Review

One year in review: the pathogenesis of rheumatoid arthritis

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ABSTRACT

The pathogenesis of rheumatoid arthritis (RA) is a complex scenario that, to date, is not fully elucidated. However, scientific progress has enabled us to understand several mechanisms underlying the development of the disease. The breakdown of self-tolerance in genetically predisposed individuals and the aberrant activation of innate and adaptive immune responses culminate in synovial hyperplasia and bone destruction. In addition, extra-articular manifestations, as well as the burden of increased cardiovascular risk (CVR), in patients with RA represent another interesting aspect of RA pathogenesis under intense investigation. The purpose of this review article is to provide an overview of the new insights in RA pathogenesis summarising the most relevant studies published over the last year.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory, autoimmune disorder characterised by a persistent joint inflammation leading to cartilage and bone damage, disability and eventually, to systemic complications including cardiovascular and pulmonary disorders.

The pathogenesis of RA is attributed to a complex interaction between genetic and environmental factors and the repeated activation of innate and adaptative immunite system evolves into the breakdown of immune tolerance, aberrant autoantigen presentation and antigen-specific T and B cells activation. All these events culminate in synovial hyperplasia and bone destruction leading to joint swelling and deformity and to systemic inflammation.

The aim of this review is to provide an overview of the new insights about RA pathogenesis, summarising the most relevant studies published over the last year.

Genetic and environmental factors

In the last few years, the identification of new genetic loci associated with RA susceptibility, articular damage progression and also the detection of genetic polymorphisms involved in RA pathogenesis have been increased (1).

In a large UK cohort of RA patients, Orozoco *et al*. identified a new RA susceptibility locus on chromosome 22q12, identified by single nucleotide polymorphism (SNP) *rs1043099*, that was previously correlated with other autoimmune diseases. The SNP is located within a gene of unknown function that is supposed to be involved in messenger RNA splicing with regulatory activity (2). A relevant role in bone damage progression in RA has been ascribed to some genetic loci codifying for matrix metalloproteinases (MMP). Following the screening of a great number of genetic susceptibility loci for autoimmunity, De Rooy *et al*. found that two loci, that confer risk for other autoimmune diseases, are also associated with radiographic damage in RA patients. One of the two genes is involved in the production of MMP-9 that degrades collagen of the extracellular matrix, playing an important role in joint damage progression (3). A correlation was also observed between radiographic progression in RA and a cluster of SNPs of SPAG16, a gene expressed in sperm and many other tissues, and also detected in synovium and fibroblast-like synoviocites (FLS) of patients with RA and that was associated with MMP-3 secretion. Based on these data, it has been hypothesised that SPAG16 could have a pathogenetic role in bone erosion by a mechanism affecting MMP-3 levels (4). In patients with anti-citrullinated protein antibodies (ACPA)-negative RA, the association between the SNP of SPP1, a gene encoding for osteopontin, and the radiographic progression was evaluated

in a study by Juge *et al*. SPP1 was previously identified as a RA susceptibility locus and in this study it was found to be correlated also with joint destruction in seronegative RA (5). An association with RA susceptibility and disease activity was also observed for genes encoding for inflammatory cytokines and their receptors. The correlation between IL-27 gene polymorphisms and RA development has been evaluated for the first time in a study by Paradowska-Gorycka *et al*. According to the higher prevalence of the IL-27 -924A/G polymorphism observed in RA patients, more so than in controls, it is reasonable to speculate its role as a RA genetic risk factor. On the other hand, the IL-27 4730 T/C polymorphism showed a significant correlation with worse clinical disease activity and erythrosedimentation rate (ESR) value (6).

Focusing on T helper (h) 17 cell development and IL-17 and IL-22 serum levels in RA patients, Leipe *et al*. identified an IL-4R SNP associated with a lack of the inhibition of Th17 development that determines increased Th17 cell frequency, enhanced clinical activity and radiographic progression in early RA (7). An association with the risk of erosion was also observed for a haplotype of 2 SNPs of the IL-2RB gene in ACPA positive RA patients (8).

