Review

One year in review 2021: pathogenesis of rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by local and systemic inflammation where the close interaction between immune cells and soluble mediators leads to amplification and perpetuation of inflammatory and remodelling processes. The research carried out in the last year in the field of RA has made it possible to identify new mechanisms involved in the pathogenesis of the disease, enabling the discovery of new potential therapeutic targets. Thus, in this review we summarise new insights in RA pathogenesis, resulting from a literature research date published in the last year.

Introduction

Rheumatoid arthritis (RA) is a complex autoimmune disease characterised by local and systemic inflammation (1-3). In the last year, advances have been made in the knowledge of RA pathogenesis, in particular in the field of genetics and environmental factors that can influence the development of the disease. Due to advanced research techniques, it has been possible to better characterise cellular and molecular processes involved in the dysregulation of innate and acquired immune responses with the identification of new pathways useful for the development of new therapeutic approaches in RA. 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, 2020.

Genetic aspects

To date, several genome-wide association studies (GWAS) and meta-analyses identified more than 100 alleles associated with RA susceptibility. Recently, it has been reported that SLAMF6, CXCL13, SWAP70, NFKBIA, ZFP36L1 and LINC00158 may be new RA-risk loci in a large Korean cohort (4) and IL12RB2, BOLL-PLCL1, CCR2, TCF7 and IQGAP1 in a Chinese cohort (5).

It is well established that among different alleles conferring RA susceptibility, the major histocompatibility complex (MHC) genes, including class I (HLA-A, B, C), class II (HLA-DR, DP, DQ), and class III sub-regions, are the most relevant ones. Within the HLA alleles, HLA-DRB1*04 has recently been confirmed to increase the risk of developing RA in a southern Mexican population, while HLA-DRB1*08 allele seems to exert a protective effect (6).

In addition to HLA loci, several genetic variants of non-HLA loci have been associated with some aspects of RA pathogenesis such as the role of immune cells (B cells, T cells and mast cells) and pro-inflammatory cytokines that actively regulate some relevant inflammatory mechanisms. The genetic variants of genes encoding for soluble mediators regulating immune cells activities have been the focus of several recent investigation.

For instance, it is well recognised that IL-1β plays a crucial role in joint inflammation and tissue damage in RA, and several polymorphisms of its gene have been widely investigated. Among these, three single nucleotide polymorphisms, rs2853550, rs1143643, and rs16944, have been recently associated with RA susceptibility in a Chinese Han population (7).

In parallel to IL-1β, the TGF-β1 +869C/T gene polymorphism was reported to increase susceptibility to RA with significant association with inflammatory parameters, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and with the disease activity (8). Interestingly, evaluation of the innate gene expression signature, including 10 Toll-like recep-

tors (TLRs), 7 IL1/IL1R family members and the chemokine CXCL8/IL8 in the peripheral blood mononuclear cells (PBMC) from active and inactive RA patients, revealed a certain heterogeneity with an association with up-regulated TLR2, TLR4, TLR6, TLR8 and down-regulated TLR10 (9).

Besides the innate immune system, lymphocytes play a fundamental role in orchestrating the production of autoantibodies in the context of the adaptive immunity. Due to their crucial role in RA, during the last year increasing attention has been given to novel variants in genes encoding for lymphocyte signalling regulating molecules, which may control cell survival and activity. For example, cytotoxic T-lymphocyte antigen-4 (CTLA4), also known as CD152, is an inhibitory glycoprotein present on the surface of T cells that regulates the activation of autoreactive T cells. The CTLA4 gene polymorphism rs11571319 was found to be associated with RA susceptibility in the Pakistani population (10). VAV1, an intracellular signal transduction protein, that plays an important role in development, proliferation and activation of T cells, was widely investigated and patients carrying VAV1 252546133T and 252617822G allele presented an increased frequency of extra-articular manifestations (11).

