Review

# One year in review 2017: pathogenesis of rheumatoid arthritis

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**ABSTRACT**

*Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease influenced by both genetic and environmental factors. It has been postulated that a high-risk genetic background, in combination with epigenetic marks and environmental exposures, leads to a cascade of events inducing synovitis and consequent destructive arthritis. The clinical picture of joint involvement in RA is the result of chronic inflammation of the synovium, characterised by interactions of resident cells such as fibroblast-like synoviocytes (FLS) with cells of the innate (e.g. macrophages, dendritic cells, mast cells and NK cells, neutrophils) and adaptive immune system (e.g. B and T lymphocytes). Currently, our understanding of the role of innate and adaptive immunity in the pathogenesis of RA is expanding. The concept of how immune responses contribute to the disease has dramatically evolved over the last 50 years. Shedding some light on the different aspects of RA pathogenesis will help to identify new targets for the development of disease-modifying therapies. Thus, in this review we report new insights in RA pathogenesis, resulting from a literature research date published in the last year.*

## Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, influenced by both genetic and environmental factors. A high-risk genetic background, in combination with epigenetic marks and environmental exposures, leads to a cascade of events inducing synovitis with consequent destructive arthritis, but also affecting a variety of extra-articular organs. The effects of environmental factors in genetically predisposed individuals are now of great significance in the pathogenesis and development of RA (1, 2). The clinical picture of joint involvement is the result of a tight interaction between resident cells such as fibroblast-like synoviocytes (FLS) and cells of the innate (*e.g.* macrophages, dendritic cells, mast cells and NK cells, neutrophils) and adaptive immune system (*e.g.* B and T lymphocytes). Soluble mediators, autoantibodies, adhesion molecules and signal transduction pathways are all involved at different stages of the diseases. Here we report a Medline search of articles in English published in the PubMed database from 1st January 2016 to 31st December 2016 giving new insights in the field of RA pathogenesis. All articles were critically analysed in order to provide an improved understanding of the mechanisms underlying RA and to contribute to identify new targets for the development of novel disease-modifying therapies.

## Genetic aspects

Recent advances in genome-wide association studies and subsequent meta-analyses have led to the identification of many alleles that govern RA susceptibility (3). Although predominant associations with the *HLA-DRB1* locus were identified decades ago, recent data have revealed additional insights into the likely causative variants within *HLA-DRB1* as well as within other *HLA* loci that contribute to disease risk. Data obtained from genome-wide association analysis are variable according to the population evaluated. In the southern Indian population, by studying the presence of the shared epitope in the *HLA-DRB1* alleles, Konda Mohan *et al.* showed that *HLA-DRB1**01, *04, *10 and *14 alleles are related to RA, while *HLA-DRB1**03, *07, *11 and *13 protect against the development of the disease (4). In a Swedish population, Jang *et al.* evaluated whether known RA susceptibility genes, HLA-DRB1 amino acids, and haplotypes either individually or integrated into a risk score, pre-

dicted treatment response to inhibitors of tumour necrosis factor (TNFi) therapy. Although all of these were strong predictors of RA, they found that a high genetic risk score was not associated with good response in either overall RA or stratified by anti-citrullinated protein antibody (ACPA) status. According to the results, the authors concluded that there is no strong evidence supporting a significant role of RA risk genes in the response to TNFi treatment. In addition, none of the single nucleotide polymorphisms (SNPs), amino acids, or haplotypes examined in their study seem to be meaningful independent predictors of response to TNFi therapy, although weak associations cannot be ruled out (5). Particular attention has been paid to the association between ACPA and *HLA-DR* alleles. These autoantibodies are highly prevalent in RA patients and are strongly associated to a specific set of *HLA-DR* alleles, including *HLA-DRB1**04:01, *04:04, and *01:01. Gerstner *et al*. demonstrated that the levels of ACPA towards the dominant citrullinated B cell epitope of α-enolase are significantly elevated in *HLA-DRB1**04:01-positive RA patients. Furthermore, they identified α-enolase-derived T cell epitopes and demonstrated that native and citrullinated versions of several peptides bind with different affinities to *HLA-DRB1**04:01, *04:04, and *01:01 (6). In addition to the *HLA-DRB1* alleles, genomic stratification by expression of *HLA-DRB4* alleles led to identify differential innate and adaptive immune transcriptional patterns, which might predict the response to therapy, namely methotrexate (MTX) in early RA (7). Some interesting results on the association between polymorphisms of *HLA* loci with RA onset or response to therapies also come from studies performed in animal models of RA. In collagen-induced arthritis (CIA) the HLA-G2 protein, involved in maternal-fetal immune tolerance, has been found to be a useful target for the treatment of RA, specifically binding paired Ig-like receptor (PIR)-B (PIR-B), only expressed by antigen presenting cells (APC). In their study, Takahashi *et al*. were able to demonstrate the immunosuppressive effect of the domain-deleted dimer of HLA-G2 isoform in the CIA model, thereby postulating that this effect might be due to the suppression of antigen presentation by HLA-G2 proteins (8).

In addition to the *HLA* loci, more than 100 common variants in non-*HLA* loci have been implicated in RA susceptibility. For example, polymorphisms in signalling transducers and activators of transcription- (*STAT)-4* and interleukin- (*IL)-10* genes confer susceptibility to RA and SNPs in *PSORS1C1*, *PTPN2* and *MIR146A* genes were associated to various extents with a severe disease phenotype in terms of autoantibody status and radiographic damage (9). In parallel, Lee *et al*. (10) investigated the associations between *K469E* and *G241R* polymorphisms of the intercellular adhesion molecule-1 (ICAM-1) and vasculitis or RA, demonstrating that the *G241R* polymorphism is associated with susceptibility to RA as well as Behçet's disease. The association of the non-coding triallelic dinucleotide polymorphism *CCR6DNP* with risk for RA has also been hypothesised. This allelic variant seems to correlate with the expression of the C-C chemokine receptor-6 (CCR6). By using transcription activator-like effector nuclease (TALEN) gene editing, Li *et al*. (11) confirmed that *CCR6DNP* regulates CCR6 and that is a causal variant through which the Poly(ADP-ribose) polymerase-1 (PARP-1), multifunctional nuclear protein involved in a variety of cellular functions, regulates this receptor.

Several candidate gene variants have been identified by genetic mapping in RA. However, RA as well as other autoimmune diseases cannot be explained by genetic susceptibility alone. The term 'epigenetic' refers to inheritable and potentially reversible changes in DNA and chromatin that regulate gene expression without altering the DNA sequence. Among the mechanisms involved in epigenetic regulation, DNA methylation, histone modification, and non-coding RNA-mediated regulation are currently under intense investigation. Therefore, a novel approach is to study the high-risk genetic background in combination with epigenetic modifications. This combined approach allows to better understand the mechanisms underlying the innate and adaptive immune responses during RA.

Gene-analysis combined with an epigenetic regulation study has been recently investigated in the regulation of FLS. These are synovial intimal lining cells which display unique aggressive behaviour, invading the articular cartilage and promoting inflammation through the production of cytokines, chemokines, and proteases. The *PTPN11* gene, encoding the tyrosine phosphatase SHP-2, is overexpressed in RA FLS compared to those from osteoarthritis (OA) patients and promotes RA FLS invasiveness. Using computational methods, Maeshima *et al*. identified a putative enhancer in *PTPN11* intron 1, which contained a glucocorticoid receptor-binding (GR-binding) motif. This region displayed enhancer function in RA FLS and contained two hypermethylation sites in RA compared with OA FLS. The authors finally demonstrated how abnormal epigenetic regulation of a pathogenic gene determines RA FLS behaviour and targeting SHP-2 or the SHP-2 pathway could be a therapeutic strategy for RA (12). This approach may be used to dissect the critical abnormalities of RA FLS and define candidate molecular or pathway targets for therapeutic intervention.

## Environmental factors

The development of RA cannot be fully explained by genetic factors and epigenetic mechanisms. It has been proposed that many environmental components, including smoking, hormones, infection and microbiota are critically involved in the induction of the disease in genetically predisposed individuals.

