Willemze A, van Beers JJ, *et al*. Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. *Annals of the rheumatic diseases* 2016; **75**(3): 578-85.

72. Vergroesen RD, Slot LM, van Schaik BDC, *et al*. N-Glycosylation Site Analysis of Citrullinated Antigen-Specific B-Cell Receptors Indicates Alternative Selection Pathways During Autoreactive B-Cell Development. *Frontiers in immunology* 2019; **10**: 2092.

73. Hafkenscheid L, de Moel E, Smolik I, *et al*. N-Linked Glycans in the Variable Domain of IgG Anti-Citrullinated Protein Antibodies Predict the Development of Rheumatoid Arthritis. *Arthritis & rheumatology (Hoboken, NJ)* 2019; **71**(10): 1626-33.

74. Rombouts Y, Ewing E, van de Stadt LA, *et al*. Anti-citrullinated protein antibodies acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of rheumatoid arthritis. *Annals of the rheumatic diseases* 2015; **74**(1): 234-41.

75. Alpizar-Rodriguez D, Brulhart L, Mueller RB, *et al*. The prevalence of anticitrullinated protein antibodies increases with age in healthy individuals at risk for rheumatoid arthritis. *Clinical rheumatology* 2017; **36**(3): 677-82.

76. Orellana C, Saevarsdottir S, Klareskog L, Karlson EW, Alfredsson L, Bengtsson

C. Postmenopausal hormone therapy and the risk of rheumatoid arthritis: results from the Swedish EIRA population-based case-control study. *European journal of epidemiology* 2015*;* **30**(5): 449-57.

77. Hensvold AH, Magnusson PK, Joshua V, *et al*. Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins. *Annals of the rheumatic diseases* 2015*;* **74**(2): 375-80.

78. Bernatsky S, Smargiassi A, Joseph L, *et al*. Industrial air emissions, and proximity to major industrial emitters, are associated with anti-citrullinated protein antibodies. *Environmental research* 2017*;* **157**: 60-3.

79. Makrygiannakis D, Hermansson M, Ulfgren AK, *et al*. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Annals of the rheumatic diseases* 2008*;* **67**(10): 1488-92.

80. Baka Z, Buzás E, Nagy G. Rheumatoid arthritis and smoking: putting the pieces together. *Arthritis research & therapy* 2009*;* **11**(4): 238.

81. Shimada N, Handa S, Uchida Y, *et al*. Developmental and age-related changes of peptidylarginine deiminase 2 in the mouse brain. *Journal of neuroscience research* 2010*;* **88**(4): 798-806.

82. Alghamdi M, Alasmari D, Assiri A, *et al*. An Overview of the Intrinsic Role of Citrullination in Autoimmune Disorders. *Journal of immunology research* 2019*;* 2019: 7592851.

83. Klareskog L, Malmström V, Lundberg K, Padyukov L, Alfredsson L. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. *Seminars in immunology* 2011*;* **23**(2): 92-8.

84. Desai MK, Brinton RD. Autoimmune Disease in Women: Endocrine Transition and Risk Across the Lifespan. *Frontiers in endocrinology* 2019*;* **10**: 265.

85. Aiello A, Farzaneh F, Candore G, *et al*. Immunosenescence and Its Hallmarks: How to Oppose Aging Strategically? A Review of Potential Options for Therapeutic Intervention. *Frontiers in immunology* 2019*;* **10**: 2247.

86. van Wesemael TJ, Ajeganova S, Humphreys J, *et al*. Smoking is associated with the concurrent presence of multiple autoantibodies in rheumatoid arthritis rather than with anti-citrullinated protein antibodies per se: a multicenter cohort study. *Arthritis research & therapy* 2016*;* **18**(1): 285.

87. McDermott G, Fu X, Stone JH, *et al*. Association of Cigarette Smoking With Antineutrophil Cytoplasmic Antibody-Associated Vasculitis. *JAMA internal medicine* 2020*;* **180**(6): 870-6.

88. Ciaffi J, van Leeuwen NM, Huizinga TWJ, de Vries-Bouwstra JK. Smoking and

systemic sclerosis: influence on microangiopathy and expression of anti-topoisomerase I antibodies in a monocentric cohort. *Clinical and experimental rheumatology* 2020*;* **38** Suppl 125(3): 25-8.

89. Rose NR. Thymus function, ageing and autoimmunity. *Immunology letters* 1994*;* **40**(3): 225-30.

90. Johnson SA, Cambier JC. Ageing, autoimmunity and arthritis: senescence of the B cell compartment - implications for humoral immunity. *Arthritis research & therapy* 2004*;* **6**(4): 131-9.

91. Johnson SA, Rozzo SJ, Cambier JC. Aging-dependent exclusion of antigen-inexperienced cells from the peripheral B cell repertoire. *Journal of immunology (Baltimore, Md : 1950)* 2002*;* **168**(10): 5014-23.

92. Duggal NA, Upton J, Phillips AC, Sapey E, Lord JM. An age-related numerical and functional deficit in CD19(+) CD24(hi) CD38(hi) B cells is associated with an increase in systemic autoimmunity. *Aging cell* 2013*;* **12**(5): 873-81.

93. Moulias R, Proust J, Wang A, *et al*. Age-related increase in autoantibodies. *Lancet* 1984*;* **1**(8386): 1128-9.

94. Kissel T, Reijm S, Slot LM, *et al*. Antibodies and B cells recognising citrullinated proteins display a broad cross-reactivity towards other post-translational modifications. *Annals of the rheumatic diseases* 2020*;* **79**(4): 472-80.

95. Johansson L, Sherina N, Kharlamova N, *et al*. Concentration of antibodies against Porphyromonas gingivalis is increased before the onset of symptoms of rheumatoid arthritis. *Arthritis research & therapy* 2016*;* **18**(1): 201.

96. Konig MF, Abusleme L, Reinholdt J, *et al*. Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Science translational medicine* 2016*;* **8**(369): 369ra176.

97. Trier NH, Holm BE, Heiden J, *et al*. Antibodies to a strain-specific citrullinated Epstein-Barr virus peptide diagnoses rheumatoid arthritis. *Scientific reports* 2018*;* **8**(1): 3684.

98. Potempa J, Mydel P, Koziel J. The case for periodontitis in the pathogenesis of rheumatoid arthritis. *Nature reviews Rheumatology* 2017*;* **13**(10): 606-20.

99. Shimada A, Kobayashi T, Ito S, *et al*. Expression of anti-Porphyromonas gingivalis peptidylarginine deiminase immunoglobulin G and peptidylarginine deiminase-4 in patients with rheumatoid arthritis and periodontitis. *Journal of periodontal research* 2016*;* **51**(1): 103-11.

100. Konig MF, Paracha AS, Moni M, Bingham CO, 3rd, Andrade F. Defining the role of Porphyromonas gingivalis peptidylarginine deiminase (PPAD) in rheumatoid arthritis through the study of PPAD biology. *Annals of the rheumatic diseases* 2015*;* **74**(11): 2054-61.

101. Quirke AM, Lugli EB, Wegner N, *et al*. Heightened immune response to autocitrullinated Porphyromonas gingivalis peptidylarginine deiminase: a potential mechanism for breaching immunologic tolerance in rheumatoid arthritis. *Annals of the rheumatic diseases* 2014; **73**(1): 263-9.

102. Lundberg K, Wegner N, Yucel-Lindberg T, Venables PJ. Periodontitis in RA-the citrullinated enolase connection. *Nature reviews Rheumatology* 2010; **6**(12): 727-30.

103. Lundberg K, Kinloch A, Fisher BA, *et al*. Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. *Arthritis and rheumatism* 2008; **58**(10): 3009-19.

104. Muñoz-Atienza E, Flak MB, Sirr J, *et al*. The P. gingivalis Autocitrullinome Is Not a Target for ACPA in Early Rheumatoid Arthritis. *Journal of dental research* 2020; **99**(4): 456-62.

105. Wegner N, Wait R, Sroka A, *et al*. Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and α-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis and rheumatism* 2010; **62**(9): 2662-72.