Furthermore, several variants may contribute to disease susceptibility by regulating molecules involved in oxidative stress or bone homeostasis. In fact, increased oxidative stress and aberrant generation of reactive oxygen species (ROS) may occur in RA pathogenesis. In particular, the NADPH-oxidase (NOX) complex is critical for ROS generation and several studies suggested that NOX gene variations could affect the risk of developing autoimmune diseases by orchestrating ROS production. NOX complex is composed of gp91phox, p22phox, IM7phox, p67phox, and p40phox encoded by CYBB, CYBA, NCF1, NCF2, and NCF4 genes. Among these, NCF4 has been proven to regulate the production of intracellular ROS by inducing the NOX complex to phagosome membranes through the binding of phosphatidylinositol 3-phosphate. In this regard, Zhang TP *et al*. demonstrated that gene polymorphisms of NCF4 rs4821544 and rs729749, but not of NCF2 and CYBA, contribute to RA susceptibility (12).

In parallel to gene polymorphisms, epigenetic mechanisms, including DNA methylation, histone modifications, and small (s) and long (l) non-coding RNAs (ncRNA) play important roles in mechanisms underlying RA pathogenesis. Among sncRNA, micro-RNAs (miRNA) are sncRNA molecules with lengths of 18–25 nucleotides regulating target messenger RNA (mRNA) and the translation of the corresponding proteins. Accumulating evidence suggests that miRNA exert important effects on some mechanisms regulating RA pathogenesis. For example, miR-150-5p is able to promote the growth of RA fibroblast-like synoviocytes (FLS) probably by inhibiting the suppressor of cytokine signalling 1 (SOCS1) molecule (13). On the other hand, a recent analysis of miRNA and mRNA expression profiles in RA PBMC, demonstrated that miR-99b-5p is a novel post-transcriptional mediator involved in different cellular activities, including T cell growth, activation, and expression of multiple pro-inflammatory cytokines (IL-2, IL-6, TNF-α and IFN-γ) (14).

However, some miRNAs may also play a protective role in RA by inhibiting pro-inflammatory mediators. For instance, miRNA-17-5p is able to reduce inflammation and bone erosions in collagen-induced arthritis (CIA) mice model, by inhibiting the IL-6 family autocrine-amplifying loop via JAK1 and STAT3 pathways (15).

As far as lncRNA are concerned, these are a heterogeneous class of molecules defined by a lack of protein-coding potential and a minimum transcribed length of 200 nucleotides. LncRNA are able to interact with RNA, DNA and proteins to regulate gene expression at multiple levels, thus critically orchestrating immune cells differentiation and activation. Notably, a recent analysis of lncRNA expression profiles demonstrated a potential role of lnc-AL928768.3 and lnc-AC091493.1 as novel biomarkers for RA susceptibility and disease activity (16). However, lncRNA may also exert their biological function by sponging target miRNA. For example, the lncRNA PVT1 was shown to regulate the proliferation and pro-inflammatory activity of RA FLS by targeting microRNA-145-5p (17), while the lncRNA Growth Arrest-Specific Transcript 5 (GAS5) is mainly involved in promoting the release of pro-inflammatory mediators in the RA-derived synovial tissues (18).

Furthermore, several genetic polymorphisms within these lncRNA genes might also contribute to RA susceptibility or disease severity. For instance, the lncRNA 00305 (LINC00305), a pro-inflammatory atherosclerosis-associated lncRNA, and its rs2850711 genetic variant, seems to contribute to the activity and severity of the disease (19). The role of various gene polymorphisms in the pathogenetic mechanisms of RA are under investigation, particularly in Asian countries. Comparing the results obtained in the different populations can contribute to better understanding the close interaction between gene profiles and inflammatory and autoimmune processes in RA.

Take home messages

- GWAS and meta-analyses of GWAS identify multiple novel susceptibility loci in RA.
- HLA-DRB1 alleles may play a protective role in RA.
- Variants of genes encoding for cytokines and lymphocyte signalling regulating molecules may contribute to disease susceptibility and/or severity.
- lnc-AL928768.3 and lnc-AC091493.1 may be novel biomarkers for RA susceptibility and activity.