### *Cigarette smoke*

Gene-environment interactions are known to play a key role in the development of RA. Exposure to cigarette smoke (CS) is probably one of the strongest environmental risk factors associated with the disease. CS might exert a range of complex modulatory effects on both innate and adaptive immune systems. In recent years, several studies have tried to clarify the mecha-

nisms involved in the effects of CS on the immune system and its contribution to the development of the disease (13). Interestingly, Sparks *at al*. performed a study to prospectively evaluate genetic, environmental, and serologic risk factors in a cohort of first-degree relatives (FDRs) of RA patients. This group of subjects is at increased risk of RA based on positive family history, but many of them were asymptomatic and seronegative, so are likely further away from developing RA. In this study, CS resulted to be an important, and potentially modifiable risk factor during preclinical transitional phases of RA. In fact, FDRs smoking more than 10 pack/years and younger than 50 years of age represent the group with the highest risk of developing inflammatory joint signs (14). Additional information on gene-smoke interaction in RA came from Immunochip (ICHIP), a custom-made Illumina Infinium array, including 195586 genetic markers from 5043 samples. In this study multiple gene-environment interactions have been identified in autoantibody-positive RA, taking into account all genetic variations in the ICHIP scan and using CS as environmental exposure. Extended gene variation patterns involved in gene-smoking interaction in ACPA-positive, but not ACPA-negative RA have been identified. Notably, variants in *HLA-DRB1* and those in additional genes within the MHC class II region, but not in any other gene regions, showed interaction with CS. Taken together, these results might be useful to design further functional studies on the potential effects of MHC class II–dependent immune activation in ACPA-positive RA following CS exposure (15). The effects of CS on the immune response have been widely studied in animal models. By using DEF6 deficiency mice, Weng *et al*. investigated the effects of CS on the function of T helper (Th)17 cells, one of the key effector subsets implicated in RA pathogenesis. They have demonstrated that the direct exposure of $CD4^+$ T cells to CS extract enhances the production of IL22, a member of the IL10 cytokine family produced by several different cellular sources including Th17 cells, Th22 cells, NK cells, lymphoid tissue inducer (LTi) cells and γδT cells. After treatment with CS extracts, they also observed decreased ROCK2 activation and phosphorylation in T cells of interferon regulatory factor-4 (IRF4), a known negative regulator of IL22. Using a GEF pull-down assay they furthermore demonstrated that this effect was associated with a reduction in the activation of ARHGEF1, an upstream regulator of the RhoA-ROCK axis (16), confirming the complex interaction between gene and environmental factors in RA and the regulatory role of CS in the development of the disease.

*Microbiota*

Growing evidence has shown that the changes in the composition and function of mucosal microbiota are closely related to RA. Several studies have demonstrated that the composition of gut microbiota is altered (dysbiosis) in RA patients. Through the analysis of faecal samples, Chen *et al*. showed that patients with RA exhibited decreased gut microbial diversity compared with healthy individuals, which correlated with disease duration and autoantibody levels. A taxon-level analysis suggested an expansion of rare taxa, namely *Actinobacteria*, in patients with RA. By using predictive models based on the random forests algorithm, they also demonstrated that the three genera, *Collinsella*, *Eggerthella*, and *Faecalibacterium* segregate with RA and that the abundance of *Collinsella* strongly correlates with high levels of alpha-aminoadipic acid and asparagine as well as with the production of the pro-inflammatory cytokine IL17A. In addition, *Collinsella* seems to modulate gut function by altering its permeability and enhancing the severity of the disease (17). Besides differences in the composition of the microbiota, the current research is focusing on characterisation of the functional role of microbial components in RA. For this purpose, Maeda *et al*. analysed the composition of faecal microbiota of patients with early RA, using 16S ribosomal RNA-based deep sequencing. Following inoculation of faecal samples obtained from RA patients into germ-free arthritis-prone SKG mice, which spontaneously develops Th17-mediated autoimmune arthritis, they studied the immune responses in this animal model. They also analysed whether the lymphocytes of SKG mice harbouring microbiota from RA patients react with the arthritis-related autoantigen 60S ribosomal protein L23a (RPL23A). These *in-vivo* and *ex-vivo* approaches allowed to demonstrate that dysbiosis increases sensitivity to arthritis via activation of Th17 cells in the intestine, and that the autoreactive SKG mouse T cells are activated by dysbiotic microbiota, leading to the development of joint inflammation. Therefore, the authors concluded that *Prevotellaceae*-dominant intestinal microbiota seems to be an environmental factor that promotes genetically susceptible T cells to induce arthritis, supporting the direct contribution of dysbiosis in triggering arthritis development in genetically susceptible mice (18). Besides Th17 cells, other T-cell subsets seem to be involved in the regulation of gut microbiota in RA. By using the K/BxN autoimmune arthritis model, Block *et al*. have shown that the gut microbiota regulates arthritis through a sub-set of Th cells, named follicular helper T cells (Tfh), and that this regulation is independent of the Th17 subsets. They have hypothesised that Tfh cells promote activation and differentiation of B cells through the production of inflammatory cytokines such as IL21 and IL4 (19). In parallel, the immune relevance of another microbial component, *Prevotella copri,* has been investigated. This is an intestinal microbe over-expanded in stool samples of patients with new-onset rheumatoid arthritis (NORA). In peripheral blood mononuclear cells (PBMC) purified from NORA patients, an HLA-DR-presented peptide from a 27-kD protein of *P. copri* (Pc-p27) has been identified. In 42% of these patients, the Pc-p27 seems to exert a regulatory role on the adaptive immune response by stimulating a Th1 phenotype. In both NORA and established RA groups, a subgroup of patients producing IgA antibodies directed to Pc-p27, which correlated with Th17

cytokines and ACPA, has been identified. The rest of patients produced IgG antibodies directed to *P. copri*, which were associated with *Prevotella* DNA in synovial fluid (SF), P. copri-specific Th1 responses, and lower prevalence of ACPA. Interestingly, the production of antibodies to *P. copri* were rarely found in patients with other rheumatic diseases or in healthy population, suggesting that the immune response to *P. copri* is specific for RA (20).

The use of various animal models to highlight mechanisms underlying RA pathogenesis often provide conflicting results. Therefore, studies in human along with studies in animal models are required in order to better understand how environmental factors modulate immune responses in RA and contribute to the development of the disease.

**Innate immune response**

*NETosis and autophagy*

In recent years, particular interest has been given to the contribution of neutrophil extracellular trap (NET) formation (NETosis) to the breaking of immunological tolerance and the maintenance of autoimmunity and inflammation in RA. NETosis is an important first line defense mechanism of neutrophils, occurring at an early step of the inflammatory cascade. NETs trap and restrain invading pathogens and use their highly localised focus of antimicrobial granular peptides to degrade virulent factors and even kill microorganisms. This process consists of the release of intracellular components, including DNA, histones and an array of proteins that all together create a net-like structure outside the cell. Although NETosis is a pathogenic mechanism shared by several autoimmune diseases, NET components and their effects on the immune system might diverge in different conditions. In RA, neutrophils from PB and SF display an increased NETosis that can be appreciated in basal conditions or after stimulation with serum antibodies or inflammatory cytokines. In RA, neutrophils exhibit increased spontaneous NETosis, associated with elevated reactive oxygen species (ROS) production, enhanced neutrophil elastase and myeloperoxidase (MPO) expression, nuclear translocation of protein arginine deiminase-4 (PAD4), PAD4-mediated citrullination of H3, and altered nuclear morphology. Among NET components, a range of cytoplasmic and extracellular citrullinated antigens, well-established targets of ACPA, have been described. These components might be the target for autoantibodies and immune complex formation and might act as inducers of further NETosis in RA as well as juvenile idiopathic arthritis (21).

Corsiero *et al*. provided novel evidence that B cells differentiated within synovial ectopic lymphoid structures (ELS) in the RA joints frequently target deiminated proteins, which could be generated during NETosis of RA synovial neutrophils, including histones.