As far as the involvement of epigenetic mechanisms in RA pathogenesis is concerned, a study by Lin *et al*. showed the role of miRNA-22 in the post-trascriptional regulation of Cyr61. The expression of Cyr61, that regulates FLS proliferation and Th17 differentiation, appears to be crucial in mediating joint inflammation in RA. In fact, Cyr61 expression was found to be regulated by p53 via miRNA-22. This observation also provides an explanation for the role of somatic mutations of p53, frequently observed in RA, in the pathogenesis of the disease (9). Concerning environmental risk factors, several studies focused on the association between cigarette smoking and both RA susceptibility and radiographic progression. With regard to the pathogenetic role of smoking in RA, Ospelt *et al*. evaluated the alterations of heat shock proteins (HSP) expression in synovial tissue of smoker patients and mice exposed to cigarette extract. An increased expression of a cluster of heat shock binding proteins (chaperones) was observed in both synovial tissue and cell cultures, and authors hypothesised their role in promoting the pathogenesis of arthritis by increasing the immunogenicity of binding proteins and by activating the innate immune system via toll-like receptors (TLRs) (10). In studies evaluating the correlation between smoking and disease activity, current smoking status was associated with both poor self assessment functional status (11) and radiographic progression in seropositive RA (12). A study by Vassalo *et al*. evaluated the effect of cigarette smoke exposure on two populations of transgenic mice, carrying one of the HLA RA-susceptible genes DR4 or DQ8, following the induction of collagene-induced arthritis. Different clinical and serological outcomes have been observed in the two groups, suggesting that the role of cigarette smoking in the induction of RA-associated autoimmunity is profoundly influenced by host genetic factors (13).

Finally, genetic factors have been also related to different response to drugs in RA. Nishimoto T *et al*. showed that the single nucleotide polymorphisms of TNF receptor-associated factor 1 (TRAF1) (+16860A/G) is a genetic predictor of the response to anti-TNF treatment in Japanese RA patients (14).

Immunopathogenesis of synovitis

Innate immune response

The innate immunity mechanisms have a key role in the pathogenesis of RA and the knowledge about the connections with other immune system components is constantly growing.

The aim of recent studies focusing on this topic was the understanding of which genetic or environment factors could influence the immune response on one hand and the identification of the molecules mainly involved in the inflammation process also in order to identify new therapeutic targets on the other.

In their study, Lubbers *et al*. (15) evaluated how expression type I interferon (IFN) response genes could influence the pathological processes in the preclinical phases of RA. The authors demonstrated a statistically significant association between high expression of IFN response genes and the risk of arthritis. This aspect could be a risk factor independent of the presence of autoantibodies and could be useful to identify individuals with a higher probability to develop arthritis. The association between IFN genes expression and RA is mainly due to a greater IFN-activity that correlates with high levels of cytokines and chemokines also in preclinical phases.

The importance of IFN as a pro-inflammatory substance was further confirmed in a study by Rodrìguez-Carrio *et al*. (16), in which it was observed that high IFN-α serum levels correlate with a more severe inflammatory state, an increased disease activity but also with a higher cardiovascular risk, probably due to an endothelial progenitor cells (EPC) imbalance, namely, greater concentration of more immature EPCs.

Regarding the identification of new inflammation substrates, the hypothesis about unknown pathogenetic pathways are numerous. In a study by Pratesi *et al*. (17), it was observed that ACPA react with citrullinated histone 4 (H4) contained in neutrophil extracellular traps (NETs). This study opens new insights into the connections between innate and adaptive immunity. If these observations were confirmed, identifying consistent differences in H4 deamination levels in RA compared to healthy subjects, NETs would be identified as a site where autoantigens interact with the autoantibodies and adaptive immune cells.

In the aberrant immune response occurring during RA pathogenesis, not only neutrophils but also monocytes play a key role in the inflammation processes. A recent study accounted for the interaction between TLR-4 and the alarmins myeloid-related protein (MRP) 8/14 as a probable mechanism of monocyte activation. Indeed, MRPs are molecules primarily involved in cell homeostasis, which also act as extracellular danger signals. The authors observed that MRP8 is able to activate the expression

of antiapoptotic pathways, thereby protecting the MRP stimulated monocytes by experimentally-induced apoptosis (18).

Another key player in the scenario of RA pathogenesis is represented by FLS, mainly through cytokine relapse. However, FLS are involved also in a variety of other pathological mechanisms, for example, they are a key site of immunological response to hypoxia, that usually characterises the microenvironment of inflamed RA joints. Recently Hu *et al*. (19) attempted to clarify the effects of hypoxia in RA pathogenesis and particularly the effects of its regulator, hypoxia-inducible factor 1 alpha (HIF-1 α) on RA-FLS. This study showed that HIF-1 α, potentiates the production of inflammatory cytokines such as MMPs and vascular endothelial growth factor (VEGF) in RA-FLS. Therefore, since HIF-1 α is able to mediate the recruitment of monocytes, T and B lymphocytes in rheumatoid synovium, and to induce cartilage destruction and angiogenesis via cytokines relapse, it seems to be a key molecule in RA pathogenesis and a promising therapeutic target.

