Pathogenesis of rheumatoid arthritis: one year in review 2025

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterised by joint destruction and extra-articular manifestations. A close interaction between both innate and adaptive immune systems leads to the development of the clinical features of the disease, characterised by inflammation and structural changes. Progress in the basic and clinical research in the field of RA has improved the current knowledge on the mechanisms underlying its pathogenesis. The data obtained from basic and clinical studies in RA has actively contributed to identifying new potential targets for novel therapeutic approaches to design personalised therapy of the disease.

Introduction

Rheumatoid arthritis (RA) is a multifactorial autoimmune disease shaped by a complex interplay of genetic susceptibility, epigenetic modifications, and immune dysregulation. Recent research continues to unravel the intricate molecular landscape that underlies disease onset and progression (1). Every year, data obtained from research activities in the field of RA adds a piece to the complex mosaic of the pathogenesis of the disease. Improving the current knowledge on the cellular and molecular mechanisms related to the innate and adaptive immune systems allows to better understand the contribution of the immune system in the development of the different stages of the disease.

In this review article we summarised the results of a Medline search of original research articles in English published in the PubMed database from January 1 to December 31, 2024.

Genetic and epigenetic advances

Key advances in the last year have further deepened our understanding of how both inherited variants and dynamic epigenetic mechanisms contribute to the development of synovial inflammation, tissue destruction, and systemic clinical manifestations in RA.

The most consistent genetic research in the pathogenesis of the disease is mainly focused in the discovery of novel polymorphisms in genes encoding components of the immune system. It has been demonstrated that variants in the signal transductor and activator of transcription-4 (STAT4) gene, particularly rs7574865, are associated with RA susceptibility in specific populations, such as those in western Mexico, and correlate with disease activity and anti-cyclic citrullinated peptide (anti-CCP) positivity (2). This supports the broader role of STAT4 in promoting Th1 and Th17 differentiation, contributing to amplified pro-inflammatory processes. In contrast, STAT3, while structurally related, plays a somewhat distinct role. In fact, its activating mutations are commonly observed in patients with RA-associated T-cell large granular lymphocytic (T-LGL) leukaemia, serving as a potential diagnostic marker distinguishing it from Felty syndrome, where such mutations are rare (3). These findings not only highlight the importance of STAT signalling in the mechanisms underlying RA pathogenesis but also suggest that somatic mutations may contribute to disease heterogeneity, particularly in patients with haematologic abnormalities.

Beyond these classical signalling pathways, attention has increasingly turned to epigenetic regulation, particularly involving miRNAs, DNA methylation, and histone modifications, which dynamically influence gene expression in key effector cells such as fibroblast-like synoviocytes (FLS). In this regard,

several miRNAs have emerged as pivotal modulators of inflammation and joint damage. Among them, miR-22 has been shown to exert anti-inflammatory effects in RA by targeting TET3, a DNA demethylation enzyme, responsible for increased transcription of *MTRNR2L2*, a gene implicated in the pro-inflammatory activity of FLS. Loss of miR-22 or up-regulation of TET3 may amplify inflammatory signalling in FLS (4). It seems to exert beneficial effects by restoring miR-22 levels, identifying in this approach a potential therapeutic strategy to dampen synovial inflammation.

Similarly, miR-100-5p, transferred through small extracellular vesicles (sEVs) derived from macrophages, has been identified as a regulator of the mTOR pathway, involved in cell proliferation and pro-inflammatory activities. Studies using *in vivo* murine models of RA showed that a reduction of miR-100-5p levels in macrophage-derived sEVs is associated with increased mTOR activation in FLS, leading to hyperplasia and pro-inflammatory cytokines production. Therefore, overexpression of miR-100-5p in these models mitigates these features, identifying in the miRNA a potential therapeutic target (5).

In parallel to mRNAs, DNA methylation and chromatin remodelling are deeply involved in the mechanisms underlying RA pathogenesis. Loss of function or down-regulation of PTEN, a negative regulator of the PI3K/Akt pathway, is observed in active FLS obtained by RA synovia, and seems to be responsible for their increased survival, migration, and pro-inflammatory activities (6). The interaction between PTEN loss and aberrant DNA demethylation (*e.g.* via TET3) further supports the hypothesis that a permissive epigenetic environment sustains the active inflammatory phenotype of FLS from RA joints.