Environmental factors

It is now well established that RA is the result of a close interaction between susceptibility genes and environmental factors, including cigarette smoking (CS), air pollutants, diet, obesity, microbiota and infections. CS is involved in autoimmunity processes due to its ability to promote the production of rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA), which are linked to joint destruction

and systemic bone loss in RA, even in the early phases of the disease (2, 3). Recently, Regueiro *et al*. confirmed that in RA patients CS is the strongest risk factor for triple-seropositivity to RF, ACPA and anti-carbamylated protein antibodies, and it is exclusively associated with the presence of RF in patients with positivity to one or two autoantibodies (20). Besides CS, exposure to air pollutants appears to affect some of the mechanisms involved in RA pathogenesis. In fact, a recent cross-sectional study demonstrated that exposure to fine particulate matter (PM 2.5) is an independent predictor of ACPA titres (21). This result has been confirmed by a meta-analysis that was also able to recognise ozone exposure and living in proximity to high-traffic roads as risk factors for developing RA (22). Furthermore, it appears that a pro-inflammatory diet may worsen the activity of the disease. This hypothesis has been supported by a recent randomised, controlled cross-over trial, showing beneficial effects of an anti-inflammatory dietary on clinical outcomes of RA. Such intervention was able to induce an improvement of the disease, in terms of Disease Activity Score 28-joint count indices (DAS28) calculated with ESR. Furthermore, the patients with the higher activity score achieved the greater disease improvement following this anti-inflammatory diet. However, some relevant information such as the ongoing pharmacological therapy of the patients, useful for a better interpretation of the results obtained, was not included in the study (23). Moreover, two independent meta-analyses, systematically reviewing all randomised clinical trials (RCTs) on the effects of anti-inflammatory dietary and RA, confirmed a moderate beneficial effect of certain foods on disease activity, although further studies are needed for better clarifying the direct contribution of nutrients to the development and severity of RA, taking in account the timing of dietary intervention, the ongoing therapy and the phase of the disease (24). Overall, dietary habits seem to be relevant in the management of RA patients. In fact, overweight and obesity have been linked to the development and activity of RA, mainly based on case-control studies. Recently, a meta-analysis, including 13 cohort studies, investigating the link between adiposity and RA, proved a correlation between body mass index (BMI) and the risk to develop the disease. In this meta-analysis the association was more significant in women than men, probably due to the lack of available accurate indicators of male adiposity. According to these results, it can be assumed that adiposity may be target for primary prevention in RA (25).

In this context, the role of microbiome has been extensively investigated. A recent comprehensive metagenome-wide association study (MWAS), conducted in Japan, demonstrated more bacteria belonging to the genus Prevotella in the stool samples of RA patients compared to healthy subjects. On the other hand, R6FCZ7, a bacterial gene involved in redox reactions and possessed by many species within the genus Bacterioides, was underrepresented in RA samples compared with controls (26). These results strongly support the role of microbiome and microbiota in the mechanisms underlying RA pathogenesis. In addition, novel therapeutic approaches can be introduced in clinical settings, such as pregnancy or lactation, where pharmacological treatments encounter limitations (27). It has been proven that a high-fat diet can promote the development of a pro-inflammatory gut microbiota, that microbial-derived short-chain fatty acids (SCFAs) are reduced in RA patients and in animal models of arthritis, and that in the CIA model butyrate supplementation was able to improve the disease activity. It has been hypothesised that this was due to the ability of SCFAs to activate regulatory B cells (Bregs), inducing IL-10 release with consequent inhibition of germinal centre B cell and plasmablast differentiation and a probable effect on autoantibodies production (28). It is well known that dysregulation of gut microbiota may affect the whole organism and may lead to RA development in patients showing preclinical evidence of autoimmunity. Furthermore, diet supplementation with butyrate, known to improve intestinal barrier function, and larazotide acetate, a zonulin antagonist, was able to attenuate arthritis. In fact, high levels of Zonula occludens toxin (zonulin), a microbial protein which disrupts epithelial tight junctions, have been recently liked to disease progression from preclinical to clinical stage of RA in both human and animal models, and treatment with a zonulin agonist peptide in CIA mice has been shown to worsen joint inflammation (29). Therefore, these results highlight the fundamental role of the intestinal epithelial barrier in protection from systemic inflammatory processes and partially explain how certain dietary components can improve microbiota homeostasis with beneficial effects on RA.