They demonstrated that 40% of RA synovial B cells from ELS displayed reactivity against citrullinated histones, mainly H2A and H2B (citH2A/H2B). Therefore, anti-citH2A/H2B-reactive RA recombinant monoclonal antibodies-rmAbs, but not anti citH2A/H2B negative, selectively recognised NET from PB and/or RA joint neutrophils. Thus, NET could represent a source of citrullinated antigens, fueling the ACPA autoimmune response within the RA synovium (22).

In parallel to human studies, new insights into the pathogenic role of NET in RA come from research conducted in the CIA model. Papadaki *et al*. have demonstrated that NET are involved not only in the innate immunity, but also in the adaptive counterpart. In fact, they showed that NET are able to promote pathogenic IFN-γ-producing Th1 immune responses by modulating the activation and maturation of dendritic cells (DC). In particular, they provided evidence for a direct immunogenic ability of NET to increase the DC co-stimulatory molecules, CD80 and CD86 as well as the secretion of the pro-inflammatory cytokine IL6 (23). It is still unclear which mechanisms are involved in the NET recognition by DC, but a role of Toll-like receptor-9 (TLR9) in this process has been postulated. The inhibition of NETosis in the *in-vivo* model using Cl-amidine (a pan-PAD inhibitor), significantly diminished the severity of arthritis and delayed disease onset, strengthening the hypothesis of a strict connection between citrullination and NETosis in RA.

In parallel to NETosis, autophagy has been proposed as a cellular mechanism involved in the pathogenesis of RA (23). This is an endogenous process necessary for the turnover of organelles, the maintenance of cellular homeostasis and the regulation of cell fate. This process seems to contribute to the progression of various rheumatic diseases, including RA. During autophagy, parts of the cytoplasm and organelles are encapsulated in double-membraned vacuoles called autophagosomes, which finally fuse with lysosomes to degrade the incorporated material using acidic hydrolases. By using *in-vitro* systems, Sorice *et al*. demonstrated a role of autophagy in the generation of citrullinated peptides. Following treatment of FLS with tunicamycin or rapamycin, both inducers of authophagy, the activation of PAD4 occurs, with consequent protein citrullination and formation of vimentin, α-enolase and filaggrin as the main citrullinated proteins (25). This cellular mechanism seems to be altered also in T cells. Van Loosdregt *et al*. investigated whether autophagy is dysregulated in $CD4^+$ T cells of RA patients, resulting in disturbed T-cell homeostasis. They demonstrated that the rate of autophagy is significantly increased in $CD4^+$ T cells from RA patients, and that increased autophagy is also a feature of *in-vitro* activated $CD4^+$ T cells. The increased apoptosis resistance observed in $CD4^+$ T cells from RA patients was significantly reversed upon autophagy inhibition (26). Therefore, according to these recent findings, understanding in detail the mechanisms of autophagy in RA and its role in the development of the disease is relevant for designing more accurate and specific therapeutic approaches.

*Innate immune cells*

A tight interaction between resident cells and innate immune cells takes play during chronic inflammation of RA synovium. This leads to structural changes of the synovial tissue and

subsequent joint destruction. Besides neutrophils, myeloid cells seem to exert relevant roles in the induction and progression of inflammatory processes (27).

Stimulation of colony stimulating factor-1 receptor (CSF1R) by colony stimulating factor-1 (CSF1), also known as macrophage colony-stimulating factor (M-CSF), promotes survival, proliferation and differentiation of myeloid cells, including monocytes, macrophages and osteoclasts. CSF1 has an essential function in joint destruction, as it is required for osteoclastogenesis and TNF-induced osteolysis. Recently, IL34 was discovered as a novel ligand of CSF1R. CSF1 and IL34 share structural but not sequence homology, and have largely overlapping effects on CSF1R downstream signalling, regulation of monocyte survival, macrophage polarisation, and osteoclastogenesis. Emerging studies indicate that IL34 levels are increased both in serum and in SF of RA patients, and are strongly associated with rheumatoid factor (RF) and ACPA levels. The contribution of CSF1 and IL34 signalling to CSF1R in RA has been recently proposed. By blocking CSF1R using specific antibodies that prevent binding of both CSF1 and IL34, it is possible to reduce inflammation in humans and murine models of RA (28). Anti-CSF1R antibodies significantly down-regulate the production of IL6 and other inflammatory mediators in synovial explants of RA, and paw swelling and joint destruction in the CIA model. On the contrary, neutralisation of CSF1 or IL34 individually had no detectable effect on inflammatory gene expression in RA synovial tissue, supporting the hypothesis that these two cytokines were redundant in inducing differentiation and survival of tissue macrophage populations. These results introduce the hypothesis that interfering with the CSF1R binding to both CSF1 and IL34 is a possible novel therapeutic strategy for RA.

It is well known that the bone tissue balance is controlled by the "RANK/RANKL/OPG/OPGL system", tightly regulated by cytokines such as TNF-α IL1β, and IL6. These mediators seem to stimulate osteoclastogenesis through up regulation of receptor activator of nuclear factor kappa-B ligand (RANKL) and down regulation of osteoprotegerin (OPG) in osteoblasts.

Therefore, in RA, chronic inflammation leads to progressive joint destruction and bone resorption due to osteoclasts' hyperactivity. Activated FLS, together with activated T and B cells, monocytes and macrophages, trigger the differentiation of osteoclasts via RANKL/RANK activation. During the process of bone loss in RA, the pro-inflammatory cytokine IL15 seems to be involved, inducing osteoclastogenesis in synergy with RANKL. IL15 does not influence osteoclast differentiation of mouse macrophage cell line RAW264.7 (RAW) by itself, whereas following co-stimulation of these cells with both IL15 and RANKL, a significant increase of genes characterising osteoclast differentiation and osteoclastogenesis were observed (29).

Innate immunity is probably involved in osteoclast differentiation also through TLRs. It is well known that TLRs are expressed in the RA joints and an increased serum and SF expression of endogenous TLR4-ligands (*e.g.* Tenascin-C, S100A8/A9, citrullinated fibrinogen (cFb) immune complexes) has been observed in patients with RA. However, their role in the pathogenesis of this disease is not fully understood. It has been recently demonstrated that joint inflammation and bone erosion in the CIA model is in part due to activation of TLR4 by various TLR4 ligands arising at different stages of disease. Some endogenous TLR4 ligands might be temporally up-regulated, and their increase seems to be associated with clinical and histological manifestation of CIA. Among endogenous TLR4 ligands, Tenascin-C was found to be up-regulated in the circulation of early phase of CIA mice. Furthermore, it positively correlated with the clinical score at day 56. S100A8/A9 was increased starting from day 28, peaking at day 42, and positively correlated with joint inflammation. Anti-cFb antibodies were increased during the late phase of CIA and positively correlated with both joint inflammation and cartilage damage (30). In addition, it was possible to prevent clinical and histological signs of arthritis by blocking the TLR4 activation in the early phase of CIA, using a neutralising monoclonal antibody against TLR4. In parallel it has been shown that Tenascin-C and cFb immune complexes promote osteoclast differentiation *in-vitro*.

Taken together, these results demonstrate the direct involvement of TLR4 activation in the pathogenesis of CIA and in osteoclast differentiation, supporting the potential beneficial effect of targeting TLR4 in RA, especially in those patients with high levels of endogenous TLR4 ligands.

