

**Identification and characterisation of anti-citrullinated protein antibody** (acpa)-producing b cells in patients with rheumatoid arthritis (ra) Kerkman, P.F.

#### Citation

Kerkman, P. F. (2017, April 18). *Identification and characterisation of anti-citrullinated protein antibody (acpa)-producing b cells in patients with rheumatoid arthritis (ra)*. Retrieved from https://hdl.handle.net/1887/47927

Version:	Not Applicable (or Unknown)
License:	<u>Licence agreement concerning inclusion of doctoral thesis in the</u> <u>Institutional Repository of the University of Leiden</u>
Downloaded from:	https://hdl.handle.net/1887/47927

Note: To cite this publication please use the final published version (if applicable).

Cover Page



## Universiteit Leiden



The handle <u>http://hdl.handle.net/1887/47927</u> holds various files of this Leiden University dissertation

Author: Kerkman, Priscilla F. Title: Identification and characterization of anti-citrullinated protein antibody (acpa)producing b cells in patients with rheumatoid arthritis (ra) Issue Date: 2017-04-18

# **Chapter 1** General introduction

### General introduction

#### **Rheumatoid arthritis**

Rheumatoid arthritis (RA) is an autoimmune disease characterised by chronic systemic inflammation. In western societies, about 1% of the population is affected, with a higher prevalence in women. The disease is characterised by systemic inflammation of joints leading to cartilage destruction and bone erosions with, as a consequence, disability of the patient.

One of the hallmarks of RA is the presence of autoantibodies. During the course of the last 50 years several autoantibodies have been identified, amongst which rheumatoid factor (RF)[1], anti-citrullinated protein antibodies (ACPA) and anti-carbamylated protein antibodies (aCARP)[2]. Following their identification, many researchers studied the characteristics, specificity and, as most interesting aspect, their (pathogenic) role in RA.

#### **Citrullination and ACPA**

In 1964, Nienhuis *et al.* were first to show that RA patient serum contains specific antibodies against keratohyaline granules, which were initially termed antiperinuclear factor (APF) due to their staining pattern on buccal mucosa cells[3]. Independently, the same group of antibodies was described as anti-keratin antibodies (AKA) in 1979[4]. It took until 1998, however, before it was found that the non-classical amino acid citrulline is essential for recognition by this group of antibodies[5]. Citrulline is the result of deimination of the amino acid arginine by the enzyme peptidyl arginine deiminase (PAD) (figure 1.1). Due to this post-translational modification (PTM), the positively charged arginine



Figure 1.1: Post-translational modification of an arginine to a citrulline.



is converted into a neutral citrulline. Loss of charge can result in structural changes on the protein level, such as differences in protein folding, change of polarity or denaturation. Under physiological conditions, citrullination is linked to various processes in different cell types[6], including apoptosis[7-9] and histone modifications[10-12]. Citrullination of histones can facilitate transcription of genes as the structure of histones becomes less condense, or it can allow extensive chromatin decondensation which facilitates the formation of neutrophil extracellular traps; NETs[12, 13]. It is unknown why tolerance against citrullinated antigens is broken in the majority of patients with RA, but it has been shown that citrullinated proteins can be found in inflamed tissues including joints of patients[14, 15]. Moreover, an intriguing observation is that a polymorphism in the gene encoding the PAD enzyme is by itself a risk factor for ACPA-positive RA[16].

#### ACPA in RA

Following the identification of citrulline as the essential part of the antigen recognized by both APF and AKA, an ELISA using a synthetic cyclic citrullinated peptide (CCP) was developed, called the anti-CCP1 test. This diagnostic test has a specificity of 98% and a sensitivity of 68% for the diagnosis RA[17]. The commercial CCP2 version of the test for routine testing, available since 2002, made it possible to use ACPA as a biomarker for RA and, in the 2010 criteria used for classification of patients, ACPA status was included[18]. In recent years, many groups studied the role of ACPA in disease pathogenesis, although the precise contribution of ACPA to disease-related processes remains still unclear. However, accumulating clinical as well as experimental data suggest that ACPA indeed contribute to inflammatory processes and chronicity of inflammation in RA.