Take home messages

- Smoking is a risk factor for triple-seropositive RA.
- PM 2.5 and ozone exposure and living near busy roads are risk factors for RA.
- An anti-inflammatory diet and probiotics supplementation may reduce disease activity.
- Overweight and obesity have been proven to be associated with RA, particularly in women, by a meta-analyses of cohort studies.

Innate immune response

Cells and soluble mediators of the innate immune system play a key role in the pathogenesis of RA and all these singular components were deeply investigated during this last year.

It is well known that neutrophils are the most abundant immune cells in RA inflamed joints and their ability to form neutrophil extracellular traps (NETs) actively contributes to RA pathogenesis, via autoantigen production and activation of FLS. Recently, NET production has been linked to cartilage damage in RA. In particular, NET-derived elastase seems to be able to disrupt cartilage matrix, leading to citrullination of cartilage components, with subsequent internalisation by FLS and presentation to $CD4^+$ T cells. In fact, transgenic mice immunised with NETs developed autoantibodies against citrullinated cartilage fragments and inhibition of NET-

derived elastase proved to rescue NET-mediated cartilage damage (30).

Besides NETs, extracellular vesicles (EVs), derived from different cell types, seem to promote inflammation in RA joints by stimulating the release of pro-inflammatory cytokines. EVs were isolated from the synovial fluid and characterised by proteomic analysis but these results were in contrast with previous data. In fact, while earlier studies pointed at B cells and platelets as the main EVs-releasing cells, new evidence identified neutrophils and FLS as major sources of EVs (31). In addition, the co-culture of CD14+ cells with small EVs containing miR-574-5p purified from RA synovial fluid , demonstrated the ability of EVs to promote osteoclast differentiation, mainly via TLR 7/8 signalling (32).

Activated FLS contribute to the formation of synovial pannus and bone destruction and it has been recently observed that FLS-derived adiponectin (AD), already known as a promoter of inflammation in RA, is able to induce *in-vitro* and *in-vivo* T-follicular helper cell (Tfh) activity via the release of soluble IL-6 (33).

In parallel, certain intracellular pathways of FLS were identified as responsible of their aggressive phenotype in RA. Particularly, WNTS5A, a member of the secreted glycoproteins family Wingless/integrase 1, up-regulated in RA-derived FLS and involved in cytoskeleton remodelling, seems to strongly enhance migration and invasion of RA FLS, but not of those isolated by patients with other types of arthritis (34). In parallel, c-Jun N-terminal kinase (JNK) was identified as one of the downstream molecules mediating the sonic hedgehog (SHH) signaling pathway in patients with RA (35), and IL-34 has been proven as one of a key soluble mediator in controlling migration and proliferation of RA-derived FLS via ERK1/2 and AKT signalling pathways (36). Furthermore, studies performed in humans and animal models demonstrated that DELR3, an endoplasmic reticulum-associated degradation protein, expressed in RA-derived FLS, is involved in inflammatory processes by increased production of cytokines and chemokines such as IL-6 and IL-8, as well as the metalloproteinases MMP-1 and MMP-13 (37). Moreover, CXCL1/CXCR2 axis (chemokine-ligand 1 and its receptor) activation may induce IL-6 production in FLS derived from both RA and osteoarthritis (38) and the alarmin IL-33 is able to modulate some mechanisms regulating proliferation and apoptosis in RA-derived FLS via NF-κB pathway (39). The central role of FLS in RA pathogenesis is also supported by recent findings showing that these cells are source of local production of CRP. It is well-known that synovial fluid from RA patients contains high levels of CRP, mainly derived from FLS. At the same time, FLS have been recognised as the major cell-type expressing CD32 and CD64, receptors for CRP, involved in FLS proliferation, invasion and production of CCL2, CXCL8, IL-6, MMP-2 and MMP-9 (40).

