How is autoimmunity against citrullinated proteins regulated?
Cantaert, T.

Citation for published version (APA):
Humoral autoimmunity

B cell development

Throughout life, B cells are generated from hematopoietic stem cells in the bone marrow. B cells with different antigen specificities are formed by somatic recombination of the immunoglobulin heavy and light chain segments. The combination of random gene segment reassortment, imprecise joining of the segments and the addition of random nucleotides during this process is a powerful mechanism to diversify the B cell receptor repertoire (1-3). A second mechanism of diversification occurs by somatic mutation of the immunoglobulin genes, a process initiated in B cells responding to antigen during T-cell dependent or T-cell independent immune responses (1;4-6). The goal of these diversification mechanisms is to recognize a large variety of foreign antigens. However, the number and diversity of antigenic epitopes which are part of our own body (autoantigens) is probably as high as the number of antigenic epitopes expressed by pathogens. To prevent the development of autoimmunity, the immune system therefore needs to discriminate between self and non-self, thereby eliminating B cells bearing self-reactive B cell receptors (figure 1) (7). How this is regulated to exclude the development of humoral autoimmunity has not been clearly defined yet.

B cell tolerance

Many studies have been conducted in transgenic or knock-out mice revealing important mechanisms to maintain self-tolerance in the B cell compartment (8-11). However, the exclusion of autoreactive B cells from the repertoire in humans is poorly investigated. One important reason for this is that the break in B cell tolerance and the generation of autoantibodies often precedes clinical disease onset with several years (12;13). Therefore, it is extremely difficult to investigate the interplay between different causal factors before the onset of autoimmunity in humans. Furthermore, we have no adequate tools to detect autoreactive B cells at our disposal and we can only measure autoantibody levels as a read out for humoral autoreactivity. Even in the case of systemic screening for autoantibodies in individuals without clinical symptoms, the presence of autoantibodies implicate that the break in B cell tolerance and the differentiation towards autoantibody producing plasma cells has already occurred.

Alternatively, we can clone the B cell receptor of single B cells at different stages during B cell development to determine the number of human B cells that display
autoreactivity. Using this exhaustive and work-intensive approach, the groups of Nussenzweig and Wardemann have showed that in healthy individuals the percentage of self reactive B cells gradually decreases during development (from 80% in early immature B cells in the bone marrow to 18% in mature naïve circulating B cells) passing discrete tolerance checkpoints (14-16). In patients with autoimmune disease such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE), self reactivity is augmented both in the new emigrant B cells and in the mature naïve B cells pointing towards defects in both central and peripheral checkpoints (17;18). Which factors contribute to these breaks in tolerance in humans still need to be elucidated.

Not all self proteins can become self antigens. Only about 1-2% of all human proteins are targets of autoimmunity (19). Therefore, immune dysregulation can be not completely and exclusively accountable for the emergence of autoantibodies. Many mechanisms including the generation of neo-epitopes by posttranslational modifications or cleavage of proteins (20), the local microenvironment which can produce danger signals (21), very high concentrations of antigen, and the

Figure 1: Schematic representation of the regulation of B cell tolerance. Autoreactive B cells which develop in the bone marrow and bind self-antigen undergo apoptosis or receptor editing, leading to expression of a new B cell receptor. B cells that react to self-antigen in the periphery are excluded from entering germinal center reactions in lymphoid tissues, become anergic, or undergo apoptosis. If self-antigen is recognized in the setting of strong co-stimulatory signals, the anergic cells may be “rescued” and become responsive to self. Furthermore, B cells that have a low-level reactivity to self-antigens may persist in the periphery, particularly in the pool of naïve B cells. Adapted from Cellular and Molecular Immunology, Elsevier.
binding of self-protein complexes to pathogen sensing receptors (such as Toll like receptors) influence the selection of protein epitopes which can eventually become autoantigenic. Furthermore, autoimmunity could be initiated exogenously by cross-reaction with an infectious agent (molecular mimicry) (22;23).