Concerning the role of ACPA as a biomarker, it is intriguing that ACPA can be detected in serum of individuals with joint pain (arthralgia) before the onset of RA. Arthralgia patients that harbour ACPA have a higher chance of developing RA compared to patients without ACPA[19]. Also, patients with undifferentiated arthritis (UA) have a higher risk for RA development when they are ACPA-positive[20]. Furthermore, patients with RA being ACPA-positive have more severe disease progression[21]. Thus, ACPA are useful diagnostic and predictive biomarkers that identify patients at risk for severe progressive disease.

#### Pathogenicity of ACPA

As ACPA are a hallmark of RA, many studies tried to identify direct ACPAmediated biological effector functions. In mice, the induction or development of ACPA is debated[22-24], but infusion of ACPA in mice can exacerbate arthritis[22, 25] suggesting that ACPA can at least enhance inflammation. Similar conclusions could be derived from in vitro studies, in which ACPA could promote inflammation by influencing different processes and cell types of the immune system. For example, ACPA can activate the classical and alternative, but not the lectin pathway of the complement system[26]. Plate-bound immune complexes (IC) of ACPA can activate mast cells, mainly via  $Fc\gamma$ RIIA[27] and, enhanced by IgM-rheumatoid factor, increase cytokine production by



macrophages[28]. As citrullinated antigens are present in the inflamed joints of patients with RA[29], ACPA-IC could be formed at these sites and contribute to inflammation. Moreover, purified ACPA skew macrophage differentiation to the inflammatory M1 phenotype, resulting in an increased M1/M2 ratio after culture[30]. Also, ACPA-containing IgG, but not control IgG, potentially induces NET formation by neutrophils, thereby creating a vicious circle where NETs contain citrullinated antigens and thereby trigger additional autoantibody formation and inflammation[31]. Furthermore, isolated ACPA-IgG but not control IgG can induce osteoclastogenesis and bone loss[32]. Focussing on signalling pathways, it was shown that ACPA can activate ERK1/2 and JNK signalling which leads to NF- $\kappa$ B activation and TNF- $\alpha$  production[33]. As ACPA have been implicated in the pathogenesis of these processes, it is very important to understand whether these effector functions are ACPA specific and if ACPA have molecular features that are important for the observations described above.

In our department, detailed characterisation of ACPA on the molecular level has been performed for many years. Studies of isotype usage and isotype subclasses showed that ACPA are expressed in most isotypes, i.e. IgG, IgA and IgM. Of interest, patients negative for ACPA-IgM can be positive for ACPA-IgM later in time indicating formation of new ACPA-expressing B cells also during the course of disease[34]. ACPA have a low avidity compared to antibodies against recall antigens and show only avidity maturation before disease onset[35, 36]. Separating ACPA-positive patients in groups according to ACPA avidity revealed that patients with the lowest avidity have more severe joint destruction[37]. In line with avidity maturation, also epitope spreading was shown to happen before the onset of RA while no changes in fine specificity were observed later in the disease course[38]. And, more recently, the majority of ACPA was found to carry N-glycans in the Fab region which modulate the binding avidity to their antigen[39]. All these data indicate that ACPA have different characteristics and dynamics compared to recall antibodies. Very interesting aspects of ACPA research include the questions on why ACPA are already present before disease onset, what makes the ACPA immune response mature just before clinical disease onset as indicated by a rise of titre, change of isotype usage and epitope spreading, and why patients in remission can have persistent high ACPA-IgG titres. Therefore, a better understanding of ACPA and the underlying immune response is required to determine why (a certain group of) ACPA show these features that are presumably related to pathogenic properties. To dissect these issues, the study of citrullinated antigen-specific B cells could give insights in the origins of this autoantigen-specific immune response and could elucidate differences between normal B cells, 'non-pathogenic' early citrullinated antigenspecific B cells, citrullinated antigen-specific B cells in remission and 'pathogenic' citrullinated antigen-specific B cells during active disease. Eventually, such knowledge could identify targets that allow interfering with the development of citrullinated antigen-specific B cells.