The contribution of epigenetic readers such as BRD4, a member of the bromodomain and extra-terminal (BET) family, has also gained attention. This regulates transcription of key osteoclastogenic genes, including the receptor activator of nuclear factor kappa-beta ligand (RANKL), by binding to acetylated histones. Inhibition of BRD4 using small-molecule inhibitors such as JQ1 has been shown to reduce bone erosion and inflammation in animal models of RA (7), suggesting that BRDA might be a potential target for controlling RA. In fact, by limiting osteoclast differentiation and dampening pro-inflammatory transcriptional programmes, BRD4 inhibitors may represent a dual-action approach to control both inflammation and joint destruction, two key processes responsible for RA development and clinical features of the disease.

From a therapeutic standpoint, targeting signalling pathways downstream of cytokine receptors has yielded promising results. JAK1, a tyrosine kinase essential for IL-6 and interferon signalling, has emerged as a clinically relevant target. Transcriptomic profiling of FLS treated with the JAK1-selective inhibitor ABT-317 revealed suppression of both canonical inflammatory pathways and previously unappreciated gene programmes involved in matrix remodelling and specific cell phenotypes (8).

Interestingly, advances in transcriptomic and spatial profiling have further refined our understanding of how structural cells contribute to the development of clinical manifestations. FLS, well known for their role in promoting inflammation and matrix degradation, have now been implicated in the direct modulation of pain perception. A gene expression module of 815 pain-associated genes was identified in FLS isolated from RA patients with minimal synovial inflammation, but persistent pain (9). This gene set, including the axon guidance factor netrin-4, was shown to promote outgrowth of CGRP+ sensory neurons, establishing a functional link between FLS gene expression and chronic pain in RA. Recent attention has been given to the role of genetic and epigenetic in extra-articular aspects of RA. In fact, it seems that interstitial lung disease (RA-ILD), is also influenced by unique genetic and epigenetic signatures. Transcriptomic analyses of circulating $CD14^+$ monocytes from RA-ILD patients revealed gene expression profiles that closely resemble those of idiopathic pulmonary fibrosis (IPF), particularly for genes regulating innate immune response and fibrosis-related markers (10). This supports the common idea that a distinct fibrotic endotype within RA takes place, with potential implications for designing personalised treatment strategies.

Moreover, broader genomic studies reaffirm the central role of both HLA and non-HLA genes in RA susceptibility. While HLA-DRB1*01, *04, *10, and *DPB1 alleles remain dominant genetic risk factors for developing RA, non-HLA variants such as PTPN22, TRAF1, PADI4, STAT4, and CXCL12 have been linked to disease onset and severity (11). These variants likely interact with epigenetic mechanisms such as DNA methylation and glycosylation changes, creating a permissive environment for activation of the mechanisms involved in the development of autoimmunity. Recently, it seems that angiogenesis, by regulating miRNA networks, is critically involved in the early RA events. Although evidence is still emerging, miRNAs are believed to modulate angiogenic processes that facilitate immune cells infiltration and synovial expansion. Advances in proteomics and transcriptomics may soon elucidate specific roles of miRNAs in this context, opening new frontiers in early intervention and disease prevention in RA (12).

Taken together, these genetic and epigenetic insights not only reveal the molecular architecture of RA, but also contribute to the development of more precise diagnostic tools and therapeutic strategies for RA patients. The convergence of genomic, transcriptomic, and epigenetic data is steadily transforming our approach to modulate different aspects of RA at different stages of the disease, from broad-spectrum immunosuppression to targeted, mechanism-based interventions.

Take home messages

- Among miRNAs, miR-22 exert anti-inflammatory effects in RA by targeting TET3, a DNA demethylation enzyme, responsible for increased transcription of *MTRNR2L2*, a gene implicated in the pro-inflammatory activity of synoviocytes (4).
- Loss of function or down-regulation

of a negative regulator of the PI3K/Akt pathway (PTEN), is observed in active RA-derived FLS from synovia, promoting their increased survival, migration, and pro-inflammatory activities (6).

- non-HLA variants such as PTPN22, TRAF1, PADI4, STAT4, and CXCL12 have been linked to RA onset and severity of the disease (11).

New insights into environmental aspects

The global prevalence of RA has increased in recent years, particularly in industrialised countries, and this seems to be partly due to the effect of some environmental factors on the development and amplification of the disease (13).