Autoantibodies

Once autoreactive B cells are stimulated to develop into plasma cells, they become able to secrete autoantibodies. Such autoantibodies are present in inflammatory conditions such as SLE and RA, in endocrine disorders such as diabetes mellitus and hyperthyroidism, and in some central nervous system disorders such as myasthenia gravis and multiple sclerosis. Some autoantibodies are very specific for one disease, making them useful diagnostic biomarkers, such as anti-islet antibodies in type I diabetes (24) or anti-citrullinated protein antibodies (ACPAs) for RA (25;26). Others can be found in a broad variety of autoimmune disorders such as anti-nuclear antibodies present in SLE, mixed connective tissue disease, scleroderma, RA, and Sjögren’s syndrome (27), pointing towards bystander phenomena of general immune activation. Some autoantibodies can be present before the onset of clinical symptoms, making them good prognostic tools and arguing for a pathophysiological role of these specific autoantibodies, such as anti-mitochondrial antibodies which have a positive predictive value of 95-100% for primary biliary cirrhosis or anti-ribonucleoprotein antibodies which have a positive predictive value of 97 – 100% for the development of SLE (28).

In general, autoantibodies are thought to cause damage when they are directed against targets on cell surfaces, such as receptors (antibodies against the nicotinic acetylcholine receptor stimulate degradation of the receptor causing myasthenia gravis (29)) or when they are specific for extracellular or circulating molecules (antibodies against GpIIb:IIIa fibrinogen receptor on platelets cause autoimmune hemolytic anemia (30)). In these cases, the autoantibodies can be the main pathogenic agent of the disease. In contrast, autoantibodies specific for intracellular components only cause clinical manifestations if the antigen is exposed to the surface (31). By this, these antibodies can generate a pathogenic loop once they are produced.

It is however not only the antigen specificity which determines the pathogenicity but also the subclass of antibody. Different subclasses are the result of isotype switch after the B cell has been stimulated by the antigen (32;33). Which subclass dominates the response to a particular antigen depends on the nature of the antigen, its route of entry, the form in which it is presented to the immune system and the local cytokine environment. IgM are low affinity antibodies produced early in the humoral immune response, mostly before the B cell has maturated in a germinal center reaction.
However, they can form pentamers which confers a high overall avidity to the antibody. IgA is the primary isotype in secretions, providing protection to mucosal layers of the intestinal and respiratory tracts. IgE is present in very low levels in the blood and extracellular fluids, but is a very potent activator of mast cells. IgG can be divided in 4 distinct subclasses, each with different functional activity such as opsonisation (IgG\(_1\)), complement activation (IgG\(_3\)), or antigen neutralization (IgG\(_4\)). IgG\(_1\) is the main isotype produced in response to protein antigens and is associated with a Th1 response; IgG\(_4\) isotype switch requires IL-4, which is associated with a Th2 type of response. The IgG\(_2\) subclass often predominates in responses to carbohydrate antigens. In systemic autoimmune conditions, such as rheumatoid arthritis and systemic lupus erythematosus, IgG\(_1\) and IgG\(_3\) autoantibodies predominate, whereas IgG\(_4\) antibodies are regularly encountered in organ-specific autoimmune diseases. The IgG subclasses bind to both inhibitory and activating receptors on a variety of cells such as dendritic cells, macrophages, and neutrophils (33). The balance between the triggering of both types of receptors regulates antibody responses such as phagocytosis, antibody-dependent cellular cytotoxicity, cytokine production, and the recruitment of inflammatory cells. Furthermore, specific sugar residues on the Fc part of the IgG have been shown to have pro- or anti-inflammatory potential. For example, sialic acid at the Asn297-linked glycan confers anti-inflammatory activity to the IgG and the presence or absence of fucose or galactose residues modifies the binding of the IgG to the different Fc\(\gamma\) receptors (34;35).

**Chronic inflammatory arthritis**

**Clinical characteristics**

In autoimmune rheumatic diseases, the presence of autoantibodies is well established. RA is the most frequent form of autoimmune arthritis, with a prevalence of approximately 1% worldwide (36). The incidence increases with age and generally shows a female predominance (37). The clinical hallmark is a chronic, symmetric polyarthritis with inflammatory pain, morning stiffness and joint swelling. Typically, the disease initially affects hands, wrists, and feet whereas full-blown disease mostly extends also to larger joints such as knees, hips and elbows. Beside the articular manifestations, features of systemic disease are also commonly present. Destruction of cartilage and bone occurs early in the disease and leads to irreversible damage of the joints. For study purposes, patients can be classified according to the American college of rheumatology revised criteria for RA (38).