106. Montgomery AB, Kopec J, Shrestha L, *et al*. Crystal structure of Porphyromonas gingivalis peptidylarginine deiminase: implications for autoimmunity in rheumatoid arthritis. *Annals of the rheumatic diseases* 2016; **75**(6): 1255-61.

107. Volkov M, Dekkers J, Loos BG, *et al*. Comment on «Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis». *Science translational medicine* 2018; **10**(433).

108. Koppejan H, Trouw LA, Sokolove J, *et al*. Role of Anti-Carbamylated Protein Antibodies Compared to Anti-Citrullinated Protein Antibodies in Indigenous North Americans With Rheumatoid Arthritis, Their First-Degree Relatives, and Healthy Controls. *Arthritis & rheumatology (Hoboken, NJ)* 2016; **68**(9): 2090-8.

109. VanDrisse CM, Escalante-Semerena JC. Protein Acetylation in Bacteria. *Annual review of microbiology* 2019; **73**: 111-32.

110. Stone M, Fortin PR, Pacheco-Tena C, Inman RD. Should tetracycline treatment be used more extensively for rheumatoid arthritis? Metaanalysis demonstrates clinical benefit with reduction in disease activity. *The Journal of rheumatology* 2003; **30**(10): 2112-22.

111. O'Dell JR, Blakely KW, Mallek JA, *et al*. Treatment of early seropositive rheumatoid arthritis: a two-year, double-blind comparison of minocycline and hydroxychloroquine. *Arthritis and rheumatism* 2001; **44**(10): 2235-41.

112. O'Dell JR, Haire CE, Palmer W, *et al*. Treatment of early rheumatoid arthritis with minocycline or placebo: results of a randomized, double-blind, placebo-controlled trial. *Arthritis and rheumatism* 1997; **40**(5): 842-8.

113. Kloppenburg M, Breedveld FC, Terwiel JP, Mallee C, Dijkmans BA. Minocycline

in active rheumatoid arthritis. A double-blind, placebo-controlled trial. *Arthritis and rheumatism* 1994; **37**(5): 629-36.

114. Tilley BC, Alarcón GS, Heyse SP, *et al*. Minocycline in rheumatoid arthritis. A 48-week, double-blind, placebo-controlled trial. MIRA Trial Group. *Annals of internal medicine* 1995; **122**(2): 81-9.

115. Ogrendik M. Antibiotics for the treatment of rheumatoid arthritis. *International journal of general medicine* 2013; **7**: 43-7.

116. Tsivkovskii R, Sabet M, Tarazi Z, Griffith DC, Lomovskaya O, Dudley MN. Levofloxacin reduces inflammatory cytokine levels in human bronchial epithelia cells: implications for aerosol MP-376 (levofloxacin solution for inhalation) treatment of chronic pulmonary infections. *FEMS immunology and medical microbiology* 2011; **61**(2): 141-6.

117. Pukhalsky AL, Shmarina GV, Kapranov NI, Kokarovtseva SN, Pukhalskaya D, Kashirskaja NJ. Anti-inflammatory and immunomodulating effects of clarithromycin in patients with cystic fibrosis lung disease. *Mediators of inflammation* 2004; **13**(2): 111-7.

118. MacLeod CM, Hamid QA, Cameron L, Tremblay C, Brisco W. Anti-inflammatory activity of clarithromycin in adults with chronically inflamed sinus mucosa. *Advances in therapy* 2001; **18**(2): 75-82.

119. van der Woude D, Houwing-Duistermaat JJ, Toes REM, *et al*. Quantitative heritability of anti–citrullinated protein antibody–positive and anti–citrullinated protein antibody–negative rheumatoid arthritis. 2009; **60**(4): 916-23.

120. Kurreeman F, Liao K, Chibnik L, *et al*. Genetic basis of autoantibody positive and negative rheumatoid arthritis risk in a multi-ethnic cohort derived from electronic health records. *American journal of human genetics* 2011; **88**(1): 57-69.

121. Suzuki A, Yamada R, Chang X, *et al*. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nature genetics* 2003; **34**(4): 395-402.

122. Julià A, González I, Fernández-Nebro A, *et al*. A genome-wide association study identifies SLC8A3 as a susceptibility locus for ACPA-positive rheumatoid arthritis. *Rheumatology (Oxford, England)* 2016; **55**(6): 1106-11.

123. Suzuki T, Ikari K, Yano K, *et al*. PADI4 and HLA-DRB1 are genetic risks for radiographic progression in RA patients, independent of ACPA status: results from the IORRA cohort study. *PloS one* 2013; **8**(4): e61045.

124. Ting YT, Petersen J, Ramarathinam SH, *et al*. The interplay between citrullination and HLA-DRB1 polymorphism in shaping peptide binding hierarchies in rheumatoid arthritis. *The Journal of biological chemistry* 2018; **293**(9): 3236-51.

125. Kurkó J, Besenyei T, Laki J, Glant TT, Mikecz K, Szekanecz Z. Genetics of rheumatoid arthritis - a comprehensive review. *Clinical reviews in allergy & immunology*

2013; **45**(2): 170-9.

126. Irigoyen P, Lee AT, Wener MH, *et al*. Regulation of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis: contrasting effects of HLA-DR3 and the shared epitope alleles. *Arthritis and rheumatism* 2005; **52**(12): 3813-8.

127. Huizinga TW, Amos CI, van der Helm-van Mil AH, *et al*. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. *Arthritis and rheumatism* 2005; **52**(11): 3433-8.

128. Linn-Rasker SP, van der Helm-van Mil AH, van Gaalen FA, *et al*. Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles. *Annals of the rheumatic diseases* 2006; **65**(3): 366-71.

129. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arthritis and rheumatism* 2006; **54**(4): 1117-21.

130. Kissel T, van Schie KA, Hafkenscheid L, *et al*. On the presence of HLA-SE alleles and ACPA-IgG variable domain glycosylation in the phase preceding the development of rheumatoid arthritis. *Annals of the rheumatic diseases* 2019; **78**(12): 1616-20.

131. Raychaudhuri S, Thomson BP, Remmers EF, *et al*. Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk. *Nature genetics* 2009; **41**(12): 1313-8.

132. Danoy P, Wei M, Johanna H, *et al*. Association of variants in MMEL1 and CTLA4 with rheumatoid arthritis in the Han Chinese population. *Annals of the rheumatic diseases* 2011; **70**(10): 1793-7.

133. Gregersen PK, Amos CI, Lee AT, *et al*. REL, encoding a member of the NF-kappaB family of transcription factors, is a newly defined risk locus for rheumatoid arthritis. *Nature genetics* 2009; **41**(7): 820-3.

134. van der Linden MP, Feitsma AL, le Cessie S, *et al*. Association of a single-nucleotide polymorphism in CD40 with the rate of joint destruction in rheumatoid arthritis. *Arthritis and rheumatism* 2009; **60**(8): 2242-7.

135. Guo Y, Walsh AM, Fearon U, *et al*. CD40L-Dependent Pathway Is Active at Various Stages of Rheumatoid Arthritis Disease Progression. *Journal of immunology (Baltimore, Md : 1950)* 2017; **198**(11): 4490-501.

136. Raychaudhuri S, Remmers EF, Lee AT, *et al*. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nature genetics* 2008; **40**(10): 1216-23.

137. Kochi Y. Genetic background of tolerance breakdown in rheumatoid arthritis. *Nihon Rinsho Men'eki Gakkai kaishi = Japanese journal of clinical immunology* 2010; **33**(2): 48-56.

138. Johansson M, Arlestig L, Hallmans G, Rantapää-Dahlqvist S. PTPN22 polymorphism and anti-cyclic citrullinated peptide antibodies in combination strongly predicts future onset of rheumatoid arthritis and has a specificity of 100% for the disease. *Arthritis research & therapy* 2006; **8**(1): R19.

139. Stahl EA, Raychaudhuri S, Remmers EF, *et al*. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nature genetics* 2010; **42**(6): 508-14.