#### B cells

B cells are key players in our adaptive immune system and provide humoral immune responses. B cells are part of the adaptive immune system, recognize a specific antigen on their surface via the B cell receptor (BCR) and can develop into highly specific memory B cells. B cells differ from T cells in antigen recognition as T cells recognize small peptides whereas B cells recognize an antigen in their native form, which can include for example lipids or sugars. Furthermore, upon differentiation to plasmablast/plasmacells (PB/PC), B cells secrete antibodies, which are part of the humoral immune system. In general, B cell development can be divided into four stages[40]: generation, elimination of self-reactive B cells, activation by foreign antigens and differentiation to antibody secreting cells (ASC) and memory B cells.

Generation of B cells starts in the bone marrow where B cells obtain their BCR. The BCR is a Y-shaped molecule comprised of two heavy and two light chains. The upper part of the V part of the Y contains the variable regions and the rest of the molecule is the constant region. The variable region contains a variable (V), a diversity (D; only in Ig heavy chain) and a joining (J) element. For all these elements of the variable region, many potential genes are available providing an enormous number of possible V(D)J combinations, thereby creating a wide range of possible BCRs. To obtain an expressed BCR the DNA needs to be rearranged. In this process, recombination activated genes (RAGs) encode enzymes which will induce cleavage of DNA at specific sites between the selected V(D)J elements. Next, the DNA of the selected V(D)J elements will become adjacent and the DNA will be repaired. As a result, the BCR gene transcript is formed and the in-between parts of the B cell's genome will be lost. Rearrangement of the BCR starts at the heavy chain locus creating a pre-B cell receptor with a surrogate light chain (LC)[41, 42]. Next, the LC is rearranged starting mostly at the kappa locus[43, 44]. When rearrangement does not result in a functional BCR the cell rearranges again until a functional BCR is formed, rescuing it from apoptosis. Finally, cells positively selected due to the expression of a functional BCR enter the stage of immature B cells and continue their development. Although the rearrangements could be completely random, it was shown that this is a tightly regulated process[45]. Immature B cells express BCRs that are often autoreactive. Under normal conditions, these autoreactive B cells will be negatively selected and undergo apoptosis, become anergic or modify (again) their receptor by receptor editing, which alters (mostly) the LC. When a cell changes its LC, the cell transcribes a kappa locus genetically further downstream, switches transcription to the second kappa containing chromosome, or it switches to one of the two lambda containing chromosomes. Choosing for a locus located downstream results in loss of the upstream loci. Thus, each time a B cell undergoes receptor editing the amount of available light chains is reduced, since part of the DNA is lost. As a final result of control checkpoints and receptor editing, mature naïve B cells can enter the periphery.

Mature naïve B cells circulate in the periphery patrolling through secondary lymphoid organs, spleen and lymph nodes, until they die from lack of survival signals or until they encounter their antigen. Upon antigen encounter in



secondary lymphoid organs and a second activation signal [46], they become activated and start proliferating and differentiating, thereby generating memory B cells and/or antibody secreting plasmablasts/plasmacells (PB/PC). Broadly, this second activation signal can be divided into two subgroups of signals: T cell dependent and T cell independent signals. T cell dependent B cell activation is characterised by the formation of germinal centres (GC). Activated T cells provide a stimulatory signal via CD40L-CD40 interaction[47, 48]. GC B cells proliferate extensively while somatic hypermutation (SHM) introduces changes in the recognition region of the BCR, a process termed affinity maturation. B cells with the highest affinity for their cognate antigen are positively selected and some will re-enter SHM resulting in B cells with even higher affinity. Other B cells can undergo class switch recombination (CSR) and become high affinity memory B cells or Ig-producing PB/PC[49]. T cell independent activation and memory formation needs activation of several receptors on one B cell. This can be achieved by combining activation of the BCR and another receptor, like toll like receptors (TLRs) or by crosslinking of several BCRs recognizing lipids and carbohydrates[50, 51]. Memory B cells can more easily be (re-)activated than naïve B cells, but both can differentiate to short-lived antibody secreting plasmablasts (PB) or long-lived plasmacells (PC). To become long-lived PC, they home to the bone marrow (BM)[52, 53] where they can become long-lived PC when they successfully compete for plasma cell survival niches[54].