The innate immune cells are closely linked to resident cells and they play an active role in the development of RA. In the synovial tissue, RA FLS play a major role in invasive joint destruction. During chronic inflammation, they are stimulated to migrate from the affected synovium to the healthy tissue, contributing to the spread of arthritis and destruction of distant joints. They invade the extracellular matrix (ECM) and secrete matrix metalloproteinases (MMPs), contributing to the destruction of the cartilage and bone tissues and leading to exacerbation of the joint damage. Activation of RA FLS may be due to the direct effect of the cytokine environment (*e.g.* TNF-α and IL6), the cell-to-cell contacts, or the activation of TLR2, TLR4, and TLR3. Following activation, these cells exert a pro-inflammatory activity by synthesising cytokines, chemokines, prostanoids, and nitric oxide (NO). Multiple signal transduction pathways might be involved, such as protein tyrosine phosphorylation, resulting from the balanced action of protein tyrosine kinases (PTKs) and phosphatases (PTPs). Recently, the transmembrane PTP RPTPκ, encoded by the *PTPRK* gene, has been found to be over-expressed in RA FLS compared with those of OA. Since *PTPRK* is a TGFβ-target gene, its over-expression in RA FLS results from an increased production of TGFβ. Therefore, RPTPκ promotes RA FLS invasiveness by regulating phosphorylation of the inhibitory Y527 of Src, enhancing in this way responsiveness to platelet-derived growth factor (PDGF), TNF

and IL1 stimulation. In fact, RPTPκ-deficient RA FLS display dramatically reduced spreading, migration, invasiveness and chemokine production. Moreover, inhibition of RPTPκ in patients with RA carrying high expression of *PTPRK* in FLS could mitigate disease severity (31). It seems that the aggressive behaviour of RA FLS was largely dependent on their ability to form "invadosomes", actin-rich structures that concentrate MMPs to focal sites of ECM degradation. In the CIA model invadosome formation and cartilage degradation capability of synovial cells were shown to depend on an autocrine activation loop that involves TGFβ, which stimulate RA FLS to release pro-inflammatory cytokines and MMPs. This effect seems to be reinforced by activation of receptor tyrosine kinase (RTK) signalling. Charbonneau *et al*. demonstrated that the PDGF receptor (PDGFR) expressed in RA FLS, promotes invadosome formation and matrix degradation through activation of PI3K/AKT pathway. PDGFR activation by phosphorylation is specifically increased in RA FLS and synovial tissues, and involves the upregulation of PDGF-B isoform induced by TGFβ. Indeed, among the PDGF isoforms, only the PDGF-B was significantly elevated in RA FLS and was related to high-invadosome forming cells by activation of PI2K/AKT pathway (32).

Together with FLS and osteoclasts, mast cells play an important role in the inflammatory processes of RA. They are tissue dwelling cells with a relevant role in inflammation, fibrosis and angiogenesis. They role in the pathogenesis of RA has been widely investigated, but some aspects of their pathological behaviour in the development of the disease are still unclear. Recent data suggest that mast cells are involved in preclinical phase of arthritis, at least in the CIA model. In this animal model, mast cells seem to affect the adaptive immune response during the development of arthritis, by inducing a more powerful anti-inflammatory T cell response. Van der Velden *et al*., indeed, by studying the role of these cells during different phases of CIA in inducible mast cell knockout mice, have shown that depletion of mast cells in established arthritis did not affect clinical outcome, whereas a depletion induced during the preclinical phase resulted in a significant improvement of arthritis. This coincided with a decrease in circulating $CD4^+$T cells and inflammatory monocytes, as well as of circulating levels of IL6 and IL17, but not of collagen-specific antibody. Furthermore, splenocytes from mast cell-depleted mice, stimulated with collagen type II, displayed reduction of IL17 and increased of IL10 production (33). Taken together, these results confirm that a close interaction of structural cells with innate immune cells takes place in RA. Therefore, targeting some signal transduction pathways in these cells might ameliorate some aspects of the disease.

## Adaptive immune response

### *T lymphocytes*

Besides the innate immune system, the adaptive counterpart plays a critical role in autoimmune diseases. Dysregulation of the adaptive immune response seems to be responsible for the development of RA. Up to now, an imbalance of effector and regulatory lymphocytes, leading to aberrant autoimmune response, is a hallmark of RA pathogenesis. As far as Th lymphocytes are concerned, recent data highlighted another aspect of the interaction between this T lymphocyte subset and cells of the innate immune system. Reynolds *et al*. described how monocytes from healthy donors can differentiate into a recently described subtype of DC, named inflammatory (INF) DC, following stimulation by granulocyte-monocyte colony stimulating factor (GM-CSF), mostly produced by SF $CD4^+$T cells and further increased after stimulation with IL12 (34). Besides $CD4^+$Th1 cells, several other T cell populations are active players in the pathogenesis of RA. The role of Th17 cells in the induction and maintenance of the inflammatory process is well established. However, the mechanisms leading to their aberrant expansion and activation are still not entirely clarified. IL23, a key cytokine for Th17 cell commitment, is increased in the circulation of RA patients (35). In addition, the recently discovered glycoprotein VSTM1-v2, over-expressed in RA PBMCs, is able to promote differentiation and activation of Th17 cells and is positively correlated with IL17 levels, disease activity, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in RA (36). Moreover, retinoic acid-related orphan receptor (ROR) γt expression in T cells, and therefore Th17 commitment, can be induced by a recently identified circulating bone-marrow-derived cell subset, named fibrocytes. These cells are precursors of FLS, are able to produce IL6, a key cytokine in the process of Th17 cell differentiation, and might be activated by Th17 cells. Of interest, fibrocyte proportion is higher in the PB of RA patients compared to the healthy subjects, suggesting an active role of these cells in RA pathogenesis (37). In addition to IL17, IL21 and IL22, through a complex network of intracellular signals, Th17 cells also produce RORγt, signal transducer and activator of STAT3 and IRF4. The protein inhibitor of activated STAT3 (PIAS3), a physiological inhibitor of STAT3, is down-regulated in RA PBMC compared to healthy subjects. Interestingly, miR-301a-3p, a PIAS3 regulating microRNA, is highly expressed in RA PBMC, accounting at least in part, for the decreased amount of PIAS3 and subsequent activation of Th17 cells (38). IL17 secretion can also be enhanced by a recently described Th cell subset, named Th9, through the secretion of IL9 (39), and stimulates T cell proliferation via the phosphatidylinositole 3-kinase (PI3K) pathway. IL17 can also hamper the chondrogenic potential of mesenchymal stem cells in the cartilage of RA, restored by blocking IL17 and TNF-α (40).

An interesting and peculiar feature of Th17 cells is their plasticity towards different T cell phenotypes such as Th1 or T regulatory (Treg) cells. Indeed, the reduction of circulating Th17 cells observed in established RA may be the result of a shift of Th17 to either Th1 (41) or Treg cells. The latter hypothesis is supported by the observation of higher proportions of $ROR\gamma t^+FoxP3^+$ (forkhead box P3) double positive (DP)

T cells in RA PB. This observation further complicates the understanding of the role of Treg cells in RA pathogenesis. Recent findings demonstrated an elevation of the levels of the Treg stabilising protein progranulin, correlating with disease activity and inflammatory markers (42). However, the majority of studies performed in the recent year agree that Treg cells maintain a suppressive activity in RA patients (43, 44), suggesting a pivotal role of these cells in the regulation of the adaptive immunity during RA.

Another T cell subset involved in RA pathogenesis is represented by Thf, which are physiologically found in secondary lymphoid structures and germinal centres. Thf cells are characterised by surface expression of the CXC-chemokine receptor-5 (CXCR5) and are able to produce its ligand CXCL13, a chemokine that orchestrates ectopic lymphoid neogenesis. This chemokine can also be produced by a $CD4^+$ cell subset, lacking its surface receptor CXCR5, that arises from naïve T-cells following TGFβ stimulation (45).

Besides $CD4^+$T lymphocytes, other T cell subsets, such as $CD8^+$ and $CD4^-$ $CD8^-$ cells were found to be involved in RA pathogenesis. A higher proportion of $CD8^+$ cells expressing the leukocyte immunoglobulin (Ig)-like receptor (LIR)-1, which is responsible for maintaining cytomegalovirus (CMV) infection in a latent state, has been detected in $CMV^+$ RA patients compared to $CMV^+$ subjects without RA, and its proportion correlated with the disease activity (46). Finally, $CD4^-CD8^-$ γδT cells, known for their potential tolerogenic effect in pregnancy, have been linked to the frequently occurring spontaneous disease remission in pregnant women. This arises from the observation that in RA woman the circulating TNF-$\alpha^+$ and IFN$\gamma^+$ γδT cells are down-regulated in pregnant compared to non-pregnant woman (47).