140. Leng RX, Liu J, Yang XK, *et al*. Evidence of epistatic interaction between DPP4 and CCR6 in patients with rheumatoid arthritis. *Rheumatology (Oxford, England)* 2016; **55**(12): 2230-6.

141. Jiang L, Yin J, Ye L, *et al*. Novel risk loci for rheumatoid arthritis in Han Chinese and congruence with risk variants in Europeans. *Arthritis & rheumatology (Hoboken, NJ)* 2014; **66**(5): 1121-32.

142. Thabet MM, Huizinga TW, Marques RB, *et al*. Contribution of Fcgamma receptor IIIA gene 158V/F polymorphism and copy number variation to the risk of ACPA-positive rheumatoid arthritis. *Annals of the rheumatic diseases* 2009; **68**(11): 1775-80.

143. Lee YH, Bae SC, Song GG. FCGR2A, FCGR3A, FCGR3B polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis. *Clinical and experimental rheumatology* 2015; **33**(5): 647-54.

144. Plenge RM, Seielstad M, Padyukov L, *et al*. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. *The New England journal of medicine* 2007; **357**(12): 1199-209.

145. Gomez-Cabrero D, Almgren M, Sjöholm LK, *et al*. High-specificity bioinformatics framework for epigenomic profiling of discordant twins reveals specific and shared markers for ACPA and ACPA-positive rheumatoid arthritis. *Genome medicine* 2016; **8**(1): 124.

146. Liu Y, Aryee MJ, Padyukov L, *et al*. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nature biotechnology* 2013; **31**(2): 142-7.

147. Terao C, Ohmura K, Kochi Y, *et al*. Anti-citrullinated peptide/protein antibody (ACPA)-negative RA shares a large proportion of susceptibility loci with ACPA-positive RA: a meta-analysis of genome-wide association study in a Japanese population. *Arthritis research & therapy* 2015; **17**(1): 104.

148. Muthana M, Hawtree S, Wilshaw A, *et al*. C5orf30 is a negative regulator of tissue damage in rheumatoid arthritis. *Proceedings of the National Academy of Sciences of the United States of America* 2015; **112**(37): 11618-23.

149. Dorris ER, Tazzyman SJ, Moylett J, *et al*. The Autoimmune Susceptibility Gene C5orf30 Regulates Macrophage-Mediated Resolution of Inflammation. *Journal of*

immunology (Baltimore, Md : 1950*)* 2019*;* **202**(4): 1069-78.

150. Dorris ER, Linehan E, Trenkmann M, Veale DJ, Fearon U, Wilson AG. Association of the Rheumatoid Arthritis Severity Variant rs26232 with the Invasive Activity of Synovial Fibroblasts. *Cells* 2019*;* **8**(10).

151. Lee WS, Kato M, Sugawara E, *et al*. Optineurin in Synovial Fibroblasts Plays a Protective Role against Joint Destructions in Rheumatoid Arthritis. Arthritis & rheumatology (Hoboken, NJ) 2020.

152. Chang SK, Noss EH, Chen M, *et al*. Cadherin-11 regulates fibroblast inflammation. *Proceedings of the National Academy of Sciences of the United States of America* 2011*;* **108**(20): 8402-7.

153. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunological reviews* 2010*;* **233**(1): 233-55.

154. Wei K, Korsunsky I, Marshall JL, *et al*. Notch signalling drives synovial fibroblast identity and arthritis pathology. Nature 2020.

155. Croft AP, Campos J, Jansen K, *et al*. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* 2019*;* **570**(7760): 246-51.

156. Martin-Mola E, Balsa A, García-Vicuna R, *et al*. Anti-citrullinated peptide antibodies and their value for predicting responses to biologic agents: a review. *Rheumatology international* 2016*;* **36**(8): 1043-63.

157. Svendsen AJ, Holm NV, Kyvik K, Petersen PH, Junker P. Relative importance of genetic effects in rheumatoid arthritis: historical cohort study of Danish nationwide twin population. *BMJ (Clinical research ed)* 2002*;* **324**(7332): 264-6.

158. Silman AJ, MacGregor AJ, Thomson W, *et al*. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *British journal of rheumatology* 1993*;* **32**(10): 903-7.

159. Jiang X, Källberg H, Chen Z, *et al*. An Immunochip-based interaction study of contrasting interaction effects with smoking in ACPA-positive versus ACPA-negative rheumatoid arthritis. *Rheumatology (Oxford, England)* 2016*;* **55**(1): 149-55.

160. Kallberg H, Padyukov L, Plenge RM, *et al*. Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis. *American journal of human genetics* 2007*;* **80**(5): 867-75.

161. Cohen S, Hurd E, Cush J, *et al*. Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis and rheumatism* 2002*;* **46**(3): 614-24.

162. Elliott MJ, Maini RN, Feldmann M, *et al*. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in

rheumatoid arthritis. *Lancet* 1994; **344**(8930): 1105-10.

163. Aletaha D, Bingham CO, 3rd, Tanaka Y, *et al*. Efficacy and safety of sirukumab in patients with active rheumatoid arthritis refractory to anti-TNF therapy (SIRROUND-T): a randomised, double-blind, placebo-controlled, parallel-group, multinational, phase 3 study. *Lancet* 2017; **389**(10075): 1206-17.

164. Burmester GR, McInnes IB, Kremer J, *et al*. A randomised phase IIb study of mavrilimumab, a novel GM-CSF receptor alpha monoclonal antibody, in the treatment of rheumatoid arthritis. *Annals of the rheumatic diseases* 2017; **76**(6): 1020-30.

165. Lee EB, Fleischmann R, Hall S, *et al*. Tofacitinib versus methotrexate in rheumatoid arthritis. *The New England journal of medicine* 2014; **370**(25): 2377-86.

166. Fleischmann R, Kremer J, Cush J, *et al*. Placebo-controlled trial of tofacitinib monotherapy in rheumatoid arthritis. *The New England journal of medicine* 2012; **367**(6): 495-507.

167. Taylor PC, Keystone EC, van der Heijde D, *et al*. Baricitinib versus Placebo or Adalimumab in Rheumatoid Arthritis. *The New England journal of medicine* 2017; **376**(7): 652-62.

168. Genovese MC, Kremer J, Zamani O, *et al*. Baricitinib in Patients with Refractory Rheumatoid Arthritis. *The New England journal of medicine* 2016; **374**(13): 1243-52.

169. Genovese MC, Kalunian K, Gottenberg JE, *et al*. Effect of Filgotinib vs Placebo on Clinical Response in Patients With Moderate to Severe Rheumatoid Arthritis Refractory to Disease-Modifying Antirheumatic Drug Therapy: The FINCH 2 Randomized Clinical Trial. *JAMA : the journal of the American Medical Association* 2019; **322**(4): 315-25.

170. Ganz T. Anemia of Inflammation. *The New England journal of medicine* 2019; **381**(12): 1148-57.

171. Lacativa PG, Farias ML. Osteoporosis and inflammation. *Arquivos brasileiros de endocrinologia e metabologia* 2010; **54**(2): 123-32.

172. Ehrenstein MR, Evans JG, Singh A, *et al*. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *The Journal of experimental medicine* 2004; **200**(3): 277-85.

173. Zhang F, Wei K, Slowikowski K, *et al*. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nature immunology* 2019; **20**(7): 928-42.

174. Darrieutort-Laffite C, Boutet MA, Chatelais M, *et al*. IL-1β and TNFα promote monocyte viability through the induction of GM-CSF expression by rheumatoid arthritis synovial fibroblasts. *Mediators of inflammation* 2014; 2014: 241840.

175. Hirota K, Hashimoto M, Ito Y, *et al*. Autoimmune Th17 Cells Induced Synovial Stromal and Innate Lymphoid Cell Secretion of the Cytokine GM-CSF to Initiate and

Augment Autoimmune Arthritis. *Immunity* 2018; **48**(6): 1220-32.e5.

176. Kishimoto T. Factors affecting B-cell growth and differentiation. *Annual review of immunology* 1985; **3**: 133-57.