Among them, dietary habits have been extensively investigated, with several clinical studies supporting the protective role of the diet on the development of RA. As already demonstrated for neoplastic diseases, also in RA the meat intake, especially red meat, seems to increase the risk of disease development (14). By using a genome-wide association study (GWAS), it was possible to analyse the effect of 30 different diets on the development of different inflammatory arthritis. Specifically, beef intake resulted positively associated with development of RA, unlike the protective factor that has been observed in groups of subjects with prevalence fresh fruit as well as iron intake (14).

As with different diet plans, also in the case of alcohol consumption, its link with RA remains controversial. Data obtained from a meta-analysis, including prospective cohort studies, supported a potential protective effect of alcohol, especially among individuals with positive autoantibodies. In addition, moderate alcohol consumption and a higher intake of fruits, oily fish, and cereals are associated with a reduced risk of RA, while tea and coffee may be linked to an increased risk of disease development (15). This has been confirmed by a recent mendelian randomisation study, showing that a genetic predisposition to alcohol consumption, independent of smoking, significantly correlates with a decreased risk of Parkinson's disease, prostate hyperplasia, as well as RA (16). A possible protective mechanism of moderate alcohol consumption in the development of RA is the action of polyphenols, such as resveratrol, which may modulate the immune response by inhibiting pro-inflammatory cytokines, altering immune cell activity, and reducing oxidative stress through free-radical neutralisation.

Regarding different foods intake, we have to take in account that several vitamins are introduced through the diet. Among different vitamins recognised to be relevant in RA pathogenesis, vitamin C has been objective of research studies at basic as well as at clinical levels. Because of its antioxidant properties, vitamin C has been widely proposed as a vitamin that contributes to modulate some immunological mechanisms involved in RA pathogenesis. In fact, it has been proved that this vitamin plays a supportive role in various enzyme-catalysed reactions and contributes to counteract inflammatory biomarkers. Given the fundamental protective effect of vitamin C on the development of RA, it has been observed that RA patients have reduced levels of circulating vitamin C and this might contribute to sustain the systemic inflammatory state of the disease. Therefore, supplementation of vitamin C could actively contribute to reduce inflammation in RA patients by acting on gut microbiota imbalance and suppressing the inflammatory response, mainly related to IL-6 and TNF-α (17). This recent study on vitamin C conducted on CIA model supports the key role of microbiota in the development of RA and how diet can alter this microbiota. One of the main responsible of changes in the microbiota in RA patients seems to be butyrate, a short-chain fatty acid, which can be obtained in small amounts from some foods, such as ruminant milk, butter, and yogurt as well as from the glycolysis of undigested carbohydrates by the intestinal microbiota. Its protective effect on RA seems due to its effect on enhancing gut immune response and permeability barriers by regulating inflammatory balance and intestinal junction proteins. In addition, butyrate is able to reduce bone erosion by inhibiting TNF and RANKL downstream factors TRAF6 and NFATc1, and to modulate the adaptive immune response by restoring the balance between pro- and anti-inflammatory lymphocyte populations (19). This finding supports the idea that the increase of the butyrate-producing ability and the restoration of the gut microbiota diversity may be a promising feature research direction for identify novel therapeutic approaches in RA.

Among different lifestyles, the one that has been most recognised to actively contribute to the development of RA and to be a well-established modifiable risk factor for the disease, is certainly the cigarette smoking. In the recent year, a study conducted in the USA on never-smoking adults showed a correlation between passive smoke exposure, measured via cotinine levels, and elevated RA risk. By monitoring levels of cotinine, a primary metabolite of nicotine that accurately reflects an individual's exposure over the previous 72 hours, it was observed a non-linear, positively saturated relationship between second-hand smoke exposure and RA development (20). Therefore, the already known pro-inflammatory effect of cigarette smoke on pro-inflammatory cytokines production and induction of oxidative stress, seems to be responsible for the development of RA even in the case of second-hand smoke in never-smokers individuals.

Taken together, food intake, consumption of alcohol, smoking of cigarettes and physical activities contribute to define the individual's lifestyle. Different lifestyle factors might therefore contribute to the development of RA, particularly those unhealthy behaviours recognised to be responsible for the development of the metabolic syndrome, a cluster of cardiovascular risk factors including central obesity, hypertension, low HDL cholesterol, high triglycerides, and hyperglycaemia. A recent study demonstrated a positive association between metabolic syndrome and increased RA risk. Data obtained from a prospective cohort study identified, among the five different components responsible for the development of the metabolic syndrome, elevated waist

circumference, elevated triglyceride level, reduced HDL cholesterol level and hyperglycaemia, to be the most responsible for increasing the developing RA risk (18). The secretion of pro-inflammatory cytokines such as leptin and adiponectin by adipose tissue, and the effect of elevated triglyceride on DNA methylation changes in the cells of the immune system are described as principal mechanisms involved in this association. In addition, low levels of HDL cholesterol might contribute to reduce the beneficial effect of HDL on the modulation of both innate and adaptive immune cells, responsible for maintaining the balance of the immune system.