*B Lymphocytes and autoantibodies*

Moving to B lymphocytes, their peculiar pathogenic role in RA is to produce autoantibodies, mainly targeting citrullinated self proteins. The large amount of peptides produced by plasma cells leads to accumulation of misfolded proteins in rough endoplasmic reticulum (RER). One of the agents responsible for eliminating these proteins, growth arrest and DNA damage-inducible gene (GADD)34, is up-regulated in circulating B cells from RA patients. GADD34 correlates with circulating ACPA (48), which display glycosylated aminoacid residues in their variable domain and influence their binding to citrullinated antigens. Such post-translational modification, likely resulting from somatic hypermutation, represents an advantage that allows specific B cell subsets to be selected (49). ACPA can occur several years before RA onset and the site where PAD-mediated protein citrullination stimulate B cells to initiate ACPA production is still unknown. It has been recently demonstrated that RA FLS can produce four different isotypes of PAD (50). Among them, PAD2 is increased in cell-free SF and correlates with serum anti-CCP and disease activity (51).

By LC-MALDI-TOF, Wang *et al*. identified 83 over-citrullinated autoantigens in the SF from RA patients, but not in the control group. In addition, they demonstrated that the sites of citrullinated PADI2 and PADI4 were different in RA compared to the control group (52).

ACPA also represent a bridge between genetic background, environmental factors and RA development. Gan *et al*. found a significant association of omega-3 fatty acid dietary intake with anti-CCP positivity in healthy subjects at increased risk of developing RA (FDRs) (53). In a similar way, Glant *et al*. characterised the structure and the localisation of citrullinated proteoglycan aggrecan (CitPG), the most abundant macromolecule of the articular cartilage (54). This antigen is found in both OA and RA cartilage, although its localisation is different in patients with RA compared to those with OA. A further work by Joshua *et al*. (55) better characterised the anti-citrullinated fibrinogen (cit-Fib) antibodies. They demonstrated that several cit-Fib peptides are targeted by autoantibodies in RA, but not in psoriatic arthritis (PsA) and ankylosing spondylitis (AS), implicating that these are more specific for RA. An association between cit-Fib autoantibodies and the *R620W PTPN22* risk allele supports the hypothesis of an altered B cell regulation, such as autoreactive B cells, which evade tolerance.

Sialylation has been proposed as a feature of RA-associated IgG in humans and in mouse models of arthritis (56). In the CIA model, genetical blockade of sialylation in activated B cells leads to exacerbation of joint inflammation. On the other hand, artificial sialylation of anti-type II collagen antibodies, including ACPA, not only attenuates arthritogenic activity, but also suppresses the development of CIA in the antibody-infused mice. On the contrary, sialylation of other IgG does not prevent features of the disease in this animal model.

Recently, some novel mechanisms involved in the citrullination of ACPA have been described. For example, the ability of periodontal disease bacterium *Porphyromonas gingivalis* to induce citrullination via PAD production is thought to be relevant in the pathogenesis of RA. In support of these observations, an association between anti-RgpB IgG, antibodies directed towards a virulence factor of the pathogen, and the development of RA, has been reported (57), as well as the link between *Porphyromonas gingivalis*-derived ACPA and RA activity (58).

The contribution of molecular mimicry between microbial antigens and autoantigens in RA is likely not limited to *Porphyromonas gingivalis*. HSP70-family binding Ig protein (BiP) is a candidate autoantigen in RA and share a substantial sequence with mycobacterial HSP70 (MycHSP70), mostly evident in the HLA-DR4 shared epitope binding sequences. MycHSP70 showed immunogenicity in RA in both humans and animal models (59).

Some studies explored other promising RA-associated subsets of autoantibodies directed against carbamylated residues (anti-CarP), which recognise the homocitrullination of lysines (60). However, the precise link between these new antibodies and ACPA is not yet fully understood. The introduction of anti-CarP antibodies allowed the researchers to identify new biomarkers, pathognomonic for the disease or re-

lated to clinical aspects of arthritis. The identification of an organ-associated antigen or a pathogen-derived epitope could elucidate the aetiology of the disease. A cross reactivity between the two groups of antibodies has been proposed. Turunen *et al*. showed that it is possible to induce a class of ureido-specific antibodies direct to both citrullinated and homocitrullinated antigens in rabbits immunised with type II collagen-related peptides, even in peptide sequences different from those used for the original immunisation (61).

Decades after the discovery of RF, its specific function in the pathogenesis of RA as well as its interplay with ACPA is still a matter of debate. An increased risk of RA development in healthy subjects with positivity of any of the three RF isotypes (IgM, IgG or IgA) in combination with any ACPA has been shown (62). However, the role of these antibodies in the mechanisms involved in RA pathogenesis is likely distinct, and this is supported by their different behaviour following pharmacological treatment. The recent study of Iannone *et al*. showed that anti-CCP levels may change during RA course, regardless of the biological drug used and the clinical response to the therapy (63).

Although B cells are considered part of the adaptive immune system, the B1 subtype does not show memory features and produces poly-reactive immunoglobulines, called natural antibodies (nIg) that are mainly of M class. They exert various physiological functions, but also show pro- and anti-inflammatory features. Interestingly, an increased amount of anti-galactosyl carbohydrate epitope (anti-Gal) nIgM (64) and a reduction in protective nIgM secretion induced by hypoxia-inducible factor (HIF)-1α were reported in RA patients (65). Finally, IgDs from RA patients seem to vigorously enhance proliferation of PBMC, activation of T and B cells and secretion of pro-inflammatory cytokines (66).

Besides the production of autoantibodies, more attention has been given to other functions of RA-derived B cells. An increase of B-cell lymphoma (Bcl)-2 protein expression seems to contribute to the reduction of B cells apoptosis, thus contributing to their expansion and pathogenic activity (67). An interesting *in-vitro* study revealed that B lymphocytes from healthy subjects can interact with OA FLS inducing the release of IL6, IL8 and MMP3, thereby inducing a RA-like destructive phenotype. On the other hand, FLS can hamper B cells, reducing their proliferation, secretion of immunoglobulins and production of pro-inflammatory cytokines (68).

B cells can also express RANKL, which is enhanced in RA following B cell receptor (BCR) binding and IFNγ stimulation. Furthermore, co-cultures of B cells and monocytes showed a RANKL-dependent increase of osteoclasto-genesis. These findings shed further light on the effect of B cells on bone damage (69, 70). This effect seems to be also exerted indirectly through secretion of different immunoglobulins. Lu *et al*. showed a positive correlation between serum antibody titre directed towards citrullinated-heat shock protein 60 (citHSP60) and bone damage scores. Anti-citHSP60 antibodies may be able to induce apoptosis in human osteoblast-generated sarcoma cell line (Saos-2) cells, after binding their citrullinated antigen expressed on Saos-2 cell membrane. These results specifically implicate the active role of ACPA and osteoblast surface-expressed citHSP60 in the pathogenesis of RA (71).