#### **Outline of this thesis**

In many autoimmune diseases including RA, there is excessive production of antibodies that can bind self-antigens. How these self-reactive B cells escape negative selection is not known. To understand this, characterisation of autoreactive B cells is required preferably by analysing these cells in an antigen-specific manner. Since we know that ACPA have different molecular characteristics and dynamics compared to recall antibodies, we hypothesised that also ACPA-expressing B cells could have different characteristics if compared to non-autoreactive B cells. How these cells develop, mature and survive is still largely unknown. The studies presented in this thesis describe the first steps to understand ACPA-expressing B cells in terms of identification, localization, isolation and characterisation.

In **chapter 2**, studies are described addressing the question where ACPAexpressing B cells are located. In contrast to conventional immune responses, ACPA-IgM in serum can appear at later stages of established disease which suggests that ACPA are continuously generated as a result of an ongoing immune response[34]. Therefore, we cultured B cells isolated from peripheral blood of ACPA-positive RA patients. We were able to identify ACPA-expressing B cells in peripheral blood of ACPA-positive RA patients but not in ACPA-negative RA patients or healthy controls. Unstimulated versus stimulated peripheral blood mononuclear cell (PBMC) cultures and cultures after FACS sorting experiments revealed that, next to ACPA-expressing B cells in the memory pool, spontaneously ACPA-secreting PB/PC are present in peripheral blood. These findings support



the hypothesis of an ongoing citrullinated antigen-specific immune response in ACPA-positive RA patients.

As we identified spontaneously ACPA-secreting PB/PC in peripheral blood in the context of the studies presented in chapter 2, we questioned if this ACPA immune response is similar near the site of inflammation, in synovial fluid (SF). To this end, and to understand the role of cells from the synovial compartment in maintaining ACPA B cells, we characterised ACPA-secreting cells from SF in detail. These studies are presented in **chapter 3**. Even though the percentage of B cells in synovial fluid mononuclear cells (SFMC) is lower than in peripheral blood, we identified an enrichment of spontaneous ACPA secretion and ACPA secreting cells in the SF compartment. Furthermore, we found that total SFMC have the potential to spontaneously form a survival niche for long-lasting (auto) antibody secretion.

Studying ACPA B cells on a single cell level could give many insights into the ACPA immune response. Therefore, we also set out to develop an antigen-specific staining for the identification of citrullinated antigen-specific B cells by flow cytometry. As peripheral blood is relatively easy to obtain from patients compared to SF, we focussed on peripheral blood despite the lower number of ACPA B cells (chapter 3). **Chapter 4** describes the successful development of a flow cytometry staining that identifies ACPA-expressing B cells. For the method to be successful, we constructed a human embryonic kidney (HEK) cell line expressing a monoclonal ACPA molecule on the cell membrane and used three streptavidin tetramers in each staining. This combination of tetramers, two containing citrullinated antigens and one an arginine control variant, is needed to identify cells recognizing citrullinated antigens but not the control antigen. This technique was validated by culturing cells isolated by flow cytometry, which yielded high amount of ACPA production in positively sorted wells, whereas higher numbers of negatively sorted cells gave very low or no detectable degree of ACPA production.

Next, we included phenotypic markers CD20 and CD27 to discriminate between the naïve, memory and PB/PC compartment, which allowed calculating the frequency of ACPA-expressing B cells for every subpopulation. This revealed that most ACPA-expressing B cells have a memory phenotype with a median of 1 in every 2600 memory B cells. Using isotype-specific antibodies, we could show that ACPA-expressing B cells in peripheral blood are mostly class-switched memory B cells expressing IgG, but also IgA, IgM and IgD ACPA B cells could be detected.