On one hand, an individual's lifestyle can contribute to increase the risk of developing RA, while on the other hand, we have to take in account that environmental exposure, mainly to environmental pollutants, can play a crucial role in these processes. Several epidemiological studies support a link between pesticide exposure and RA development and recently some of these pesticides have been identified. In fact, the use of nine substances, four insecticides including malathion, phorate, carbaryl, carbofuran, four herbicides, including alachlor, metolachlor, S-ethyl dipropylthiocarbamate, metribuzin, and one fungicide (benomyl), was associated with an increased risk of RA development (21), carrying substantial implications as many of that pesticides are currently approved for general residential or public health uses in the USA. In parallel to pesticides, heavy metals have been recognised as another impacting factor in the development of RA. Recently an increase levels of Lead, Cadmium and Chromium in the sera of RA patients, but not in the healthy subjects, has been reported. Chronic exposure to heavy metals, especially lead and Cadmium, might exert negative effects on the immune system, mainly on the adaptive one, reducing the number of B and T lymphocytes, contributing to promote and amplify the autoimmune response in RA patients (22).

As recently reported in the *Journal of Environmental Rheumatology* (23) we have to take in account the serious methodological limitations that in many cases call into question the validity of studies on environment and its effect on RA pathogenesis. Currently, the introduction of the Artificial Intelligence (AI) would allow to reduce these limitations by utilising deep learning algorithms, increasing the possibility of validating the data obtained so far.

Take home messages

- A possible protective mechanism of moderate alcohol consumption in the development of RA is the action of polyphenols, such as resveratrol, which may modulate the immune response by inhibiting pro-inflammatory cytokines, altering immune cell activity, and reducing oxidative stress through free-radical neutralisation (16).
- Vitamin C supplementation in the diet of RA patients could actively contribute to reduce inflammation by acting on gut microbiota imbalance and suppressing the inflammatory response, mainly related to IL-6 and TNF-α (17).
- The already known effect of cigarette smoke on pro-inflammatory cytokines production and induction of oxidative stress, seems to be responsible for the development of RA even in the case of second-hand smoke in never-smokers individuals (20).
- Exposure to heavy metals, including lead and Cadmium, might exert negative effects on the immune system, mainly on the adaptive one, reducing the number of B and T lymphocytes, contributing to promote and amplify the autoimmune response in RA patients (22).

Novelties in the innate immune response

The role of the innate immune system in the pathogenesis of RA has been well established for years. Recent scientific research has further elucidated the involvement of innate immune cells such as neutrophils, mast cells, fibroblasts, as well as the role of soluble mediators such cytokines, chemokines and growth factors. Several studies have focused on the regulation of neutrophil activation, particularly on neutrophil extracellular traps (NETs), web-like structures composed of DNA and neutrophil-derived proteins. NETs are formed in response to pro-inflammatory signals, and when their formation becomes dysregulated, they contribute to disease pathogenesis by exacerbating inflammation and host tissue damage.

A key regulator of neutrophil activation and NET formation is the inhibitory C-type lectin receptor, myeloid inhibitory C-type lectin-like receptor (MICL). MICL directly recognises DNA within NETs, limiting their formation and preventing uncontrolled neutrophil activation via the ROS-PAD4 pathway. Loss-of-function mutations in MICL or the presence of anti-MICL autoantibodies, which have been detected in patients with RA, systemic lupus erythematosus (SLE), and severe COVID-19, lead to excessive NETs formation and a self-sustaining inflammatory cycle. According to the recent research activities, MICL thus emerges as a critical regulator of the inflammatory homeostasis (24). Some mediators involved in the innate immune system seems to be involved in the formation and activation of NETs in RA, including the alarmin IL-33. In fact, a novel pathogenic mechanism of RA, involving a tight interaction between IL-33, NETs, and FLS has been proposed. In synovial fluid from RA patients, elevated IL-33 levels promote NETs formation, which in turn activates neutrophils and FLS. Activated FLS then produce IL-33 and CXCL8, recruiting additional neutrophils and amplifying of production of additional NETs. It has been recognised that this pro-inflammatory feedback loop involves TLR9 signalling, and downstream intracellular pathways including MAPK and NF-κB (25).