In addition to FLS, several studies demonstrated the pivotal role of macrophages in joint inflammation and bone erosion. Synovial macrophages (SM) are a type of tissue macrophages deriving from bone marrow cells orchestrating joint inflammation and tissue remodeling. Immunofluorescent comparison of synovial tissue from CIA and control mice proved that a tight crosstalk between SM and FLS takes place in RA. Under inflammatory conditions, the tight interaction between these two cell types in murine arthritis leads to metabolic reprogramming of SM with consequent increase of their cell viability. Several soluble mediators, such as IL-26 (41), and receptors, such as Angiotensin II Type 2 receptor (AT2R) (42), are able to modulate SM polarisation in RA-derived synovia, contributing to the amplification and perpetuation of tissue inflammation.

Furthermore, different SM subsets, particularly MerTKposTREM2high and MerTKposLYVE1pos, seems to regulate inflammatory processes in the active as well as in the remission phase of the disease. *In-vitro*, these two SM types producing inflammation-resolving lipid mediators are responsible for repair response of FLS, and low proportion of MerTKpos SMs in the remission phase of RA was associated with increased risk of disease flare, suggesting that modulation of these two macrophages subsets could be a potential therapeutic strategy in RA (43). Given the key role of macrophages in joint inflammation, modulation of certain apoptotic processes has been proposed as alternative therapeutic approach. In particular, the pro-apoptotic BCL-2 family protein BAD has been suggested as potential pharmacological target in RA, given that its inactivation confers the apoptosis resistance on synovial sub-lining macrophages, thereby contributing to the development of arthritis (44).

Monocytes have also been investigated in order to better characterise their phenotype in different mechanisms underlying RA pathogenesis. Monocytes are the main PB cells that can differentiate in macrophages or dendritic cells. Monocyte subpopulations can be divided, based on the different expression of CD14 and CD16, into classical (CD14++CD16-), intermediate (CD14++CD16+/++) and nonclassical (CD14-/+CD16++) monocytes. Recently, differences in monocytes subpopulations with a shift towards nonclassical subset have been detected in individuals at high risk of developing RA, especially in ACPA+ subjects, priming the progression from early phase to the active disease (45).

Further studies aimed to characterise inflammatory and structural cells of the innate immune system will allow to better understand the mechanisms underlying RA pathogenesis.

Take home messages

- NETs actively contribute to RA pathogenesis, via autoantigens production and activation of FLS.
- EVs, produced mainly by neutrophils and FLS in RA, promote inflammation and osteoclast differentiation.
- Intracellular pathways such as WNTS5A, JNK, ERK1/2, AKT and DELR3 actively regulate FLS activities in inflammatory and remodelling processes.
- Synovial macrophages (SM) interact with FLS through IL-26 and AT2R, perpetuating tissue inflammation.

Adaptive immune response

As far as B cells are concerned, Rivellese *et al*. observed that B cell rich synovitis is associated with high levels of disease activity and seropositivity for RF and ACPA, particularly in early RA and in patients with treatment-resistant disease (46). When comparing B cells from the synovial tissue with those from the PB, the latter showed a more pronounced complexity in their clonal families, supporting the hypothesis that the small subset of clonal families present in the synovial tissue and the antibodies produced here, mainly ACPA, may have a role in RA pathogenesis due to both the persistent peripheral antigen stimulation in this site and the capability to indirectly stimulate the production of TNF-α (47).