To better understand the provenance of ACPA-expressing B cells in terms of B cell development, the usage of lambda and kappa light chains was studied in the context of the studies presented in **chapter 5**. In general, B cells start with the expression of a kappa light chain-containing BCR[43, 44]. Prior to entering the periphery the cells need to pass the checkpoint of being nonautoreactive. Although this is a very important checkpoint, this checkpoint in itself is incomplete and some autoreactive cells 'escape' and enter the periphery. We hypothesised that a combination of starting with a kappa LC, more rounds of failing the autoreactivity checkpoint and more additive chance of escaping the checkpoint generates, on average, more lambda usage in autoreactive B cells. If ACPA B cells were generated this way we expected that ACPA would more



frequently carry lambda light chains. To this end, we characterised ACPA-IgG antibodies isolated from serum and SF and studied the usage of lambda light chains by ACPA-expressing B cells. We found a shift in frequency of lambda LC usage, as serum ACPA-IgG and ACPA(-IgG<sup>+</sup>) B cells more frequently use lambda light chains. This could indicate that ACPA-expressing B cells underwent, on average, more rounds of receptor editing.

As we hypothesised that long-lived plasma cells could be important in (ACPA-positive) RA, we also analysed the expression of CD28 on B cells in RA patients in the context of the studies presented in **chapter 6**. As has been described for mice, expression of CD28 on B cells is involved in the live span of PC[55]. For human B cells, this has not been shown so far, but the expression of CD28 on B cells has been reported. Therefore, we studied the expression of CD28 on the different B cell subsets in peripheral blood (and SF) of RA patients compared to patients with systemic lupus erythematosus (SLE) and healthy controls, as well as on ACPA-expressing B cells. We identified CD28 expression on PB/PC in both patient groups and heathy controls and this was found to be comparable. In PB/PC in SF, this percentage was increased. ACPA-expressing PB also expressed CD28. Yet also here, the expression level was found to be comparable for peripheral blood as well as SF PB/PC.

Finally, **chapter** 7 summarizes and discusses the results and describes possible directions for future research.

#### References

- 1. Waaler E. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. Apmis 2007;115:422-438.
- 2. Shi J, Knevel R, Suwannalai P, *et al.* Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. Proc Natl Acad Sci 2011;108:17372-17377.
- 3. Nienhuis RL, Mandema E. A New Serum Factor in Patients With Rheumatoid Arthritis; the Antiperinuclear Factor. Ann Rheum Dis 1964;23:302-5.
- 4. Young BJJ, Mallya RK, Leslie RDG, Clark CJM, Hamblin TJ. Antikeratin antibodies in rheumatoid arthritis. Br Med J 1979;97-99.
- Schellekens GA, De Jong BAW, Van Den Hoogen FHJ, Van De Putte LBA, Van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998;101:273-281.
- Gyorgy B, Toth E, Tarcsa E, Falus A, Buzas EI. Citrullination: A posttranslational modification in health and disease. Int J Biochem Cell Biol 2006;38:1662-1677.
- Asaga H, Yamada M, Senshu T. Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages. Biochem Biophys Res Commun 1998;243:641-646.
- Hsu P-C, Liao Y-F, Lin C-L, Lin W-H, Liu G-Y, Hung H-C. Vimentin is involved in peptidylarginine deiminase 2-induced apoptosis of activated Jurkat cells. Mol Cells 2014;37:426-34.