177. Greven DE, Cohen ES, Gerlag DM, *et al*. Preclinical characterisation of the GM-CSF receptor as a therapeutic target in rheumatoid arthritis. *Annals of the rheumatic diseases* 2015; **74**(10): 1924-30.

178. Tanaka Y, Martin Mola E. IL-6 targeting compared to TNF targeting in rheumatoid arthritis: studies of olokizumab, sarilumab and sirukumab. *Annals of the rheumatic diseases* 2014; **73**(9): 1595-7.

179. Genovese MC, Fleischmann R, Furst D, *et al*. Efficacy and safety of olokizumab in patients with rheumatoid arthritis with an inadequate response to TNF inhibitor therapy: outcomes of a randomised Phase IIb study. *Annals of the rheumatic diseases* 2014; **73**(9): 1607-15.

180. Netea MG, Kullberg BJ, Van der Meer JW. Circulating cytokines as mediators of fever. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2000; **31** Suppl 5: S178-84.

181. Atzeni F, Nucera V, Masala IF, Sarzi-Puttini P, Bonitta G. Il-6 Involvement in pain, fatigue and mood disorders in rheumatoid arthritis and the effects of Il-6 inhibitor sarilumab. *Pharmacological research* 2019; **149**: 104402.

182. Louati K, Berenbaum F. Fatigue in chronic inflammation - a link to pain pathways. *Arthritis research & therapy* 2015; **17**: 254.

183. Ogata A, Kato Y, Higa S, Yoshizaki K. IL-6 inhibitor for the treatment of rheumatoid arthritis: A comprehensive review. *Modern rheumatology / the Japan Rheumatism Association* 2019; **29**(2): 258-67.

184. Cope AP, Schulze-Koops H, Aringer M. The central role of T cells in rheumatoid arthritis. *Clinical and experimental rheumatology* 2007; **25**(5 Suppl 46): S4-11.

185. Zvaifler NJ, Boyle D, Firestein GS. Early synovitis--synoviocytes and mononuclear cells. *Seminars in arthritis and rheumatism* 1994; **23**(6 Suppl 2): 11-6.

186. Constantine GM, Lionakis MS. Lessons from primary immunodeficiencies: Autoimmune regulator and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Immunological reviews* 2019; **287**(1): 103-20.

187. Rao DA, Gurish MF, Marshall JL, *et al*. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* 2017; **542**(7639): 110-4.

188. van der Lubbe PA, Dijkmans BA, Markusse HM, Nässander U, Breedveld FC. A randomized, double-blind, placebo-controlled study of CD4 monoclonal antibody therapy in early rheumatoid arthritis. *Arthritis and rheumatism* 1995; **38**(8): 1097-106.

189. Moreland LW, Pratt PW, Mayes MD, *et al*. Double-blind, placebo-controlled

multicenter trial using chimeric monoclonal anti-CD4 antibody, cM-T412, in rheumatoid arthritis patients receiving concomitant methotrexate. *Arthritis and rheumatism* 1995; **38**(11): 1581-8.

190. Choy EH, Chikanza IC, Kingsley GH, Corrigall V, Panayi GS. Treatment of rheumatoid arthritis with single dose or weekly pulses of chimaeric anti-CD4 monoclonal antibody. *Scandinavian journal of immunology* 1992; **36**(2): 291-8.

191. Tak PP, van der Lubbe PA, Cauli A, *et al*. Reduction of synovial inflammation after anti-CD4 monoclonal antibody treatment in early rheumatoid arthritis. *Arthritis and rheumatism* 1995; **38**(10): 1457-65.

192. Scheerens H, Su Z, Irving B, *et al*. MTRX1011A, a humanized anti-CD4 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: a phase I randomized, double-blind, placebo-controlled study incorporating pharmacodynamic biomarker assessments. *Arthritis research & therapy* 2011; **13**(5): R177.

193. Mason U, Aldrich J, Breedveld F, *et al*. CD4 coating, but not CD4 depletion, is a predictor of efficacy with primatized monoclonal anti-CD4 treatment of active rheumatoid arthritis. *The Journal of rheumatology* 2002; **29**(2): 220-9.

194. Choy EH, Panayi GS, Emery P, *et al*. Repeat-cycle study of high-dose intravenous 4162W94 anti-CD4 humanized monoclonal antibody in rheumatoid arthritis. A randomized placebo-controlled trial. *Rheumatology (Oxford, England)* 2002; **41**(10): 1142-8.

195. Kerschbaumer A, Sepriano A, Smolen JS, *et al*. Efficacy of pharmacological treatment in rheumatoid arthritis: a systematic literature research informing the 2019 update of the EULAR recommendations for management of rheumatoid arthritis. *Annals of the rheumatic diseases* 2020; **79**(6): 744-59.

196. Dokoupilová E, Aelion J, Takeuchi T, *et al*. Secukinumab after anti-tumour necrosis factor-α therapy: a phase III study in active rheumatoid arthritis. *Scandinavian journal of rheumatology* 2018; **47**(4): 276-81.

197. König M, Rharbaoui F, Aigner S, Dälken B, Schüttrumpf J. Tregalizumab - A Monoclonal Antibody to Target Regulatory T Cells. *Frontiers in immunology* 2016; **7**: 11.

198. van Vollenhoven RF, Keystone EC, Strand V, *et al*. Efficacy and safety of tregalizumab in patients with rheumatoid arthritis and an inadequate response to methotrexate: results of a phase IIb, randomised, placebo-controlled trial. *Annals of the rheumatic diseases* 2018; **77**(4): 495-9.

199. Silverman HA, Johnson JS, Vaughan JH, McGlamory JC. Altered lymphocyte reactivity in rheumatoid arthritis. *Arthritis and rheumatism* 1976; **19**(3): 509-15.

200. Mirza NM, Relias V, Yunis EJ, Pachas WN, Dasgupta JD. Defective signal transduction via T-cell receptor-CD3 structure in T cells from rheumatoid arthritis patients. *Human immunology* 1993; **36**(2): 91-8.

201. Allen ME, Young SP, Michell RH, Bacon PA. Altered T lymphocyte signaling in rheumatoid arthritis. *European journal of immunology* 1995; **25**(6): 1547-54.

202. Ali M, Ponchel F, Wilson KE, *et al*. Rheumatoid arthritis synovial T cells regulate transcription of several genes associated with antigen-induced anergy. *The Journal of clinical investigation* 2001; **107**(4): 519-28.

203. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nature reviews Immunology* 2015; **15**(8): 486-99.

204. Pfeifle R, Rothe T, Ipseiz N, *et al*. Regulation of autoantibody activity by the IL-23-T(H)17 axis determines the onset of autoimmune disease. *Nature immunology* 2017; **18**(1): 104-13.

205. Cohen SB, Emery P, Greenwald MW, *et al*. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis and rheumatism* 2006; **54**(9): 2793-806.

206. Edwards JC, Szczepanski L, Szechinski J, *et al*. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *The New England journal of medicine* 2004; **350**(25): 2572-81.

207. Leandro MJ. B-cell subpopulations in humans and their differential susceptibility to depletion with anti-CD20 monoclonal antibodies. *Arthritis research & therapy* 2013; **15** Suppl 1(Suppl 1): S3.

208. Martin VG, Wu YB, Townsend CL, *et al. Transitional B Cells in Early Human B Cell Development - Time to Revisit the Paradigm? Frontiers in immunology* 2016; **7**: 546.

209. Tavakolpour S, Alesaeidi S, Darvishi M, *et al*. A comprehensive review of rituximab therapy in rheumatoid arthritis patients. *Clinical rheumatology* 2019; **38**(11): 2977-94.

210. Sellam J, Hendel-Chavez H, Rouanet S, *et al*. B cell activation biomarkers as predictive factors for the response to rituximab in rheumatoid arthritis: a six-month, national, multicenter, open-label study. *Arthritis and rheumatism* 2011; **63**(4): 933-8.

211. Sellam J, Rivière E, Courties A, *et al*. Serum IL-33, a new marker predicting response to rituximab in rheumatoid arthritis. *Arthritis research & therapy* 2016; **18**(1): 294.