Spondyloarthritis (SpA), the second most frequent form of chronic joint
inflammation, is a group of chronic inflammatory arthritides that share a number of clinical, radiological and genetic features. SpA includes ankylosing spondylitis, psoriatic arthritis, reactive arthritis, joint disease associated with inflammatory bowel disorders, and undifferentiated SpA. The prevalence is 0.5-2% worldwide and it mostly affects males in their 3\textsuperscript{rd} or 4\textsuperscript{th} life decade (39;40). Clinically, SpA is characterized by both axial and peripheral joint disease. The most important hallmark is the involvement of the axial skeleton. By forming new bony bridges between the vertebrae, the spine becomes ankylosed. The peripheral inflammatory arthritis is mostly asymmetric and pauciarticular and affects predominantly the lower limbs. Also here, patients can be classified for study purposes according to the European Spondyloarthritis study group criteria (41).

\textbf{Genetic predisposition}

Both diseases have a strong association with HLA genotypes. In RA, there is an association with MHC II genes, the so-called shared epitope. This is a conserved motif of amino acid residues (QKRAA, QRRAA or RRRAA) at the third hypervariable region of the DR\beta(1) chain, shared by all human HLA-DRB1 alleles associated with RA (such as DRB1*0101, *0102, *0104, *0404, *0405, *0408, *1001, *1402) (42;43). However, the presence of the shared epitope is neither necessary nor sufficient for the development of RA. In SpA, a genetic association with MHC I genes is observed as the majority of SpA patients is HLA-B27 positive (39). This genotype is also observed in 8% of the healthy population, of whom 90% will never develop SpA (44). On the other hand, the risk of SpA among HLA-B27 positive persons who have a first degree relative with ankylosing spondylitis is increased threefold, illustrating the genetic predisposition and familial clustering in SpA (44).

\textbf{Synovial inflammation}

The synovial tissue is a major target organ of inflammation both in RA and SpA. The synovial membrane lines the joint cavity and provides nutrients to cells of the articular cartilage and produces lubricating fluid to ensure low friction between the joint components (figure 2) (45). It consists of 2 distinct compartments which are not separated by a basal membrane: the thin intimal lining layer and the synovial sublining of connective tissue (45). The intimal lining layer is in direct contact with the intraarticular cavity and plays an important role in the production of synovial fluid. Normally, this layer is merely one or two cell layers thick and consists of macrophage-like and fibroblast-like synoviocytes. The synovial sublining consists
of connective tissue which is sparsely populated with fibroblasts, macrophages, and fat cells. Under inflammatory conditions, the synovial tissue changes dramatically. Endothelium is activated and new vessels are formed (46;47). Inflammatory cells infiltrate the synovial tissue, with virtually all types of lymphocytes and leukocytes protruding into the tissue (48-52). The lymphocytic infiltrate consists of CD4+ and CD8+ T cells, memory B cells, plasma cells, and natural killer cells. Different macrophage subsets (such as CD68+ and CD163+ macrophages), dendritic cells, neutrophils, and mast cells are also abundantly present in the inflamed synovium (figure 2). Many of these cell types produce pro-inflammatory cytokines such as TNF alpha and IL-1, which enhance the inflammatory cascade and have an important proliferative and activating effect on the fibroblast-like synoviocytes of the intimal lining layer (53-56). Together with an increased influx of macrophages from the sublining, this leads to
an increased lining layer thickness of 3 to more than 10 cell layers (figure 2).

**Anti-citrullinated protein antibodies**

**Rheumatoid factor**

The presence of the rheumatoid factor (RF) is one of the criteria for classification of RA (38). RF is directed against the Fc part of human IgG. In addition to the RF of the IgM isotype (which is routinely detected in clinical practice), the occurrence of IgA and IgG RF is well established. The majority of patients with RA (ranging from 60 – 75%) exhibit elevated levels of serum RF (57;58). The specificity is, however, quite low as RF can be found in other rheumatic disorders (such as systemic lupus erythematosus and Sjögren’s syndrome), in chronic inflammatory or infectious diseases, in malignancies, and even in 5% of the healthy population (59).

**Anti-citrullinated protein antibodies**

Another autoantibody system consists of the anti-citrullinated protein antibodies (ACPAs). These autoantibodies have been known for a long time as anti-filaggrin, anti-keratin, or anti-perinuclear factor antibodies, without the knowledge of the specific epitope targeted by these autoantibodies (60;61). Pivotal work has demonstrated that ACPA are directed against epitopes in which the L-arginine amino acid has been posttranslationally modified to a L-citrulline amino acid (figure 3) (25;26). These antibodies are present in 65 – 85% of established RA patients (57;62;63) and have a much higher specificity for RA (92 – 96%) than RF. Therefore, these autoantibodies are a helpful diagnostic tool for RA. Currently, these antibodies are detected by ELISA having a cyclic citrullinated peptide or citrullinated fibrinogen as substrate (25;64). They are mainly of the IgG\textsubscript{1} and IgG\textsubscript{4} subclass (64).