- U KP, Subramanian V, Nicholas AP, Thompson PR, Ferretti P. Modulation of calcium-induced cell death in human neural stem cells by the novel peptidylarginine deiminase – AIF pathway. BBA – Mol. Cell Res. 2014, 1843: 1162-1171.
- Hagiwara T, Nakashima K, Hirano H, Senshu T, Yamada M. Deimination of arginine residues in nucleophosmin/B23 and histones in HL-60 granulocytes. Biochem Biophys Res Commun 2002;290:979-983.
- 11. Cuthbert GL, Daujat S, Snowden AW, *et al.* Histone deimination antagonizes arginine methylation. Cell 2004;118:545-553.
- Wang S, Wang Y. Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. Biochim Biophys Acta – Gene Regul Mech 2013;1829:1126-1135.
- 13. Leshner M, Wang S, Lewis C, *et al.* PAD4 mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures. Front Immunol 2012.
- 14. Klareskog L, Rönnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. Annu Rev Immunol 2008;26:651-75.
- 15. Baeten D, Peene I, Union A, *et al.* Specific presence of intracellular citrullinated proteins in rheumatoid arthritis synovium: Relevance to antifilaggrin autoantibodies. Arthritis Rheum 2001;44:2255-2262.
- 16. Suzuki A, Yamada R, Chang X, *et al.* Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 2003;10:520-527.
- 17. Schellekens GA, Visser H, De Jong BAW, *et al.* The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-163.
- Aletaha D, Neogi T, Silman AJ, *et al.* 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569-2581.
- 19. Van de Stadt LA, Van der Horst AR, de Koning MHMT, *et al.* The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. Ann Rheum Dis 2011;70:128-133.
- 20. Van Gaalen FA, Linn-Rasker SP, Van Venrooij WJ, et al. Autoantibodies to Cyclic Citrullinated Peptides Predict Progression to Rheumatoid Arthritis in Patients with Undifferentiated Arthritis: A Prospective Cohort Study. Arthritis Rheum 2004;50:709-715.
- 21. Van Gaalen FA, Van Aken J, Huizinga TWJ, *et al.* Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum 2004;50:2113-2121.



- 22. Kuhn KA, Kulik L, Tomooka B, *et al.* Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. J Clin Invest 2006;116:961-973.
- 23. Stoop JN, Liu BS, Shi J, *et al.* Antibodies specific for carbamylated proteins precede the onset of clinical symptoms in mice with collagen induced arthritis. PLoS One 2014;9:1-9.
- 24. Stoop JN, Fischer A, Hayer S, *et al.* Anticarbamylated protein antibodies can be detected in animal models of arthritis that require active involvement of the adaptive immune system. Ann Rheum Dis 2015;0:2014-2016.
- 25. Uysal H, Bockermann R, Nandakumar KS, *et al.* Structure and pathogenicity of antibodies specific for citrullinated collagen type II in experimental arthritis. J Exp Med 2009;206:449-62.
- 26. Trouw LA, Haisma EM, Levarht EWN, *et al.* Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. Arthritis Rheum 2009;60:1923-1931
- 27. Suurmond J, Rivellese F, Dorjée AL, *et al.* Toll-like receptor triggering augments activation of human mast cells by anti-citrullinated protein antibodies. Ann Rheum Dis 2014;1915-1923.
- 28. Sokolove J, Johnson DS, Lahey LJ, *et al.* Rheumatoid factor as a potentiator of anti-citrullinated protein antibody-mediated inflammation in rheumatoid arthritis. Arthritis Rheumatol 2014;66:813-821.
- 29. Kinloch A, Lundberg K, Wait R, *et al.* Synovial fluid is a site of citrullination of autoantigens in inflammatory arthritis. Arthritis Rheum 2008;58:2287-2295
- Zhu W, Li X, Fang S, *et al.* Anti-Citrullinated Protein Antibodies Induce Macrophage Subset Disequilibrium in RA Patients. Inflammation 2015;38:2067-2075.
- 31. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, *et al.* NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. Sci Transl Med 2013;5:178ra40.
- 32. Harre U, Georgess D, Bang H, *et al.* Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. J Clin Invest 2012;122:1791-1802.
- 33. Lu MC, Lai NS, Yin WY, et al. Anti-citrullinated protein antibodies activated ERK1/2 and JNK mitogen-activated protein kinases via binding to surfaceexpressed citrullinated GRP78 on mononuclear cells. J Clin Immunol 2013;33:558-566.
- 34. Verpoort KN, Jol-Van Der Zijde CM, Papendrecht-Van Der Voort EAM, *et al.* Isotype distribution of anti-cyclic citrullinated peptide antibodies in undifferentiated arthritis and rheumatoid arthritis reflects an ongoing immune response. Arthritis Rheum 2006;54:3799-3808.
- 35. Suwannalai P, Scherer HU, Van der Woude D, *et al.* Anti-citrullinated protein antibodies have a low avidity compared with antibodies against recall antigens. Ann Rheum Dis 2011;70:373-9.