212. Couderc M, Mathieu S, Pereira B, Glace B, Soubrier M. Predictive factors of rituximab response in rheumatoid arthritis: results from a French university hospital. *Arthritis care & research* 2013; **65**(4): 648-52.

213. Gardette A, Ottaviani S, Tubach F, *et al*. High anti-CCP antibody titres predict good response to rituximab in patients with active rheumatoid arthritis. *Joint, bone, spine : revue du rhumatisme* 2014; **81**(5): 416-20.

214. Talabot-Ayer D, McKee T, Gindre P, *et al*. Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Joint,*

bone, spine : revue du rhumatisme 2012*;* **79**(1): 32-7.

215. Miller AM. Role of IL-33 in inflammation and disease. *Journal of inflammation (London, England)* 2011*;* **8**(1): 22.

216. Owczarczyk K, Lal P, Abbas AR, *et al*. A plasmablast biomarker for nonresponse to antibody therapy to CD20 in rheumatoid arthritis. *Science translational medicine* 2011*;* **3**(101): 101ra92.

217. Thurlings RM, Boumans M, Tekstra J, *et al*. Relationship between the type I interferon signature and the response to rituximab in rheumatoid arthritis patients. *Arthritis and rheumatism* 2010*;* **62**(12): 3607-14.

218. Raterman HG, Vosslamber S, de Ridder S, *et al*. The interferon type I signature towards prediction of non-response to rituximab in rheumatoid arthritis patients. *Arthritis research & therapy* 2012*;* **14**(2): R95.

219. Sellam J, Marion-Thore S, Dumont F, *et al*. Use of whole-blood transcriptomic profiling to highlight several pathophysiologic pathways associated with response to rituximab in patients with rheumatoid arthritis: data from a randomized, controlled, open-label trial. *Arthritis & rheumatology (Hoboken, NJ)* 2014*;* **66**(8): 2015-25.

220. Cantaert T, van Baarsen LG, Wijbrandts CA, *et al*. Type I interferons have no major influence on humoral autoimmunity in rheumatoid arthritis. *Rheumatology (Oxford, England)* 2010*;* **49**(1): 156-66.

221. Lübbers J, Brink M, van de Stadt LA, *et al*. The type I IFN signature as a biomarker of preclinical rheumatoid arthritis. *Annals of the rheumatic diseases* 2013*;* **72**(5): 776-80.

222. Castañeda-Delgado JE, Bastián-Hernandez Y, Macias-Segura N, *et al*. Type I Interferon Gene Response Is Increased in Early and Established Rheumatoid Arthritis and Correlates with Autoantibody Production. *Frontiers in immunology* 2017*;* **8**: 285.

223. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. *Science (New York, NY)* 2003*;* **301**(5638): 1374-7.

224. Brink R, Phan TG. Self-Reactive B Cells in the Germinal Center Reaction. *Annual review of immunology* 2018*;* **36**: 339-57.

225. Wardemann H, Hammersen J, Nussenzweig MC. Human autoantibody silencing by immunoglobulin light chains. *The Journal of experimental medicine* 2004*;* **200**(2): 191-9.

226. Burnett DL, Reed JH, Christ D, Goodnow CC. Clonal redemption and clonal anergy as mechanisms to balance B cell tolerance and immunity. *Immunological reviews* 2019*;* **292**(1): 61-75.

227. 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. *Proceedings of the National Academy of Sciences of the United States of America* 2014*;* **111**(25): E2567-75.

228. Yang G, Holl TM, Liu Y, *et al*. Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. *The Journal of experimental medicine* 2013; **210**(2): 241-56.

229. Haynes BF, Verkoczy L, Kelsoe G. Redemption of autoreactive B cells. *Proceedings of the National Academy of Sciences of the United States of America* 2014; **111**(25): 9022-3.

230. Verkoczy L, Diaz M. Autoreactivity in HIV-1 broadly neutralizing antibodies: implications for their function and induction by vaccination. *Current opinion in HIV and AIDS* 2014; **9**(3): 224-34.

231. Han S, Zheng B, Dal Porto J, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. IV. Affinity-dependent, antigen-driven B cell apoptosis in germinal centers as a mechanism for maintaining self-tolerance. *The Journal of experimental medicine* 1995; **182**(6): 1635-44.

232. Shokat KM, Goodnow CC. Antigen-induced B-cell death and elimination during germinal-centre immune responses. *Nature* 1995; **375**(6529): 334-8.

233. Pulendran B, Kannourakis G, Nouri S, Smith KG, Nossal GJ. Soluble antigen can cause enhanced apoptosis of germinal-centre B cells. *Nature* 1995; **375**(6529): 331-4.

234. Wardemann H, Busse CE. Expression Cloning of Antibodies from Single Human B Cells. *Methods in molecular biology (Clifton, NJ)* 2019; 1956: 105-25.

235. Tiller T, Tsuiji M, Yurasov S, Velinzon K, Nussenzweig MC, Wardemann H. Autoreactivity in human IgG+ memory B cells. *Immunity* 2007; **26**(2): 205-13.

236. Hwang JY, Randall TD, Silva-Sanchez A. Inducible Bronchus-Associated Lymphoid Tissue: Taming Inflammation in the Lung. *Frontiers in immunology* 2016; **7**: 258.

237. Alsughayyir J, Pettigrew GJ, Motallebzadeh R. Spoiling for a Fight: B Lymphocytes As Initiator and Effector Populations within Tertiary Lymphoid Organs in Autoimmunity and Transplantation. *Frontiers in immunology* 2017; **8**: 1639.

238. Weinstein JS, Nacionales DC, Lee PY, *et al*. Colocalization of antigen-specific B and T cells within ectopic lymphoid tissue following immunization with exogenous antigen. *Journal of immunology (Baltimore, Md : 1950)* 2008; **181**(5): 3259-67.

239. Morissette MC, Jobse BN, Thayaparan D, *et al*. Persistence of pulmonary tertiary lymphoid tissues and anti-nuclear antibodies following cessation of cigarette smoke exposure. *Respiratory research* 2014; **15**(1): 49.

240. Titcombe PJ, Wigerblad G, Sippl N, *et al*. Pathogenic Citrulline-Multispecific B Cell Receptor Clades in Rheumatoid Arthritis. *Arthritis & rheumatology (Hoboken, NJ)* 2018; **70**(12): 1933-45.

241. Hafkenscheid L, Bondt A, Scherer HU, *et al*. Structural Analysis of Variable Domain Glycosylation of Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis Reveals the Presence of Highly Sialylated Glycans. *Molecular & cellular proteomics : MCP*

2017; **16**(2): 278-87.

242. Lübbers J, Rodríguez E, van Kooyk Y. Modulation of Immune Tolerance via Siglec-Sialic Acid Interactions. *Frontiers in immunology* 2018; **9**: 2807.

243. Seda V, Mraz M. B-cell receptor signalling and its crosstalk with other pathways in normal and malignant cells. *European journal of haematology* 2015; **94**(3): 193-205.

244. Meffre E. The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. *Annals of the New York Academy of Sciences* 2011; 1246: 1-10.

245. Lau A, Avery DT, Jackson K, *et al*. Activated PI3Kδ breaches multiple B cell tolerance checkpoints and causes autoantibody production. *The Journal of experimental medicine* 2020; **217**(2).

246. Smith KG, Clatworthy MR. FcgammaRIIB in autoimmunity and infection: evolutionary and therapeutic implications. *Nature reviews Immunology* 2010; **10**(5): 328-43.

247. Radbruch A, Muehlinghaus G, Luger EO, *et al*. Competence and competition: the challenge of becoming a long-lived plasma cell. *Nature reviews Immunology* 2006; **6**(10): 741-50.

248. Mackay M, Stanevsky A, Wang T, *et al*. Selective dysregulation of the FcgammaIIB receptor on memory B cells in SLE. *The Journal of experimental medicine* 2006; **203**(9): 2157-64.

249. Hansen A, Gosemann M, Pruss A, *et al*. Abnormalities in peripheral B cell memory of patients with primary Sjögren's syndrome. *Arthritis and rheumatism* 2004; **50**(6): 1897-908.