Parallel to neutrophils, in the recent year particular attention has been given to the role of macrophages in RA pathogenesis. In fact, macrophages have been shown to contribute to the pathogenesis of the disease by internalising NETs and by activating the Rab5a-NF-κB pathway, which enhances the synthesis and release of pro-inflammatory cytokines and bone destruction. Neutrophil elastase (NE) and the GTPase dynamin, along with Rab5a, are essential for NETs uptake and subsequent sig-

nalling. Additionally, NET-associated DNA can directly activate NF-κB and other inflammatory pathways through receptors such as TLRs and the cGAS-STING axis (26). Collectively, these findings underscore the multifaceted and complex roles of NETs in promoting RA pathogenesis, involving not only neutrophils, but also macrophages.

Among the innate immune system components, several cells are responsible for the regulation of different mechanisms underlying RA pathogenesis. Among them, mast cells, which are normally resident in the human synovium, are increased in the joints of RA patients and actively contribute to both inflammation and tissue remodelling (27). These cells play a complex role in the inflammatory processes as well as in the tissue remodelling phase of RA, acting at the interface of innate and adaptive immunity. In early disease, mast cells are involved in B-cell activation, anti-citrullinated protein antibody (ACPA) production, and the release of pro-inflammatory cytokines. Despite their pro-inflammatory functions, these cells can also produce anti-inflammatory mediators such as IL-10. Moreover, mast cells support fibroblast survival and promote cartilage degradation and osteoclast activation. *In-vivo* studies using animal models have yielded conflicting results regarding mast cells function, suggesting that the role of mast cells in RA may vary depending on the stage of the disease (27). We have to take in account that the survival and activation of mast cells in the synovia is regulated mainly by FLS. Therefore, changes in mast cell function might be fundamental for several mast cells activities and survival. Recently it has been demonstrated phenotypic changes in fibroblasts during the inflammatory resolution, using positron emission tomography to track fibroblast activation protein (FAP). This approach revealed a transition from pro-inflammatory fibroblasts ($MMP3^+/IL6^+$) to pro-resolving fibroblasts ($CD200^+/DKK3^+$), promoting tissue healing through interactions with type 2 innate lymphoid cells (ILC2) via CD200–CD200R1 signalling (28). Further research into fibroblast function has explored the ac4C RNA modification mediated by N-acetyltransferase 10 (NAT10) in FLS derived from RA patients. It has been proved that high expression of NAT10 is associated with increased FLS aggressiveness and immune cell infiltration and inhibition of NAT10 seems to reduce FLS migration and inflammation in animal models of the disease, by regulating PTX3 mRNA stability (29).

Taken together these recent findings support the dynamic state of fibroblasts and their active interaction with ILCs, particularly ILC1, recognised to be implicated in the mechanisms underlying RA pathogenesis. Recent studies show that the JAK/STAT signalling pathway regulates ILC1 function and that the JAK inhibitor tofacitinib reduces both ILC1 frequency and IFN-γ production in patients with active RA. This effect appears to be specific, as TNF-α inhibitors do not significantly affect ILC1. Therefore, targeting ILC1 may contribute to the clinical efficacy of tofacitinib and highlight the importance of ILC1 in the mechanism involved in the development of RA (30). In the complex scenario of cytokines and chemokines several novel mediators seem to play an active role in the mechanisms involved in RA pathogenesis. For example, elevated levels of IL-40 have been observed in the sera of RA patients compared to healthy individuals and patients with osteoarthritis and SLE. It has been demonstrated that this cytokine is implicated in inflammatory processes and seems to be regulated by other cytokines including IL-1β and TNF-α. In addition, IL-40 itself can activate neutrophils to release additional pro-inflammatory cytokines. Notably, serum IL-40 correlates with RA-specific autoantibodies such as RF, anti-MCV and anti-CCP, suggesting a role of Il-40 not only in the inflammatory processes, but also in the mechanisms involved in autoimmunity. A positive association has also been observed between IL-40 and coagulation markers, indicating a possible link between inflammation and thrombosis in RA. In addition, IL-40 levels decrease following anti-TNF therapy, implying that TNF may drive its expression (31). However, no correlation has been found between IL-40 levels and clinical disease activity (DAS28, CRP, ESR) in RA patients, supporting the need for further studies on the potential role of IL-40 in the development of the clinical features of the disease.