In addition, Kissel *et al*. demonstrated that ACPA have a broad cross-reactivity towards several post-translationally modified antigens, not only citrullinated, but also carbamylated and/or acetylated antigens that can be present in the inflammatory sites and could be involved in the autoimmune processes. These findings shed some light on B cells tolerance (48) and the production of these autoantibodies was explained by the classical theory of "shared epitope". However, Auger *et al*. put forward a new hypothesis and speculated that peptidyl arginine deiminase (PAD)4-specific T cells may help PAD4-specific B cells leading to the production of anti-PAD4 antibodies and ACPA. This is explained by the hapten-carrier mechanism, where PAD4 acts as carrier and haptens are the proteins bound and citrullinated by PAD. Moreover, it has been demonstrated that the peptide of PAD4 that triggers T cells proliferation in 40% of the RA patients, peptide 8, is associated with PAD4-antibodies, HLA-DRB1 shared epitope and RA, giving a chance to prevention in these high-risk individuals (49).

B cells are involved also in the production of RF, and a longitudinal study across 5 years demonstrated that in the first stage of RA T helper cells are required for IgG RF production, while after 5 years there is a dissociation between T cell reactivity and RF status, suggesting that B cells may become able to independently produce RF over time (50).

A peculiar B cell subset, $CD19^{+}$ CD24hiCD38hi regulatory B cells (cTr B cells), was found to be increased in naïve early RA (51). cTr B cells display anti-inflammatory properties, such as the production of IL-10, and are associated with ACPA titres and the response to methotrexate at 1 year. Another IL-10 producing B cell subset characterised by the co-expression of CD27 and high levels of CD24, are reduced as a consequence of disease activity. In fact, pro-inflammatory cytokines like TNF-α and IL-1β cause the overexpression of the CD70 on both CD4+T cells and CD8+B cells, causing the CD27/CD70 interaction and the increase of the soluble form of CD27 (52).

As far as T lymphocytes are concerned, a relationship between signaling lymphocytic activation molecule, SLAM associated protein (SAP) and programmed cell death (PD)-1 was observed. SAP is an adaptor molecule important in T cells signalling and in the contest of self-tolerance, while PD-1 is a receptor that has a role in the prevention of autoimmunity. RA T cells display higher levels of SAP and since it renders the PD-1 ineffective, this ultimately prevents the PD-1 mediated T-cell exhaustion. Despite its function is hampered, PD-1 expression is increased in RA synovial fluid and PB T cells with a positive correlation with disease activity (53).

CD4+Foxp3+regulatory T cells (Treg) absolute number, percentage and activation are reduced in the PB of early untreated RA patients in comparison to healthy controls a negative correlation between the percentage of these cells and the disease activity, the ESR, the CRP and the RF (IgM) levels has also been described. The importance of Treg cells is further underlined by the observation that a specific Treg subset expressing TNF receptor (TNFRII) and associated with a Foxp3 TSDR (forkhead box P3 Treg specific demethylation region) hypomethylation, the latter determining Treg cells stability, is increased after treatment with methotrexate (54). In addition, an exacerbation of inflammatory state that ameliorates after the adoptive transfer of WT (wild-type) mouse Treg and an hypermethylation of Foxp3 gene in comparison of WT mice was detected in experimental models of TNFRII deletion (homozygous mice TNFRII-/-) (55).

The involvement in RA pathogenesis of phospholipase (PL) D1, a molecule responsible for Treg cells immunosuppressive action, was observed in CIA model. PLD1 inhibition leads to suppression of autoantibodies and inflammatory cytokines production, ultimately determining a reciprocal regulation of Th17/Treg balance. The Th17/Treg cell ratio was decreased in RA, especially in early, active and severe disease (56). PLD1 inhibition determined an increase of Treg cells with enhanced anti-inflammatory effect, and reduction of Th17 effector cells, leading to a decrease of the inflammatory state. PLD1 effect on the Th17/Treg balance also affects osteoclastogenesis and bone resorption. Furthermore, Treg cells were found to be negatively correlated with signs of high disease activity like DAS28, but also white blood cells count, percentage of neutrophils, platelet count, while Th17 cells were increased in the same disease stage (57).