*Cytokines and chemokines*

An array of soluble mediators contribute at different levels to the regulation of both innate and adaptive immune responses during RA. Among them, TNF-α is the main cytokine involved in the inflammatory response of RA, whereas cartilage and bone destruction is mostly directed by IL1. In addition to TNF-α and IL1, particular attention has been directed to IL6. This cytokine, produced by a variety of cells, mainly activated macrophages and FLS, has both pro- and anti-inflammatory properties. High levels of IL6 have been detected both in the circulation and SF of RA patients and a correlation of its levels with radiological joint destruction has been reported. Indeed, IL6 is probably involved in joint destruction by acting on neutrophils and on pre-osteoclasts in which promotes differentiation by either a RANKL-dependent or RANKL-independent mechanism. IL6 also acts in synergism with IL1β and TNF-α in producing vascular endothelial growth factor (VEGF), the main pro-angiogenic mediator, contributing to the maintenance of the synovitis. IL6 not only plays a pivotal role in regulating innate immunity, but it is also essential in modulating the adaptive immune response. This cytokine induces Th17 cell generation from naive T cells, by acting in synergism with TGFβ and inhibiting TGFβ-induced Treg (iTreg) differentiation. In normal conditions, Th17 and Treg cells remain in a dynamic balance just like the "Ying and Yang". Recently, it has been shown that the "Yin Yang" transcription factor (YY1) may regulate the balance of Th17 cell and Treg cell via IL6 signalling pathway (72). This transcription factor, related to cancer development and progression, either activates or represses gene transcription, depending on the stimuli. An over-expression of YY1 in RA was found both in humans and in the animal model of CIA (DBA/1J mice). Its activity is mainly due to the binding to the promoter region of the IL6 gene, whereas IL6 and JAK/Stat signalling pathways were significantly inhibited by YY1 shRNA lentivirus (LV-YY1-shRNA) treatment. The blocking of the IL6 activity with LV-YY1-shRNA, down-regulates Th17 cells and ameliorates inflammation and disease progression in CIA mice as a consequence of the suppression of IL6 signalling pathway (73).

Among the family of IL6/IL12 (IL2 superfamily), IL27 has been recently proposed as another mediator involved in RA pathogenesis. This cytokine is secreted by macrophages, epithelial cells and DC through IFNγ, and its receptor is present on B cells, $CD4^+$T cells, NK cells, mast cells and monocytes. IL27 might induce monocytes to release IL1β, TNF-α, IL18 and IL12 and it might contribute to the differentiation of Th1 in naive $CD4^+$T cells, which can secrete IL10 and IL17. Therefore, modulating the expression of IL10 and IL17, IL27 can promote Treg cell differentiation and exert broad inhibitory

effects on Th1, Th2 and Th17 cells. In addition, IL27 inhibits the formation of mature osteoclasts from pre-osteoclasts, by suppressing the expression of calcineurin-dependent 1 NFAT, which in turn down-regulates RANK signalling. Recently, an association of IL27 with disease activity and response to therapy has been hypothesised (74). In fact, an increase of IL27 levels in the circulation of RA patients positively correlates with the disease activity and seems to decrease following immunosuppressant treatment. Therefore, this cytokine could be a potential biomarker of disease activity and response to therapy in RA.

Among the IL1 family, IL33 plays a dual role both as an alarmin and a stimulus for Th2 response, exerting both positive and negative regulation of inflammatory processes, depending on the tissue environment. Increased levels of IL33 have been detected in serum, synovial tissue and cultured RA FLS, however its role in the pathogenesis of the disease is not fully elucidated. For example, exogenous IL33 could inhibit arthritis in CIA model, and mice deficient in the IL33 receptor ST2 show reduced susceptibility to arthritis, whereas the disease is not modified in IL33-deficient mice. Recent data have shown that the absence of IL33 does not affect the shift of T cells toward Th1, Th17, or Treg subpopulations (75). By evaluating two mouse models of chronic inflammatory diseases, CIA and imiquimod (IMQ)-induced psoriasis, Athari *et al*. showed that both arthritis and psoriasis developed independently of IL33 expression and does not affect the frequencies of Treg, Th1, and Th17 cells in both animal models.

Among IL12 family cytokines, the anti-inflammatory cytokine IL35 is able to attenuate arthritis in the CIA mice and this seems to be due to its stimulatory effect on Treg and suppressing effect on Th17 cells. Due to its role in tumour angiogenesis, some researchers speculated that IL35 might also play a role in the angiogenic processes during RA. In both *in-vitro* and *ex-vivo* models, IL-35 might reduce synovial neovascularisation and inflammation by interfering on VEGF/Ang2 crosstalk. Its inhibitory effect on Ang2 expression and disruption of Ang2/Tie2 signal transduction seems to be responsible for down-regulation of angiogenesis and inflammation in the synovial tissue (76).

Finally, a number of other soluble mediators such as soluble adhesion molecules have been recently identified as potential modulators of RA. The soluble form of integrin CD18 (sCD18), secreted by leukocytes, is reduced in RA and is capable of competing with its membrane counterpart for the binding to ICAM-1 (77).

Interestingly, Boutet *et al*. investigated in RA the different isoforms of IL36 (IL-36α, IL-36β and IL-36γ), pro-inflammatory cytokines known to be involved in the pathogenesis of psoriasis. In CIA mice model and in the synovium of patients with RA, IL-36α, β, γ and their antagonists (IL-36Ra and IL-38) were all increased and correlated with the levels of IL1β, CCL3, CCL4 and M-CSF, but not with Th17 cytokines. However, they found differences in the ratio of these cytokines and their antagonists among patients with RA and other chronic conditions such as Crohn's disease and psoriasis, and in some subgroups of RA patients. The rationale of these distinct expression profiles is currently debated, and further studies are required (78).

## Conclusion

In the pathogenesis of RA several processes are involved. Dysregulation of the innate and adaptive immune responses occur at different stages of the disease. Innate and adaptive immune cells, soluble mediators, adhesion molecules and autoantibodies contribute to the development of inflammation and structural changes of joints and internal organs. The understanding of the mechanisms underlying the pathogenesis of RA will enable the development of novel and more specific disease-modifying therapies.