Increasing evidence defines the ACPA positive RA patients as a distinct subtype of RA. Firstly, these antibodies can be detected in RA patients before onset of clinical disease and very few RA patients develop ACPA after disease onset (13;65;66). Furthermore, the presence of ACPA is associated with more severe joint destruction and higher disease activity and ACPA positive RA patients appear to respond better to B cell depletion by rituximab than ACPA negative RA patients (57;66;67). Secondly, the long established genetic association between RA and the shared epitope can be refined to a genetic association with ACPA positive RA and not with ACPA negative RA (68). Two other RA-predisposing genes, PTPN22 and IRF-5, are
CHAPTER 1

confined to ACPA positive and ACPA negative RA, respectively (69;70). Thirdly, a
gene-environment interaction between smoking and the shared epitope has been
shown in the development of ACPA positive RA and not ACPA negative RA (71).
Taken together, these studies indicate that ACPA are not only a useful diagnostic
biomarker but also divides RA in two clinically and pathophysiologically distinct
subsets of disease.

The antigen: citrullinated proteins

ACPA are directed against epitopes in which the L-arginine amino acid has been
posttranslationally modified to a L-citrulline amino acid (figure 3) (25;26). This is
a normal physiological process which can lead to partial unfolding of the target
protein due to the change in charge (72;73). Citrullination of keratin and filaggrin is
an important process during the terminal differentiation of keratinocytes (74;75). As
a consequence, it is believed that the flexibility of the keratin cytoskeleton is reduced
upon citrullination, stimulating the cornification of the epidermis (75;76). It has also
been shown that citrullination of both methylated and normal arginine residues
of histones can take place in vivo (77;78). This can modify the chromatin structure
and counteracts the methylation of the histones which has been shown to be an
important regulator of gene transcription. Finally, in 18% of the myelin basic protein
(MBP) molecules arginine residues are converted into citrulline in healthy adult
individuals (79). The proportion of citrulline-containing MBP molecules is increased
in patients with multiple sclerosis, causing partial unfolding of the MBP, a weakened
interaction with phospholipids, and more rapid degradation of the protein (80).

Figure 3: Deimination of protein bound L-arginine to protein bound L-citrulline by the enzyme peptidyl arginine
deiminase (PAD). This posttranslational modification leads to the loss of the positive charge of the L-arginine
residue. This causes changes in intra- en intermolecular interactions which can lead to enhanced protein unfolding
and protein degradation (124).
However, these patients do not develop ACPA. Citrullinated proteins are not only present in normal physiological conditions; citrullination is augmented in a variety of inflammatory conditions (81). Recently, it has been shown in vitro that inflammatory stimuli cause activation of PAD4 in neutrophils (82). Also in inflamed synovial tissue, citrullinated proteins are abundantly present. It has been shown that citrullinated fibrinogen is a major component of the inflamed synovial tissue of both RA patients and patients with other inflammatory arthritis such as SpA (83;84). Other possible citrullinated synovial proteins include vimentin, fibronectin or alpha-enolase (84-89). An intracellular protein has been detected which colocalizes with ACPA reactivity in the synovial tissue of exclusively RA patients (90). However, the biochemical nature of this protein remains unknown.