- 36. Suwannalai P, Van De Stadt LA, Radner H, *et al.* Avidity maturation of anticitrullinated protein antibodies in rheumatoid arthritis. Arthritis Rheum 2012;64:1323-1328.
- 37. Suwannalai P, Britsemmer K, Knevel R, *et al.* Low-avidity anticitrullinated protein antibodies (ACPA) are associated with a higher rate of joint destruction in rheumatoid arthritis. Ann Rheum Dis 2014;73:270-276.
- 38. Van der Woude D, Rantapää-Dahlqvist S, Ioan-Facsinay A, *et al.* Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. Ann Rheum Dis 2010;69:1554-1561.
- 39. Rombouts Y, Willemze A, Van Beers JJBC, *et al.* Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. Ann Rheum Dis 2015;1-8.
- 40. Parham P. The immune system, 2nd ed. 2005.
- 41. Ehlich A, Schaal S, Gu H, Kitamura D, Müller W, Rajewsky K. Immunoglobulin heavy and light chain genes rearrange independently at early stages of B cell development. Cell 1993;72:695-704.
- 42. Ghia P, Ten Boekel E, Rolink AG, Melchers F. B-cell development: a comparison between mouse and man. Immunol Today 1998.
- Mehr R, Shannon M, Litwin S. Models for antigen receptor gene rearrangement. I. Biased receptor editing in B cells: implications for allelic exclusion. J Immunol 1999;163:1793-1798.
- 44. Van der Burg M, Tumkaya T, Boerma M, De Bruin-Versteeg S, Langerak AW, Van Dongen JJM. Ordered recombination of immunoglobulin light chain genes occurs at the IGK locus but seems less strict at the IGL locus. Blood 2001;97:1001-1008.
- 45. Ehlich A, Martin V, Müller W, Rajewsky K. Analysis of the B-cell progenitor compartment at the level of single cells. Curr Biol 1994;4:573-583.
- 46. Bretscher P, Cohn M. A theory of self-nonself discrimination. Science 1970;169:1042-9.
- 47. Liu YJ, Joshua DE, Williams GT, Smith CA, Gordon J, MacLennan IC. Mechanism of antigen-driven selection in germinal centres. Nature 1989;342:929-31.
- 48. Foy TM, Laman JD, Ledbetter JA, Aruffo A, Claassen E, Noeue RJ. gp39-CD40 Interactions Are Essential for Germinal Center Formation and the Development of B Cell Memory. J Exp Med 1994;180.
- 49. De Silva NS, Klein U. Dynamics of B cells in germinal centres. Nat Rev Immunol 2015;15:137-148.
- 50. Obukhanych TV, Nussenzweig MC. T-independent type II immune responses generate memory B cells. J Exp Med 2006;203:305-310.
- 51. Fagarasan S, Honjo T. T-Independent immune response: new aspects of B cell biology. Science 2000;290:89-92.



- 52. Benner R, Hijmans W, Haaijman JJ. The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. Clin Exp Immunol 1981;46:1-8.
- 53. Slifka MK, Matloubian M, Ahmed R. Bone marrow is a major site of long-term antibody production after acute viral infection. J Virol 1995;69:1895-1902
- 54. Radbruch A, Muehlinghaus G, Luger EO, *et al.* Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol 2006;6:741-50.
- 55.Rozanski CH, Arens R, Carlson LM, *et al.* Sustained antibody responses depend on CD28 function in bone marrow-resident plasma cells. J Exp Med 2011;208:1435-1446.