## References


1. BELLUCCI E, TERENZI R, LA PAGLIA GM *et al*.: One year in review 2016: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol* 2016; 34: 793-801.
2. PICERNO V, FERRO F, ADINOLFI A *et al*.: One year in review: the pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol* 2015; 33: 551-8.
3. SUZUKI A, YAMAMOTO K: From genetics to functional insights into rheumatoid arthritis. *Clin Exp Rheumatol* 2015; 33 (Suppl. 92): S40-3.
4. KONDA MOHAN V, GANESAN N, GOPALAKRISHNAN R *et al*.: HLA-DRB1 shared epitope alleles in patients with rheumatoid arthritis: relation to autoantibodies and disease severity in a south Indian population. *Int J Rheum Dis* 2016 [Epub ahead of print].
5. JIANG X, ASKLING J, SAEVARSDOTTIR S *et al*.: A genetic risk score composed of rheumatoid arthritis risk alleles, HLA-DRB1 haplotypes, and response to TNFi therapy - results from a Swedish cohort study. *Arthritis Res Ther* 2016; 18: 288.
6. GERSTNER C, DUBNOVITSKY A, SANDIN C *et al*.: Functional and structural characterization of a novel HLA-DRB1*04:01-restricted α-enolase T cell epitope in rheumatoid arthritis. *Front Immunol* 2016; 7: 494.
7. STUHLMÜLLER B, MANS K, TANDON N *et al*.: Genomic stratification by expression of HLA-DRB4 alleles identifies differential innate and adaptive immune transcriptional patterns - A strategy to detect predictors of methotrexate response in early rheumatoid arthritis. *Clin Immunol* 2016; 171: 50-61.
8. TAKAHASHI A, KUROKI K, OKABE Y *et al*.: The immunosuppressive effect of domain-deleted dimer of HLA-G2 isoform in collagen-induced arthritis mice. *Hum Immunol* 2016; 77: 754-9.
9. CICCACCI C, CONIGLIARO P, PERRICONE C *et al*.: Polymorphisms in STAT-4, IL-10, PSORS1C1, PTPN2 and MIR146A genes are associated differently with prognostic factors in Italian patients affected by rheumatoid arthritis. *Clin Exp Immunol* 2016; 186: 157-163.
10. LEE YH, BAE SC: Intercellular adhesion molecule-1 polymorphisms, K469E and G261R and susceptibility to vasculitis and rheumatoid arthritis: a meta-analysis. *Cell Mol Biol* (Noisy-le-grand) 2016; 62: 84-90.
11. LI G, CUNIN P, WU D *et al*.: The rheumatoid arthritis risk variant CCR6DNP regulates CCR6 via PARP-1. *PLoS Genet* 2016; 12: e1006292.
12. MAESHIMA K, STANFORD SM, HAMMAKER D *et al*.: Abnormal PTPN11 enhancer methylation promotes rheumatoid arthritis fibroblast-like synoviocyte aggressiveness and joint inflammation. *JCI Insight* 2016;1: pii: e86580.
13. DAMGAARD D, FRIBERG BRUUN NIELSEN M, QUISGAARD GAUNSBAEK M *et al*.: Smoking is associated with increased levels of extracellular peptidylarginine deiminase 2 (PAD2) in the lungs. *Clin Exp Rheumatol* 2015; 33: 405-8.
14. SPARKS JA, CHANG SC, DEANE KD *et al*.: Associations of smoking and age with inflammatory joint signs among unaffected first-degree relatives of rheumatoid arthritis patients: results from studies of the etiology of rheumatoid arthritis. *Arthritis Rheumatol* 2016; 68: 1828-38.
15. 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) 2016; 55: 149-55.
16. WENG CH, GUPTA S, GERAGHTY P *et al*.: Cigarette smoke inhibits ROCK2 activation in T cells and modulates IL-22 production. *Mol Immunol* 2016; 71: 115-22.
17. CHEN J, WRIGHT K, DAVIS JM *et al*.: An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. *Genome Med* 2016; 8: 43.
18. MAEDA Y, KURAKAWA T, UMEMOTO E *et al*.: Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the Intestine. *Arthritis Rheumatol* 2016; 68: 2646-61.
19. BLOCK KE, ZHENG Z, DENT AL *et al*.: Gut microbiota regulates K/BxN autoimmune arthritis through follicular helper T but not Th17 cells. *J Immunol* 2016; 196: 1550-7.
20. PIANTA A, ARVIKAR S, STRLE K *et al*.: Evidence for immune relevance of prevotella copri, a gut microbe, in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2016 Nov 18. [Epub ahead of print].
21. GIAGLIS S, HAHN S, HASLER P: "The NET outcome": are neutrophil extracellular traps of any relevance to the pathophysiology of autoimmune disorders in childhood? *Front Pediatr* 2016; 4: 97.
22. 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 ofNETs. *Ann Rheum Dis* 2016; 75: 1866-75.
23. PAPADAKI G, KAMBAS K, CHOULAKI C *et al*.: Neutrophil extracellular traps exacerbate Th1-mediated autoimmune responses in rheumatoid arthritis bypromoting DC maturation. *Eur J Immunol* 2016; 46: 2542-54.
24. ZHONG Z, SANCHEZ-LOPEZ E, KARIN M: Autophagy, NLRP3 inflammasome and auto-inflammatory/immune diseases. *Clin Exp Rheumatol* 2016; 34: 12-6.
25. SORICE M, IANNUCCELLI C, MANGANELLI V *et al*.: Autophagy generates citrullinated peptides in human synoviocytes: a possible trigger for anti-citrullinated peptide antibodies. *Rheumatology* (Oxford) 2016; 55: 1374-85.
26. VAN LOOSDREGT J, ROSSETTI M, SPREAFICO R *et al*.: Increased autophagy in CD4$^+$ T cells of rheumatoid arthritis patients results in T-cell hyperactivation and apoptosis resistance. *Eur J Immunol* 2016; 46: 2862-70.
27. AVCI AB, FEIST E, BURMESTER GR: Targeting GM-CSF in rheumatoid arthritis. *Clin Exp Rheumatol* 2016; 34: 39-44.
28. GARCIA S, HARTKAMP LM, MALVAR-FERNANDEZ B *et al*.: Colony-stimulating factor (CSF) 1 receptor blockade reduces inflammation in human and murine models of rheumatoid arthritis. *Arthritis Res Ther* 2016; 18: 75.
29. OKABE I, KIKUCHI T, MOGI M *et al*.: IL-15 and RANKL play a synergistically important role in osteoclastogenesis. *J Cell Biochem* 2017; 118: 739-47.
30. KIYEKO GW, HATTERER E, HERREN S *et al*.: Spatiotemporal expression of endogenous TLR4 ligands leads to inflammation and bone erosion in mouse collagen-induced arthritis. *Eur J Immunol* 2016; 46: 2629-38.
31. STANFORD SM, ALEMAN MUENCH GR, BARTOK B *et al*.: TGFβ responsive tyrosine phosphatase promotes rheumatoid synovial fibroblast invasiveness. *Ann Rheum Dis* 2016; 75: 295-302.
32. CHARBONNEAU M, LAVOIE RR, LAUZIER A *et al*.: Platelet-derived growth factor receptor activation promotes the prodestructive invadosome-forming phenotype of synoviocytes from patients with rheumatoid arthritis. *J Immunol* 2016; 196: 3264-75.
33. VAN DER VELDEN D, LAGRAAUW HM, WEZEL L *et al*.: Mast cell depletion in the preclinical phase of collagen-induced arthritis reduces clinical outcome by lowering the inflammatory cytokine profile. *Arthritis Res Ther* 2016; 18: 138.
34. REYNOLDS G, GIBBON JR, PRATT AG *et al*.: Synovial CD4$^+$ T-cell-derived GM-CSF supports the differentiation of an inflammatory dendritic cell population in rheumatoid arthritis. *Ann Rheum Dis* 2016; 75: 899-907.
35. ZAKY DS, EL-NAHRERY EM: Role of interleukin-23 as a biomarker in rheumatoid arthritis patients and its correlation with disease activity. *Int Immunopharmacol* 2016; 31: 105-8.
36. WANG D, LI Y, LIU Y *et al*.: Expression of VSTM1-v2 is increased in peripheral blood mononuclear cells from patients with rheumatoid arthritis and is correlated with disease activity. *PLoS One* 2016; 11: e0146805.
37. GALLIGAN CL, KEYSTONE EC, FISH EN: Fibrocyte and T cell interactions promote disease pathogenesis in rheumatoid arthritis. *J Autoimmun* 2016; 69: 38-50.
38. TANG X, YIN K, ZHU H *et al*.: Correlation between the expression of microRNA-301a-3p and the proportion of Th17 cells in patients with rheumatoid arthritis. *Inflammation* 2016; 39: 759-67.
39. KUNDU-RAYCHAUDHURI S, ABRIA C, RAYCHAUDHURI SP: IL-9, a local growth factor for synovial T cells in inflammatory arthritis. *Cytokine* 2016; 79: 45-51.
40. SCHMINKE B, TRAUTMANN S, MAI B *et al*.: Interleukin 17 inhibits progenitor cells in rheumatoid arthritis cartilage. *Eur J Immunol* 2016; 46: 440-5.
41. KOTAKE S, NANKE Y, YAGO T *et al*.: Elevated ratio of Th17 cell-derived Th1 cells (CD161(+)Th1 cells) to CD161(+)Th17 cells in peripheral blood of early-onset rheumatoid arthritis patients. *Biomed Res Int* 2016; 2016: 418602.
42. CHEN J, LI S, SHI J *et al*.: Serum progranulin irrelated with Breg cell levels, but elevated in RA patients, reflecting high disease activity. *Rheumatol Int* 2016; 36: 359-64.
43. WALTER GJ, FLESKENS V, FREDERIKSEN KS *et al*.: Phenotypic, functional, and gene expression profiling of peripheral CD45RA$^+$ and CD45RO$^+$ CD4$^+$CD25$^+$CD127(low) treg cells in patients with chronic rheumatoid arthritis. *Arthritis Rheumatol* 2016; 68: 103-16.
44. ZARAGOZA B, CHEN X, OPPENHEIM JJ *et al*.: Suppressive activity of human regulatory T cells is maintained in the presence of TNF. *Nat Med* 2016; 22: 16-7.
45. KOBAYASHI S, WATANABE T, SUZUKI R *et al*.: TGF-β induces the differentiation of human CXCL13-producing CD4(+) T cells. *Eur J Immunol* 2016; 46: 360-71
46. ROTHE K, QUANDT D, SCHUBERT K *et al*.: Latent cytomegalovirus infection in rheumatoid arthritis and increased frequencies of cytolytic lir-1+cd8+ t cells. *Arthritis Rheumatol* 2016; 68: 337-46.
47. THAM M, SCHLÖR GR, YERLY D *et al*.: Reduced pro-inflammatory profile of γδT cells in pregnant patients with rheumatoid arthritis. *Arthritis Res Ther* 2016; 18: 26.
48. CLAVARINO G, ADRIOUACH S, QUESADA JL *et al*.: Unfolded protein response gene GADD34 is overexpressed in rheumatoid arthritis and related to the presence of circulating anti-citrullinated protein antibodies. *Autoimmunity* 2016; 49: 172-8.
49. ROMBOUTS Y, WILLEMZE A, VAN BEERS JJ *et al*.: Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. *Ann Rheum Dis* 2016; 75: 578-85.
50. OLIVARES-MARTÍNEZ E, HERNÁNDEZ-RAMÍREZ DF, NÚÑEZ-ÁLVAREZ CA *et al*.: The amount of citrullinated proteins in synovial tissue is related to serum anti-cyclic citrullinated peptide (anti-CCP) antibody levels. *Clin Rheumatol* 2016; 35: 55-61.
51. DAMGAARD D, SENOLT L, NIELSEN CH: Increased levels of peptidylarginine deiminase 2 in synovial fluid from anti-CCP-positive rheumatoid arthritis patients: Association with disease activity and inflammatory markers. *Rheumatology* (Oxford) 2016; 55: 918-27.
52. WANG, F. CHEN FF, GAO WB *et al*.: Identification of citrullinated peptides in the synovial fluid of patients with rheumatoid arthritis using LC-MALDI-TOF/TOF. *Clin Rheumatol* 2016; 35: 2185-94.
53. GAN RW, YOUNG KA, ZERBE GO *et al*.: Lower omega-3 fatty acids are associated with the presence of anti-cyclic citrullinated peptide autoantibodies in a population at risk for future rheumatoid arthritis: a nested case-control study, *Rheumatology* (Oxford) 2016; 55: 367-76.
54. GLANT TT, OCSKO T, MARKOVICS A *et al*.: Characterization and localization of citrullinated proteoglycan aggrecan in human articular cartilage. *PLoS One* 2016; 11: e0150784.
55. JOSHUA, V. SCHOBERS L, TITCOMBE PJ *et al*.: Antibody responses to de novo identified citrullinated fibrinogen peptides in rheumatoid arthritis and visualization of the corresponding B cells. *Arthritis Res Ther* 2016; 18: 284.
56. OHMI Y, ISE W, HARAZONO A *et al*.: Sialylation converts arthritogenic IgG into inhibitors of collagen-induced arthritis. *Nat Commun* 2016; 7: 11205.
57. KHARLAMOVA N, JIANG X, SHERINA N *et al*.: Antibodies to porphyromonas gingivalis indicate interaction between oral infection, smoking, and risk genes in rheumatoid arthritis etiology. *Arthritis Rheum* 2016; 68: 604-13.
58. LI S, YU Y, YUE Y: Autoantibodies from single circulating plasmablasts react with citrullinated antigens and porphyromonas gingivalis in rheumatoid arthritis. *Arthritis Rheumatol* 2016; 68: 614-26.