**Peptidyl arginine deiminase, the enzyme responsible for citrullination**

Conversion of the guanidine group of protein-bound arginine into the ureido group of citrulline is performed by peptidyl arginine deiminase enzymes (PAD) (protein–L-arginine iminohydrolase, EC 3.5.3.15). Five isoforms of the enzyme have been described (75;91). All have slightly different substrate specificities and are expressed in different tissues. In vitro, most proteins with accessible arginine residues can be citrullinated. However, in vivo only a few proteins have been shown to be substrates for the PAD enzymes. PAD1 is mainly expressed in the epidermis and citrullinates keratin and filaggrin (74;92). PAD2 is expressed in muscle tissue, brain, spleen and secretory glands (93;94). This isoform has been shown to be expressed in macrophages and citrullinates myelin basic protein and vimentin (95-97). The expression of PAD3 is restricted to hair follicles and its natural substrate is trychohialin (98;99). PAD4 is expressed in granulocytes and monocytes and can therefore be detected in a variety of tissues (100-102). This isoform is expressed in the nucleus and citrullinates histones (77;78;101). A functional haplotype of PAD4 has been shown to be associated with RA in a Japanese population (103). However, this could not be confirmed in a French and a UK population (104;105). Of the last isoform, PAD6, only mRNA has been shown in variable tissues such as ovary or testes (106;107). One feature all PAD isoforms have in common is the dependence on high Ca²⁺ levels for their activation (108;109). Normally, intracellular Ca²⁺ concentrations are around 10⁻⁷ M, whereas the concentration needed for PAD is 100 fold higher. Therefore, citrullination only occurs when intracellular Ca²⁺ levels rise, for example during terminal differentiation of epidermal cells, apoptosis or after strong stimuli. Alternatively, citrullination could occur when the enzyme is present in the extracellular environment (75;82;97).
Immune regulation towards citrullinated antigens

As citrullination is a normal physiological process, human B and T cells should be tolerant for the antigen. PAD mRNA expression has been shown in the thymus (92) suggesting that T cells could have encountered the citrulline epitope during their development.

One important remark is that immunoreactive proteins are not per definition autoantigens. Not all citrullinated proteins or citrullinated epitopes are autoantigens. On the contrary, probably there are limited citrullinated epitopes which can induce the humoral autoimmune response. Subsequently, epitope spreading and cross-reactivity can induce B cells to react against other citrullinated proteins. This concept is supported by the observation that not all patients sera react with the same panel of citrullinated epitopes (25;26;63;110). Therefore, ACPA can be considered as a group of autoantibodies, directed against not only citrulline, but also the flanking amino acids.

An interesting recent finding is that proteins undergoing processing in antigen presenting cells can be citrullinated before presentation to T cells (111). However, the functional consequence of this intriguing finding is not yet fully understood. For a citrullinated peptide of vimentin, higher binding affinity to MHC II genotypes of the SE family has been demonstrated (112). However, this could not be confirmed by an in depth study of peptides derived from both the alpha and beta chain of fibrinogen (113). Until today, no in vivo T cells specific for citrullinated epitopes are identified from humans or arthritis animal models.

Do ACPA play a role in the induction of rheumatoid arthritis?

Many attempts have been made to directly investigate the pathogenicity of ACPA in animal models but direct evidence for such a role is still scarce. Firstly, no ACPA can be detected in classical arthritis animal models such as collagen induced arthritis or in MRL/lpr mice (a model for SLE) (114;115). Secondly, there are some conflicting data regarding the fact that ACPA may contribute to the enhancement of arthritis. Immunization with deiminated collagen rather than native collagen can slightly aggravate collagen-induced arthritis in mice (116). Accordingly, passive transfer of monoclonal antibodies specific for citrullinated fibrinogen in combination with anti-CII antibodies can aggravate subclinical arthritis (117), although further control experiments with antibodies to non-citrullinated fibrinogen should confirm the specificity of this observation. In contrast, however, antibodies against citrullinated fibrinogen failed to enhance adjuvant-induced arthritis in Lewis rats (118). Finally, the experimental evidence for a role of citrullinated proteins and/or ACPA in the
induction phase of the arthritis is still weak. Immunization with citrullinated proteins such as fibrinogen failed to induce arthritis in most studies, although they were clearly immunogenic (118). In one recent study, immunisation of HLA-DR4 transgenic animals with human citrullinated fibrinogen did, however, induce arthritis. Of note, the arthritis occurred in only 30% of the animals, had a late onset 10 weeks after immunization, was restricted to the ankle joints and was histologically mild, and could not be induced with autologous citrullinated fibrinogen (119;120). Further studies on this and novel models should confirm the implication of ACPA in the pathogenesis of RA.