Finally, a recent study investigating IL-18 signalling pathway in antibody-induced arthritis used the K/BxN serum-transfer mouse model. Mice lacking the IL-18 receptor alpha (IL-18Rα) exhibited significantly attenuated arthritis compared to wild-type controls, with reduced neutrophil infiltration, mast cells activation, and IL-1β expression in synovial tissue, strongly supporting the role of IL-18/IL-18Rα axis in amplifying the effector-phase inflammation and to support the activation of the innate immunity in RA (32).

Take home messages

- The myeloid inhibitory C-type lectin-like receptor (MICL), a key regulator of neutrophil activation and NET formation, directly recognises DNA within NETs, limiting their formation and preventing uncontrolled neutrophil activation via the ROS-PAD4 pathway (24).
- High expression of N-acetyltransferase 10 (NAT10) is associated with increased FLS aggressiveness and immune cell infiltration in RA and inhibition of NAT10 seems to reduce FLS migration and inflammation in animal models of RA, by regulating PTX3 mRNA stability (29).
- IL-18/IL-18Rα axis exert an active role in amplifying the effector-phase inflammation and in regulating the innate immune response in RA (32).

New insights in the adaptive immune response

It is now well established that $CD4^+CD8^+$ T cells are expanded in both the peripheral blood and the synovial tissue of RA patients. These cells have also been previously described in seropositive individuals at-risk of developing RA and have been associated with erosive manifestations in patients with overt disease (33). Building on existing work demonstrating a pathogenic role of $CD4^+CD8\alpha^+$ T cells in RA and by means of single-cell RNA and single-cell T cell receptor sequencing, Beck *et al*. showed that CD4+CD8αlow T cells,

RA specific and potentially detected very early in the course of RA, are part of T cell clones, clustering separately from $CD4^+CD8^-$ T cells, and are associated with RA disease activity (34). Therefore, their targeting in RA patients since early phases of the disease is certainly intriguing.

In the setting of T cell clones, by analysing paired samples of seropositive RA in the peripheral blood and in synovial tissue, clonally expanded $CD4^+$ T cells, including $CCL5^+$ cells and T peripheral helper (Tph) cells characterised by a prominent transcriptomic signature of recent activation and effector function, has been identified (35). One major strength of this study was the availability of paired analysis of T and B cells from the same synovial samples allowing to directly link clonally expanded T cell populations with synovial tissue expanded B cells. The most outstanding predicted interactions were those between proliferating T cells and Tph with activated B cells, ABCs, and plasmablasts. In more detail, Tph and ABCs had among the largest numbers of incoming and outgoing interactions, suggesting an elevated signalling potential for these populations. In addition, $CD8^+$ T cells displayed higher oligoclonality than $CD4^+$ T cells, with the largest synovial clones enriched in $GZMK^+$ cells. These findings were complemented by those of another study revealing that Tph cells were significantly expanded in early RA and, a peculiar B cell population, namely $CXCR5^-CD11c^-CD38^+$ naive B cells, were significantly increased in the peripheral blood of early RA (36).

With regard to T cell subsets involved in RA flares, analysis of data from a clinical trial of csDMARD cessation via high-dimensional mass cytometry and single-cell RNA sequencing to identify flare-associated cellular clusters, revealed distinct lymphocyte subsets, including cytotoxic and exhausted $CD4^+$ memory T cells, memory $CD8^+CXCR5^+$ T cells and $IGHA1^+$ plasma cells, flagged as the clusters undergoing significant changes at flare onset compared to baseline. Of interest, regulatory memory $CD4^+$ T cells (Treg cells) increased at flare onset, but with dysfunctional regulatory marker expression (37). Among these cells, only some are able to carry immuno-suppressive properties and therefore are those required to counteract aberrant immune responses (38). This suppressive Treg cell subset, expressing high levels of the transcription factor FOXP3, is significantly smaller in RA patients with active disease requiring bDMARD therapy compared to normal individuals. Of interest, the possibility that this phenomenon depends to IL-6 was postulated since treatment with IL-6 inhibitors restored normal proportions of these suppressive Treg cells. In addition, IL-6 blockade was also able to expand resting Treg cells, suggesting that the elevated concentrations of this cytokine hamper the broader pool of Treg cells and inhibit their beneficial effects in preventing/dampening abnormal inflammatory/autoimmune responses (39).