250. Hansen A, Odendahl M, Reiter K, *et al*. Diminished peripheral blood memory B cells and accumulation of memory B cells in the salivary glands of patients with Sjögren's syndrome. *Arthritis and rheumatism* 2002; **46**(8): 2160-71.

251. Sellam J, Rouanet S, Hendel-Chavez H, *et al*. CCL19, a B cell chemokine, is related to the decrease of blood memory B cells and predicts the clinical response to rituximab in patients with rheumatoid arthritis. *Arthritis and rheumatism* 2013; **65**(9): 2253-61.

252. Pickens SR, Chamberlain ND, Volin MV, Pope RM, Mandelin AM, 2nd, Shahrara S. Characterization of CCL19 and CCL21 in rheumatoid arthritis. *Arthritis and rheumatism* 2011; **63**(4): 914-22.

253. Vossenaar ER, Smeets TJ, Kraan MC, Raats JM, van Venrooij WJ, Tak PP. The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. *Arthritis and rheumatism* 2004; **50**(11): 3485-94.

254. Scheel T, Gursche A, Zacher J, Häupl T, Berek C. V-region gene analysis of locally defined synovial B and plasma cells reveals selected B cell expansion and accumulation of plasma cell clones in rheumatoid arthritis. *Arthritis and rheumatism* 2011; **63**(1): 63-72.

255. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, *et al*. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Science translational medicine* 2013; **5**(178): 178ra40.

256. Kerkman PF, Kempers AC, van der Voort EI, *et al*. Synovial fluid mononuclear cells provide an environment for long-term survival of antibody-secreting cells and promote the spontaneous production of anti-citrullinated protein antibodies. *Annals of the rheumatic diseases* 2016; **75**(12): 2201-7.

257. Corsiero E, Bombardieri M, Carlotti E, *et al*. Single cell cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal frequent targeting of citrullinated histones of NETs. *Annals of the rheumatic diseases* 2016; **75**(10): 1866-75.

258. Mehrishi JN, Szabó M, Bakács T. Some aspects of the recombinantly expressed humanised superagonist anti-CD28 mAb, TGN1412 trial catastrophe lessons to safeguard mAbs and vaccine trials. *Vaccine* 2007; **25**(18): 3517-23.

259. Weissmann G, Korchak H. Rheumatoid arthritis. The role of neutrophil activation. *Inflammation* 1984; **8** Suppl: S3-14.

260. Venuprasad K, Chattopadhyay S, Saha B. CD28 signaling in neutrophil induces T-cell chemotactic factor(s) modulating T-cell response. *Human immunology* 2003; **64**(1): 38-43.

261. Woerly G, Roger N, Loiseau S, Dombrowicz D, Capron A, Capron M. Expression of CD28 and CD86 by human eosinophils and role in the secretion of type 1 cytokines (interleukin 2 and interferon gamma): inhibition by immunoglobulin a complexes. *The Journal of experimental medicine* 1999; **190**(4): 487-95.

262. Di Ceglie I, Ascone G, Cremers NAJ, *et al*. Fcγ receptor-mediated influx of S100A8/A9-producing neutrophils as inducer of bone erosion during antigen-induced arthritis. *Arthritis research & therapy* 2018; **20**(1): 80.

263. Alenius GM, Jonsson S, Wållberg Jonsson S, Ny A, Rantapää Dahlqvist S. Matrix metalloproteinase 9 (MMP-9) in patients with psoriatic arthritis and rheumatoid arthritis. *Clinical and experimental rheumatology* 2001; **19**(6): 760.

264. Barr TA, Shen P, Brown S, *et al*. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *The Journal of experimental medicine* 2012; **209**(5): 1001-10.

265. Sun W, Meednu N, Rosenberg A, *et al*. B cells inhibit bone formation in rheumatoid arthritis by suppressing osteoblast differentiation. *Nature communications* 2018; **9**(1): 5127.

266. Meednu N, Zhang H, Owen T, *et al*. Production of RANKL by Memory B Cells: A Link Between B Cells and Bone Erosion in Rheumatoid Arthritis. *Arthritis & rheumatology (Hoboken, NJ)* 2016; **68**(4): 805-16.

267. Ota Y, Niiro H, Ota S, *et al*. Generation mechanism of RANKL(+) effector memory

B cells: relevance to the pathogenesis of rheumatoid arthritis. *Arthritis research & therapy* 2016; **18**: 67.

268. Alemao E, Postema R, Elbez Y, Mamane C, Finckh A. Presence of anti-cyclic citrullinated peptide antibodies is associated with better treatment response to abatacept but not to TNF inhibitors in patients with rheumatoid arthritis: a meta-analysis. *Clinical and experimental rheumatology* 2020; **38**(3): 455-66.

269. Thurlings RM, Vos K, Wijbrandts CA, Zwinderman AH, Gerlag DM, Tak PP. Synovial tissue response to rituximab: mechanism of action and identification of biomarkers of response. *Annals of the rheumatic diseases* 2008; **67**(7): 917-25.

270. Boeters DM, Burgers LE, Toes RE, van der Helm-van Mil A. Does immunological remission, defined as disappearance of autoantibodies, occur with current treatment strategies? A long-term follow-up study in rheumatoid arthritis patients who achieved sustained DMARD-free status. *Annals of the rheumatic diseases* 2019; **78**(11): 1497-504.

271. Cambridge G, Torre Ide L. *Response to rituximab: has the original hypothesis been confirmed? Current pharmaceutical design* 2015; **21**(2): 212-20.

272. Cambridge G, Leandro MJ, Lahey LJ, Fairhead T, Robinson WH, Sokolove J. B cell depletion with rituximab in patients with rheumatoid arthritis: Multiplex bead array reveals the kinetics of IgG and IgA antibodies to citrullinated antigens. *Journal of autoimmunity* 2016; **70**: 22-30.

273. Cambridge G, Perry HC, Nogueira L, *et al*. The effect of B-cell depletion therapy on serological evidence of B-cell and plasmablast activation in patients with rheumatoid arthritis over multiple cycles of rituximab treatment. *Journal of autoimmunity* 2014; **50**: 67-76.

274. Endo Y, Koga T, Kawashiri SY, *et al*. Anti-citrullinated protein antibody titre as a predictor of abatacept treatment persistence in patients with rheumatoid arthritis: a prospective cohort study in Japan. *Scandinavian journal of rheumatology* 2020; **49**(1): 13-7.

275. Koorella C, Nair JR, Murray ME, Carlson LM, Watkins SK, Lee KP. Novel regulation of CD80/CD86-induced phosphatidylinositol 3-kinase signaling by NOTCH1 protein in interleukin-6 and indoleamine 2,3-dioxygenase production by dendritic cells. *The Journal of biological chemistry* 2014; **289**(11): 7747-62.

276. Hammarlund E, Thomas A, Amanna IJ, *et al*. Plasma cell survival in the absence of B cell memory. *Nature communications* 2017; **8**(1): 1781.

277. Purtha WE, Tedder TF, Johnson S, Bhattacharya D, Diamond MS. Memory B cells, but not long-lived plasma cells, possess antigen specificities for viral escape mutants. *The Journal of experimental medicine* 2011; **208**(13): 2599-606.

278. Teng YK, Wheater G, Hogan VE, *et al*. Induction of long-term B-cell depletion in refractory rheumatoid arthritis patients preferentially affects autoreactive more than

protective humoral immunity. *Arthritis research & therapy* 2012; **14**(2): R57.

279. Kerkman PF, Fabre E, van der Voort EI, *et al*. Identification and characterisation of citrullinated antigen-specific B cells in peripheral blood of patients with rheumatoid arthritis. *Annals of the rheumatic diseases* 2016; **75**(6): 1170-6.

280. Zheng J, Ibrahim S, Petersen F, Yu X. Meta-analysis reveals an association of PTPN22 C1858T with autoimmune diseases, which depends on the localization of the affected tissue. *Genes & Immunity* 2012; **13**(8): 641-52.