Research objectives

Until today, no convincing animal model has been developed where we can study the induction and immune regulation of ACPA. Therefore, translational studies in human RA are still pivotal to elucidate some of the mechanisms regulating autoimmunity against citrullinated proteins (figure 4). In the present thesis, we combined four strategic approaches to address this question. Firstly, other autoimmune diseases with more readily defined autoantigens, such as anti-nuclear antibodies in SLE, served as an inspiration for this research. Secondly, we used the existing knowledge coming from different animal models and assessed if similar mechanisms were also relevant for B cell tolerance in humans. Thirdly, we focused our attention to citrullinated antigens and immunological mechanisms in the inflamed synovial membrane as ACPA are produced locally in the joint (89;121). Finally, both RA and SpA are characterized by extensive peripheral joint inflammation but only RA displays known autoantibodies. It can thus be hypothesized that, in contrast with RA and SLE, the B cell tolerance checkpoints active in healthy individuals are functional in SpA. Therefore, we compared throughout this thesis both diseases in order to investigate factors contributing to the regulation of immune tolerance towards citrullinated proteins in humans.

In the first chapter we describe a human model in which we investigated which factors could contribute to the induction of anti-nuclear antibodies. In human SLE, these autoantibodies are present before clinical symptoms and are part of a positive feedback loop, making it impossible to study the induction phase of this humoral autoimmune response. We discovered, however, that anti-nuclear antibodies are also markedly induced by tumor necrosis factor (TNF) blockade in SpA. We used this model to investigate the contribution of excessive antigen release, defective antigen clearance, and type I interferons to the development of anti-nucleosome autoimmunity.
Type I interferons (IFN) have been shown to be very important regulators of humoral immune responses in both murine and human SLE. Also in RA, a type I IFN signature has been demonstrated in a subset of patients. In the second chapter, we investigated if type I IFN influence ACPA serum levels. As it has been hypothesised that RA (and other inflammatory diseases) results from an imbalance between TNF alpha and type I IFN, we also investigated how APCA levels change with respect to type I IFN after TNF blockade. Furthermore, we investigated if type I IFN could play a role in the initiation of the ACPA response before the onset of disease.

In the third chapter, we turned our attention to the characteristics of synovial
citrullinated antigens. As not only immune dysregulation but also the characteristics of the antigen influence the induction of autoimmunity, we investigated if distinct citrullinated epitopes are present in the major target organ of RA, the synovial tissue. Furthermore, we analysed their pathophysiological relevance in the induction or perpetuation of the ACPA and their colocalisation with different PAD isoforms in the synovial tissue.

In the fourth chapter, we explored the relation between PAD4 haplotypes, the presence of RA specific citrullinated epitopes in the synovial tissue, and ACPA levels. The functional PAD4 haplotype described in a Japanese cohort was proposed to have increased activity and therefore could augment the amount or the diversity of citrullinated proteins in the synovium. We investigated if the described haplotype was associated with higher levels of both synovial citrullinated proteins and ACPA in our cohort.

Augmented citrullination of proteins can be observed during inflammatory processes. As RA can be associated with extra-articular manifestations such as interstitial pneumonia or rheumatoid nodules, we investigated the presence of citrullinated proteins in these tissues in the fifth chapter. To investigate their possible role in the induction of ACPA, we compared the RA samples to ACPA negative patients with idiopathic interstitial pneumonia and healthy control lung samples.

In chapter 6 we reviewed the current knowledge and viewpoints on the characteristics and specificity of the antigens, citrullinated proteins.

In chapter 7 and 8, we performed detailed analysis of the immunological microenvironment in the inflamed synovium in relation to the ACPA response. In the seventh chapter, we analysed extensively the presence, structure, and function of synovial ectopic lymphoid neogenesis. Ectopic lymphoid neogenesis and germinal-center like reactions in tertiary lymphoid organs have been proposed to promote humoral immunity and, eventually, autoimmunity. However, it is not known if tolerance checkpoints are fully functional in these structures. We explored the relevance of ectopic lymphoid neogenesis for the expansion of clonally related B cells and the production of ACPA in the synovial tissue.

In chapter 8, we assessed the involvement of T lymphocytes in ACPA positive RA. Both the fact that ACPA are directed against protein epitopes and the genetic association between the MHC II shared epitope and ACPA positive RA suggest the involvement of T cells in the development of the ACPA response. Here, we used an advanced method of spectratyping by combining both qualitative and quantitative
Vβ CDR3 analysis to study T lymphocyte clonal alterations in the peripheral blood and the inflamed synovium of ACPA positive RA versus ACPA negative RA and SpA.
References

213.
28. Shoenfeld Y, Blank M, bu-Shakra M, Amital H, Barzilai O, Berkun Y et al. The mosaic of autoimmunity: prediction, autoantibodies, and therapy in
136(12):896-907.


2006; 65(9):1219-1222.