Treg cells are important not only in the pathogenesis of RA, but also in the Treg/Th17 imbalance. By identifying similarities and differences in this scenario in patients with seropositive or seronegative RA, Li *et al*. observed that patients with seronegative RA display an immunological profile more similar to PsA rather than to seropositive RA. This is related not only to a broad range of pro-inflammatory cytokines that are significantly higher in seropositive RA, but also to immune cell subsets since the Th17/Treg ratio was significantly lower in seronegative, but not seropositive RA. Of interest, when performing multivariable logistic regression, the only variable remaining significant was IL-4 that therefore seemed the key immunological discrepancy between seronegative and seropositive RA (40). The results of this study fit well with those obtained by De Stefano *et al*., demonstrating that the degree of B lymphocyte infiltration and the frequency of lympho-myeloid synovitis in patients with seronegative RA was different from that of seropositive RA and more similar that observed in PsA (41). Therefore, the results obtained may open new intriguing research avenues in the area of therapeutic management of seronegative versus seropositive RA. Additional differences between these two different phenotypes of RA patients emerged in the study performed by Neys *et al*. who were able to describe dissimilar involvement of dysregulated BCR signalling (42).

In the scenario of cells involved in the adaptive immune response of RA, Th9 is recognised as a relatively new pro-inflammatory T cells subset mainly characterised by the production of IL-9 and know able to interact and affect Th17 and Treg cells (43). Although some studies pointed to Th9 as a pathogenic cell subset in RA (44), the mechanisms involved are not yet fully elucidated. Therefore, Tu *et al*. explored into the effects of PU.1, the peculiar transcription factor or Th9 cells, showing that a PU.1/IL-9 positive feedback loop occurs in experimental arthritis and is directly involved in the disease progression. To note, since Th9 can interact with Th17 cells, suggesting that this PU.1/IL-9 positive loop may also target these cells (45). Therefore, by co-culturing ILC3 obtained by CIA mice and naïve CD4+ T cells, it has been observed that under Th17 cell differentiation conditions ILC3 are able to promote Th17 differentiation and IL-17 secretion mainly via cell-to-cell contact (46). In parallel, some light has been shed on the role of CD21-/low memory B cells in the pathogenesis of RA. Specifically, the expansion of CD21-/low CD27-IgD- double-negative B cells was associated with cartilage destruction in early RA (47) and CD21-/lowTbet+CD11c+ are able to promote bone destruction through antigen presentation, T cell activation, and Th17 polarisation (48). In the last year several advances have been done in the role of B cells in RA pathogenesis. In this setting, Hu *et al*. demonstrated for the first time that B10 and B10pro-regulatory cells exert and active role in some mechanisms of RA pathogenesis. Specifically, TNF-α seems to be able to trigger the pro-inflammatory functions of B10 and B10pro cells, by affecting cell metabolism (namely acting on glycolysis), cell proliferation and cell cytokine profile by up-regulating IL-17 and INF-γ, and down-regulating IL-10. In addition, the inhibition of TNF was able to revert these phenomena (49).

Take home messages

- Peculiar B cell populations, namely CXCR5-CD11c-CD38^{+} naive B cells, were significantly increased in the peripheral blood of early RA (36).
- Seronegative RA patients display an immunological profile more similar to PsA rather than to seropositive RA (40).
- CD21-/low memory B cells play an active role in RA pathogenesis. CD21-/low CD27-IgD- double-negative B cells expansion was associated with cartilage destruction in early RA (47) and CD21-/lowTbet^{+}CD11c^{+} promote bone destruction through antigen presentation, T cell activation, and Th17 polarisation (48).

Conclusions

The mechanisms of RA pathogenesis are complex, and several factors are involved, contributing to the development of different features of the disease. Basic and clinical research in the field of RA, particularly on genetic, epigenetic and molecular biology, has allowed us to better characterise new pathways of RA pathogenesis. In the last year, several research activities have been focused on the role that the environment at different levels influences several aspects of the disease. Novel components of the innate and immune systems, including cytokines, chemokines, growth factors and cells have been deeply investigated, and proposed as novel mechanisms involved in the pathogenesis of RA potentially targets for further therapeutic approaches, useful for developing a patient tailored therapy.

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