281. Metzler G, Dai X, Thouvenel CD, *et al*. The Autoimmune Risk Variant PTPN22 C1858T Alters B Cell Tolerance at Discrete Checkpoints and Differentially Shapes the Naive Repertoire. *Journal of immunology (Baltimore, Md : 1950)* 2017; **199**(7): 2249-60.

282. Menard L, Saadoun D, Isnardi I, *et al*. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *The Journal of clinical investigation* 2011; **121**(9): 3635-44.

283. Zhang J, Zahir N, Jiang Q, *et al*. The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness. *Nature genetics* 2011; **43**(9): 902-7.

284. Ruiz-Noa Y, Hernández-Bello J, Llamas-Covarrubias MA, *et al*. PTPN22 1858C>T polymorphism is associated with increased CD154 expression and higher CD4+ T cells percentage in rheumatoid arthritis patients. *J Clin Lab Anal* 2019; **33**(3): e22710.

285. Di Paolo JA, Huang T, Balazs M, *et al*. Specific Btk inhibition suppresses B cell- and myeloid cell-mediated arthritis. *Nature chemical biology* 2011; **7**(1): 41-50.

286. Rawlings DJ, Saffran DC, Tsukada S, *et al*. Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. *Science (New York, NY)* 1993; **261**(5119): 358-61.

287. Corneth OBJ, Verstappen GMP, Paulissen SMJ, *et al*. Enhanced Bruton's Tyrosine Kinase Activity in Peripheral Blood B Lymphocytes From Patients With Autoimmune Disease. *Arthritis & rheumatology (Hoboken, NJ)* 2017; **69**(6): 1313-24.

288. Wang SP, Iwata S, Nakayamada S, *et al*. Amplification of IL-21 signalling pathway through Bruton's tyrosine kinase in human B cell activation. *Rheumatology (Oxford, England)* 2015; **54**(8): 1488-97.

289. Jellusova J, Rickert RC. The PI3K pathway in B cell metabolism. *Critical reviews in biochemistry and molecular biology* 2016; **51**(5): 359-78.

290. Abdelrasoul H, Werner M, Setz CS, Okkenhaug K, Jumaa H. PI3K induces B-cell development and regulates B cell identity. *Scientific reports* 2018; **8**(1): 1327.

291. Safina BS, Baker S, Baumgardner M, *et al*. Discovery of novel PI3-kinase δ specific inhibitors for the treatment of rheumatoid arthritis: taming CYP3A4 time-dependent inhibition. *Journal of medicinal chemistry* 2012; **55**(12): 5887-900.

292. Greaves SA, Peterson JN, Strauch P, Torres RM, Pelanda R. Active PI3K abrogates central tolerance in high-avidity autoreactive B cells. *The Journal of experimental medicine* 2019; **216**(5): 1135-53.

293. Camps M, Rückle T, Ji H, *et al*. Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nature medicine* 2005; **11**(9): 936-43.

294. Barber DF, Bartolomé A, Hernandez C, *et al*. PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. *Nature medicine* 2005; **11**(9): 933-5.

295. Azzi J, Moore RF, Elyaman W, *et al*. The novel therapeutic effect of phosphoinositide 3-kinase-γ inhibitor AS605240 in autoimmune diabetes. *Diabetes* 2012; **61**(6): 1509-18.

296. Feng FB, Qiu HY. Effects of Artesunate on chondrocyte proliferation, apoptosis and autophagy through the PI3K/AKT/mTOR signaling pathway in rat models with rheumatoid arthritis. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2018; **102**: 1209-20.

297. Chen K, Lin ZW, He SM, *et al*. Metformin inhibits the proliferation of rheumatoid arthritis fibroblast-like synoviocytes through IGF-IR/PI3K/AKT/m-TOR pathway. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2019; **115**: 108875.

298. Durham SR, Leung DY. One hundred years of allergen immunotherapy: time to ring the changes. *The Journal of allergy and clinical immunology* 2011; **127**(1): 3-7.

299. Furie R. Abetimus sodium (riquent) for the prevention of nephritic flares in patients with systemic lupus erythematosus. *Rheumatic diseases clinics of North America* 2006; **32**(1): 149-56, x.

300. Abetimus: Abetimus sodium, LJP 394. *BioDrugs : clinical immunotherapeutics, biopharmaceuticals and gene therapy* 2003; **17**(3): 212-5.

301. Wallace DJ, Tumlin JA. LJP 394 (abetimus sodium, Riquent) in the management of systemic lupus erythematosus. *Lupus* 2004; **13**(5): 323-7.

302. Chataway J, Martin K, Barrell K, Sharrack B, Stolt P, Wraith DC. Effects of ATX-MS-1467 immunotherapy over 16 weeks in relapsing multiple sclerosis. *Neurology* 2018; **90**(11): e955-e62.

303. Pearce SHS, Dayan C, Wraith DC, *et al*. Antigen-Specific Immunotherapy with Thyrotropin Receptor Peptides in Graves' Hyperthyroidism: A Phase I Study. *Thyroid : official journal of the American Thyroid Association* 2019; **29**(7): 1003-11.

304. Wraith DC. Designing antigens for the prevention and treatment of autoimmune diseases. *Current Opinion in Chemical Engineering* 2018; **19**: 35-42.

305. Chistiakov DA, Bobryshev YV, Kozarov E, Sobenin IA, Orekhov AN. Intestinal mucosal tolerance and impact of gut microbiota to mucosal tolerance. *Front Microbiol* 2015;

5: 781-.

306. Mansouri S, Katikaneni DS, Gogoi H, *et al*. Lung IFNAR1(hi) TNFR2(+) cDC2 promotes lung regulatory T cells induction and maintains lung mucosal tolerance at steady state. Mucosal immunology 2020.

307. Bresson D, Togher L, Rodrigo E, *et al*. Anti-CD3 and nasal proinsulin combination therapy enhances remission from recent-onset autoimmune diabetes by inducing Tregs. *The Journal of clinical investigation* 2006*;* **116**(5): 1371-81.

308. Tao R, de Zoeten EF, Ozkaynak E, *et al*. Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nature medicine* 2007*;* **13**(11): 1299-307.

309. Wang J, Yang J, Yan Y, *et al*. Effect of adoptive transfer of CD4(+)CD25(+)Foxp3(+) Treg induced by trichostatin A on the prevention of spontaneous abortion. *Journal of reproductive immunology* 2019*;* **131**: 30-5.

310. Arpaia N, Campbell C, Fan X, *et al*. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013*;* **504**(7480): 451-5.

311. Luu M, Pautz S, Kohl V, *et al*. The short-chain fatty acid pentanoate suppresses autoimmunity by modulating the metabolic-epigenetic crosstalk in lymphocytes. *Nature communications* 2019*;* **10**(1): 760.

312. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *The Journal of experimental medicine* 2007*;* **204**(8): 1765-74.

313. Saadoun D, Rosenzwajg M, Joly F, *et al*. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. *The New England journal of medicine* 2011*;* **365**(22): 2067-77.

314. Koreth J, Matsuoka K, Kim HT, *et al*. Interleukin-2 and regulatory T cells in graft-versus-host disease. *The New England journal of medicine* 2011*;* **365**(22): 2055-66.

315. Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo MG. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. *Journal of immunology (Baltimore, Md : 1950)* 2006*;* **177**(12): 8338-47.

316. Battaglia M, Stabilini A, Roncarolo MG. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. *Blood* 2005*;* **105**(12): 4743-8.

317. Tsai S, Shameli A, Yamanouchi J, *et al*. Reversal of autoimmunity by boosting memory-like autoregulatory T cells. *Immunity* 2010*;* **32**(4): 568-80.

318. Clemente-Casares X, Blanco J, Ambalavanan P, *et al*. Expanding antigen-specific regulatory networks to treat autoimmunity. *Nature* 2016*;* **530**(7591): 434-40.

319. Feitsma AL, van der Helm-van Mil AH, Huizinga TW, de Vries RR, Toes RE. Protection against rheumatoid arthritis by HLA: nature and nurture. *Annals of the rheumatic*

diseases 2008*;* **67** Suppl 3: iii61-3.