With regard to the imbalance of Treg and other T cell subsets, Cao *et al*. investigated the role of an imbalance between a subpopulation of Treg cells, follicular regulatory T cells (Tfreg), found in germinal centres, and Tfh that resulted in the expansion of autoreactive B cells and autoantibody production. The Tfreg/Tfh ratio is decreased in RA patients and it is negatively correlated with ESR, CRP, RF, IL-21 and disease activity while being positively correlated with TGF-β, produced by Tfreg. In normal subjects, TGF-β can inhibit the function of Tfh cells, preventing their germinal centre response and antibodies production (58).

Developmental plasticity of T cell subsets is a pivotal phenomenon in the field of autoimmunity. As far as RA is concerned, Leipe *et al*. detected that Th1/Th2 subsets can differentiate in Th17 cells in early RA, while Th17 cells do not change their phenotype towards Th1/Th2 cells (59). Furthermore, when assessing classic (CD161-/CCR6-) and

Table I. Studies on gene susceptibility.

Genetic features	Associations or roles	References
SLAMF6	Susceptibility	(4)
CXCL13	Susceptibility	(4)
SWAP70	Susceptibility	(4)
NFKBIA	Susceptibility	(4)
ZFP36L1	Susceptibility	(4)
LINC00158	Susceptibility	(4)
IL12RB2	Susceptibility	(5)
BOLL-PLCL1	Susceptibility	(5)
CCR2	Susceptibility	(5)
TCF7	Susceptibility	(5)
IQGAP1	Susceptibility	(5)
HLA-DRB1*04	Susceptibility	(6)
HLA-DRB1*08	Protective role	(6)
IL1B (rs2853550)	Susceptibility	(7)
IL1B (rs1143643)	Susceptibility	(7)
IL1B (rs16944)	Susceptibility	(7)
TGF-β1 +869C/T polymorphism	Susceptibility	(8)
CTLA4 (rs11571319)	Susceptibility	(10)
VAV1 (rs2617822)	Susceptibility	(11)
NCF4 (rs4821544)	Susceptibility	(12)
miR-150-5p	Pro-inflammatory role	(13)
miR-99b-5p	Pro-inflammatory role	(14)
miRNA-17-5p	Protective role	(15)
lnc-AL928768.3	Susceptibility and disease activity	(16)
lnc-AC091493.1	Susceptibility and disease activity	(16)
lncRNA PVT1	Pro-inflammatory role	(17)
lncRNA GAS5	Pro-inflammatory role	(18)
LINC00305 (rs2850711)	Severity and disease activity	(19)

non-classic (CD161+/CCR6+) Th1 cell subtypes, they observed that CCR6+ cells display an increased plasticity towards IL17 producing cells, while CCR6- seemed to show higher plasticity towards IL-4 producing cells (60).

This finding is of particular importance also in light of the demonstration that Th1 and Th17 cells not only are increased in RA compared to normal subjects but that among patients, increased frequencies were found in carriers of HLA-DRB1 genotypes that have a shared epitope (HLA-SE), the strongest genetic risk factor for RA, involved in ACPA production but also in the induction of innate and adaptive response, through IL-17a and IFN-γ producing $CD4^+$T cells (61).

Other studies have also assessed the mechanism of Th17 regulation in RA showing that the expression of USF2, a transcription factor fundamental in maintaining inflammatory Th17 cells, is increased in RA patients non-responders to treatment (62). Furthermore, Bai *et al*. have assessed the role of an endothelial receptor EPCR (endothelial protein C receptor) in its membranous (mEPCR) and soluble (sEPCR) forms, the former being expressed by $CD4^+$ T cells. Lower levels of $CD4^+EPCR^+$Tcells and higher levels of sEPCR have been observed in RA compared to osteoarthritis and sEPCR showed a positive correlation with clinical and laboratory parameters of RA (63). *In-vitro* experiments demonstrated that sEPCR inhibited Th17 activation, modifying the Th17/Treg balance alleviating disease progression. Hence, higher levels of sEPCR in RA may explain a compensatory, albeit not sufficient, compensatory mechanism leading to limit disease progression (51).