59. SHODA H, HANATA N, SUMITOMO S *et al*.: Immune responses to Mycobacterial heat shock protein 70 accompany self-reactivity to human BiP in rheumatoid arthritis. *Sci Rep* 2016; 6: 22486.
60. ALESSANDRI C, BARTOSIEWICZ I, PENDOLINO M *et al*.: Anti-carbamylated protein antibodies in unaffected first-degree relatives of rheumatoid arthritis patients: lack of correlation with anti-cyclic citrullinated protein antibodies and rheumatoid factor. *Clin Exp Rheumatol* 2015; 33: 824-30.
61. TURUNEN S, KOIVULA MK, RISTELI L *et al*.: Ureido group-specific antibodies are induced in rabbits immunized with citrulline- or homocitrulline-containing antigens. *Autoimmunity* 2016; 49: 459-65.
62. BRINK M, HANSSON M, MATHSSON-ALM L *et al*.: Rheumatoid factor isotypes in relation to antibodies against citrullinated peptides and carbamylated proteins before the onset of rheumatoid arthritis. *Arthritis Res Ther* 2016; 18: 43.
63. IANNONE F, TAMPOIA M, GIANNINI M *et al*.: Changes in anti-cyclic citrullinated peptide antibodies and rheumatoid factor isotypes serum levels in patients with rheumatoid arthritis following treatment with different biological drugs. *Clin Exp Rheumatol* 2016; 34: 424-9.
64. NGUYEN TG, MCKELVEY KJ, MARCH LM *et al*.: Aberrant levels of natural IgM antibodies in osteoarthritis and rheumatoid arthritis patients in comparison to healthy controls. *Immunol Lett* 2016; 170: 27-36.
65. HU F, LIU H, XU L *et al*.: Hypoxia-inducible factor-1α perpetuates synovial fibroblast interactions with T cells and B cells in rheumatoid arthritis. *Eur J Immunol* 2016; 46: 742-51.
66. WU Y, CHEN W, CHEN H *et al*.: The elevated secreted immunoglobulin D enhanced the activation of peripheral blood mononuclear cells in rheumatoid arthritis. *PLoS One* 2016; 11: e0147788.
67. YANG J, ZHAO S, YANG X *et al*.: Inhibition of B-cell apoptosis is mediated through increased expression of Bcl-2 in patients with rheumatoid arthritis. *Int J Rheum Dis* 2016; 19: 134-40.
68. STÖRCH H, ZIMMERMANN B, RESCH B *et al*.: Activated human B cells induce inflammatory fibroblasts with cartilage-destructive properties and become functionally suppressed in return. *Ann Rheum Dis*. 2016; 75: 924-32.
69. 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 Rheumatol* 2016; 68: 805-16.
70. OTA Y, NIIRO H, OTA S *et al*.: Generation mechanism of RANKL(+) effector memory B cells: relevance to the pathogenesis of rheumatoid arthritis. *Arthritis Res Ther* 2016; 18: 67.
71. LU MC, YU CL, YU HC *et al*.: Anti-citrullinated protein antibodies promote apoptosis of mature human Saos-2 osteoblasts via cell-surface binding to citrullinated heat shock protein 60. *Immunobiology* 2016; 221: 76-83.
72. LIN J, HE Y, CHEN J *et al*.: Datasets of YY1 expression in rheumatoid arthritis patients. *Data Brief* 2016; 9: 1034-38.
73. LIN J, HE Y, CHEN J: A critical role of transcription factor YY1 in rheumatoid arthritis by regulation of interleukin-6. *J Autoimmun* 2016 Epub 2016 Nov 6.
74. LAI X, WANG H, CAO J *et al*.: Circulating IL-27 is elevated in rheumatoid arthritis patients. *Molecules* 2016; 21(11). pii: E1565.
75. ATHARI SK, POIRIER E, BITON J *et al*.: Collagen-induced arthritis and imiquimod-induced psoriasis develop independently of interleukin-33. *Arthritis Res Ther* 2016; 18: 143.
76. JIANG S, LI Y, LIN T *et al*.: IL-35 inhibits angiogenesis through VEGF/Ang2/Tie2 pathway in rheumatoid arthritis. *Cell Physiol Biochem* 2016; 40: 1105-16.
77. KRAGSTRUP TW, JALILIAN B, KELLER KK *et al*.: Changes in soluble CD18 in murine autoimmune arthritis and rheumatoid arthritis reflect disease establishment and treatment response. *PLoS One* 016; 11: e0148486.
78. BOUTET MA, BART G, PENHOAT M *et al*.: Distinct expression of interleukin (IL)-36α, β and γ, their antagonist IL-36Ra and IL-38 in psoriasis, rheumatoid arthritis and Crohn's disease. *Clin Exp Immunol* 2016; 184: 159-73.