320. Kanaan SB, Sensoy O, Yan Z, Gadi VK, Richardson ML, Nelson JL. Immunogenicity of a rheumatoid arthritis protective sequence when acquired through microchimerism. 2019*;* **116**(39): 19600-8.

321. Khare A, Viswanathan B, Gund R, *et al*. Role of Bruton's tyrosine kinase in macrophage apoptosis. *Apoptosis : an international journal on programmed cell death* 2011*;* **16**(4): 334-46.

322. Müller J, Nitschke L. The role of CD22 and Siglec-G in B-cell tolerance and autoimmune disease. *Nature reviews Rheumatology* 2014*;* **10**(7): 422-8.

323. Yuasa T, Kubo S, Yoshino T, *et al*. Deletion of fcgamma receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. *The Journal of experimental medicine* 1999*;* **189**(1): 187-94.

324. Poe JC, Fujimoto Y, Hasegawa M, *et al*. CD22 regulates B lymphocyte function *in vivo* through both ligand-dependent and ligand-independent mechanisms. *Nature immunology* 2004*;* **5**(10): 1078-87.

325. Anania JC, Chenoweth AM, Wines BD, Hogarth PM. The Human FcγRII (CD32) Family of Leukocyte FcR in Health and Disease. *Frontiers in immunology* 2019*;* **10**: 464.

326. Bednar KJ, Nycholat CM, Rao TS, Paulson JC, Fung-Leung WP, Macauley MS. Exploiting CD22 To Selectively Tolerize Autoantibody Producing B-Cells in Rheumatoid Arthritis. *ACS chemical biology* 2019*;* **14**(4): 644-54.

327. Veri MC, Burke S, Huang L, *et al*. Therapeutic control of B cell activation via recruitment of Fcgamma receptor IIb (CD32B) inhibitory function with a novel bispecific antibody scaffold. *Arthritis and rheumatism* 2010*;* **62**(7): 1933-43.

328. Lelieveldt L, Kristyanto H, Pruijn GJM, Scherer HU, Toes REM, Bonger KM. Sequential Prodrug Strategy To Target and Eliminate ACPA-Selective Autoreactive B Cells. *Molecular pharmaceutics* 2018*;* **15**(12): 5565-73.

329. Taddeo A, Gerl V, Hoyer BF, *et al*. Selection and depletion of plasma cells based on the specificity of the secreted antibody. *European journal of immunology* 2015*;* **45**(1): 317-9.

330. Neilson EG, Zakheim B. T cell regulation, anti-idiotypic immunity, and the nephritogenic immune response. *Kidney international* 1983*;* **24**(3): 289-302.

331. Hampe CS. Protective role of anti-idiotypic antibodies in autoimmunity--lessons for type 1 diabetes. *Autoimmunity* 2012*;* **45**(4): 320-31.

332. Schaub A, von Gunten S, Vogel M, *et al*. Dimeric IVIG contains natural anti-Siglec-9 autoantibodies and their anti-idiotypes. *Allergy* 2011*;* **66**(8): 1030-7.

333. Tzioufas AG, Routsias JG. Idiotype, anti-idiotype network of autoantibodies: pathogenetic considerations and clinical application. *Autoimmunity reviews* 2010*;* **9**(9): 631-3.

334. Martínez D, Pupo A, Cabrera L, Raymond J, Holodick NE, Hernández AM. B-CD8(+) T Cell Interactions in the Anti-Idiotypic Response against a Self-Antibody. *Journal of immunology research* 2017*;* 2017: 2860867.

335. Brown CA, Carey K, Colvin RB. Inhibition of autoimmune tubulointerstitial nephritis in guinea pigs by heterologous antisera containing anti-idiotype antibodies. *Journal of immunology (Baltimore, Md :* 1950*)* 1979*;* **123**(5): 2102-7.

336. Ellebrecht CT, Bhoj VG, Nace A, *et al*. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science (New York, NY)* 2016*;* **353**(6295): 179-84.

337. Benham H, Nel HJ, Law SC, *et al*. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. *Science translational medicine* 2015*;* **7**(290): 290ra87.

338. Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. *Nature reviews Immunology* 2011*;* **11**(2): 119-30.

339. Liu MF, Wang CR, Fung LL, Lin LH, Tsai CN. The presence of cytokine-suppressive CD4+CD25+ T cells in the peripheral blood and synovial fluid of patients with rheumatoid arthritis. *Scandinavian journal of immunology* 2005*;* **62**(3): 312-7.

340. Cao D, van Vollenhoven R, Klareskog L, Trollmo C, Malmström V. CD25brightCD4+ regulatory T cells are enriched in inflamed joints of patients with chronic rheumatic disease. *Arthritis research & therapy* 2004*;* **6**(4): R335-46.

341. van Amelsfort JM, Jacobs KM, Bijlsma JW, Lafeber FP, Taams LS. CD4(+) CD25(+) regulatory T cells in rheumatoid arthritis: differences in the presence, phenotype, and function between peripheral blood and synovial fluid. *Arthritis and rheumatism* 2004*;* **50**(9): 2775-85.

342. Herrath J, Müller M, Amoudruz P, *et al*. The inflammatory milieu in the rheumatic joint reduces regulatory T-cell function. *European journal of immunology* 2011*;* **41**(8): 2279-90.

343. Tang Q, Henriksen KJ, Bi M, *et al*. *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *The Journal of experimental medicine* 2004*;* **199**(11): 1455-65.

344. Zhou X, Kong N, Wang J, *et al*. Cutting edge: all-trans retinoic acid sustains the stability and function of natural regulatory T cells in an inflammatory milieu. *Journal of immunology (Baltimore, Md :* 1950*)* 2010*;* **185**(5): 2675-9.

345. Thornton AM, Shevach EM. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. *Journal of immunology (Baltimore, Md :* 1950*)* 2000*;* **164**(1): 183-90.

346. Stephens LA, Malpass KH, Anderton SM. Curing CNS autoimmune disease with

myelin-reactive Foxp3+ Treg. *European journal of immunology* 2009; **39**(4): 1108-17.

347. Wright GP, Notley CA, Xue SA, *et al*. Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. *Proceedings of the National Academy of Sciences of the United States of America* 2009; **106**(45): 19078-83.

348. Morgan ME, Flierman R, van Duivenvoorde LM, *et al*. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cells. *Arthritis and rheumatism* 2005; **52**(7): 2212-21.

349. Chen FH, Tuan RS. Mesenchymal stem cells in arthritic diseases. *Arthritis research & therapy* 2008; **10**(5): 223.

350. de Kleer I, Vastert B, Klein M, *et al*. Autologous stem cell transplantation for autoimmunity induces immunologic self-tolerance by reprogramming autoreactive T cells and restoring the CD4+CD25+ immune regulatory network. *Blood* 2006; **107**(4): 1696-702.

351. Gonzalez-Rey E, Gonzalez MA, Varela N, *et al*. Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells *in vitro* in rheumatoid arthritis. *Annals of the rheumatic diseases* 2010; **69**(1): 241-8.

352. Wang L, Wang L, Cong X, *et al*. Human umbilical cord mesenchymal stem cell therapy for patients with active rheumatoid arthritis: safety and efficacy. *Stem cells and development* 2013; **22**(24): 3192-202.

353. Álvaro-Gracia JM, Jover JA, García-Vicuña R, *et al*. Intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells in refractory rheumatoid arthritis (Cx611): results of a multicentre, dose escalation, randomised, single-blind, placebo-controlled phase Ib/IIa clinical trial. *Annals of the rheumatic diseases* 2017; **76**(1): 196-202.

354. Liang J, Zhang H, Hua B, *et al*. Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. *Annals of the rheumatic diseases* 2010; **69**(8): 1423-9.

355. Papadopoulou A, Yiangou M, Athanasiou E, *et al*. Mesenchymal stem cells are conditionally therapeutic in preclinical models of rheumatoid arthritis. *Annals of the rheumatic diseases* 2012; **71**(10): 1733-40.