RA patients also displayed an increase of the γδTcells, a small T cell subset that has a role in RA pathogenesis through release of pro-inflammatory cytokines such as IL-17 and the support of antibody production by B cells. These effects seem to be mediated by prostaglandin (PG) E2 since the addition of this molecule leads to an increased expression of CD80 and CD86 on γδTcells and, consequently, of secreted IL-17 *in-vitro* (64). Another subset of CD4+ T cells, the CD45RA+CD62L+CD95+ memory stem T cells (Tscm), may play a role in RA pathogenesis since their number, and particularly that of the arthritogenic clones specific for a citrullinated vimentin epitope, is significantly increased in RA patients while being reduced by treatment with anti-TNF agents (65).

CCL21, a chemokine that binds to CCR7, is abundantly present in RA synovial tissue being expressed by dendritic cells, macrophages and endothelial cells. CCR7+ cells are therefore recruited to the synovial microenvironment modulating, among other tasks, the immune response. In the early phase of RA, the presence of CCL21 in the synovial fluid induces the formation of an inflammatory infiltrate mainly constituted by CCR7+ monocytes that, as RA progresses, are committed towards an M1 phenotype (classically activated macrophages), able to produce IL-6 and IL-23. Such mediators ultimately promote the differentiation of Th17 cells that are strongly involved in RA pathogenesis. A positive feedback mechanism has also been identified in this scenario since IL-17 can further promote the function of the CCL21/CCR7 axis. In the erosive, final stages, these cytokines and CCL21 directly and indirectly participate in osteoclastogenesis and bone resorption (66).

Another study reported that hypoxic conditions in RA synovial tissue led to reduced indoleamine 2,3-dioxygenase-1 expression, namely tryptophan metabolism, thereby interfering with the interaction between FLS and T cells (67). In particular, while normally FLS act as immunomodulators, suppressing T cell responses, hypoxia may represent a pathogenic mechanism preventing such interaction. In addition, a relationship between anaerobic metabolism, through glycolysis, as it happens in hypoxic microenvironment like the synovial membrane and the inflammatory features of CD8+ T cells has been reported (68). In fact, the inhibition of glycolysis leads to a of the proinflammatory CD8+ T cell phenotype in RA. Furthermore, CD4+ T cells directly impact the metabolic profile of RA-derived FLS, promoting a glycolytic phenotype to meet increased metabolic demand in a reciprocal activating re-

lationship (69). Taken together, these findings suggest glycolytic manipulation as possible new therapeutic strategy in RA.

Take home messages

- B cell rich synovitis is associated with high levels of disease activity and seropositivity for RF and ACPA.
- peptidyl arginine deiminase (PAD)4-specific T cells may help PAD4-specific B cells leading to the production of anti-PAD4 antibodies and ACPA.
- Tfreg/Tfh ratio is decreased in RA patients and it is negatively correlated with ESR, CRP, RF, IL-21 and disease activity while being positively correlated with TGF-β, produced by Tfreg.
- CCR6+ cells display an increased plasticity towards IL17 producing cells, while CCR6- seemed to show higher plasticity towards IL-4 producing cells.

Conclusions

In the last year several studies have been published in order to better understand the pathogenic mechanisms underlying RA. In particular, studies on gene susceptibility (Table I), epigenetic mechanisms and different environmental factors allowed us to recognise additional mechanisms underlying the disease, useful for building new therapeutical strategies. In parallel, interesting and promising results for future development of therapeutic approaches have been reported in the context of the innate and adaptive immune systems with particular attention to novel pathways regulating the activity of FLS, different phenotypes of monocytes and macrophages and different subsets of B and T lymphocytes.

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