As far as other cytokines are concerned, it is worth mentioning the new insights into the role of IL-23/IL17 axis in RA. The IL-23/IL 17 axis is able to induce a positive inflammatory loop promoting the secretion of IL-6, IL-8 and tumour necrosis factor (TNF)-α. IL-6 itself is a stimulus that further boosts Th17 response. Lee *et al*. (20) have recently observed a higher IL-17 concentration in serum and synovial fluid from RA patients with respect to OA patients. These data were confirmed by immunohistochemical staining that showed greater IL-17 and TLR expression in synovial tissues from RA subjects. The authors also demonstrated that the expression of these molecules increases after the incubation of FLS with IL-23. It appears clear that IL-23-Il-17 axis plays an important role in joint inflammation and TLR expression. On the basis of these data, the authors provided additional evidence regarding this positive feedback mechanism that perpetuates synovial inflammation in RA and underlined that the blocking of this pathway may be an interesting therapeutic target.

In a recent study by Müller *et al*. (21) it has been reported that pro-inflammatory cytokines, including IL-6 and TNF-α, are also able to differentially regulate the long non-coding RNAs (IncRNA) transcipts in cells of the innate immune system, namely RA monocyte. It appeared that the treatment with anti-TNF-α or anti-IL6 was able to induce a consistent up-regulation of IncRNA, and such effect was more pronounced in the TNF-α treated group. Taken together, these findings may suggests that IncRNA may be anti-inflammatory regulators, which can prevent monocyte differentiation into activated macrophages.

Adaptive immune pathways

The adaptive immune system is another leading actor in of RA pathogenesis and knowledge about the main pathways abnormally activated in this disease has progressively improved.

The most recent data show that among the several subpopulation of dendritic cells (DCs), the myeloid DCs are significantly increased in RA-SF with respect to peripheral blood (PB) and are involved in the activation of self-reactive T-cells through the increased production of IFNγ, IL-17, IL-4 and chemokines (22). The chemokine stromal cell- derived factor (SDF)-1, also known as CXCL12, appears to play a role in the process of bone destruction in RA through RANKL-mediated up-regulation of osteoclast and the expression of TNF-α by $CD4^+$T-cells FLS. Furthermore, SDF-1, RANKL and TNF-α are abundantly expressed in RA synovium compared to OA synovium (23). Given that B-cells may play the role of antigen presenting cells (APC) and expose a variety of acquired antigens, including autoantigens, Knight *et al*. generated B-cells expressing a unique B-cell receptor (BCR) specific for aggrecan (A20-agg, a component of cartilage extracellular matrix) and demonstrated that A20-agg B-cells acquire aggrecan and drive the activation of $CD4^+$ T-cells specific for the major arthritogenic epitopes within the G1 domain of aggrecan (24).

T-cell costimulation through the interaction between inducible T-cell costimulator (ICOS), a member of the CD28 superfamily, and ICOSL, expressed on B cells, macrophages and DCs, is required for the development of proteoglycan-induced arthritis since the complete inhibition of the arthritis progression has been observed in both ICOSL-/- mice and B-cell-specific ICOSL -/- chimeric mice (25). Recently Chu *et al*. demonstrated the presence of specific staining of T-follicular-helper cells (Tfh, also called $CD4^+CXCR5^+ICOS^+$ Tcells) in rheumatoid synovium that were completely absent in OA synovium and in normal synovial tissue (26).

T cell-mediated adaptive immunity results in the subsequent generation of autoantibodies by B cells and/or activation of different T subsets. Recently, attention has been more and more focused on the role of Th17 cells as the differentiation of naïve T cells into Th17 and the suppressed differentiation into regulatory T-cells (Treg) determine a shifting of T-cell homeostasis toward inflammation.

Monocyte-derived DCs (mo-DCs) seem to play a role in the enhanced generation of Th17 lymphocytes in RA patients through the release of different cytokines including IL-1β, IL-6 and IL-23, while tolerogenic moDCs show a defective capability to induce the generation of Treg cells (27). Some data showed that the percentage of $CD4^+CD161^+$ Th17 cells are increased in RA PB compared to normal subjects and are positively correlated with disease activity parameters like C-reactive protein (CRP), ESR, and disease activity score (DAS) on 28 joints (28) and with the circulating levels of IL-17 and IL-6 (29). In addition, the intriguing observation that $CD161^+$ Th17 cells were increased in SF from late-stage disease patients and in PB of patients at risk for developing RA, may support the hypothesis of their homing and recirculation in the bloodstream in different phases of the disease (30). Recently, the debate concerning the presence of non-T cellular sources of IL-17 was further animated by the observation by Schlegel *et al*., who reported significantly higher proportions of IL-17 producing non-T lymphocytes, mainly B

cells, but also NK cells and monocytes, in RA patients compared to healthy controls (31). In addition, besides conventional Th17 cells, also Th17/Th1 cell subsets, that are able to produce both IL-17 and IFNγ, have been described. However, while patients with active disease display higher levels of Th17 cells and lower levels of Th1 cells compared with patients with low/inactive disease, no differences in the percentage of Th17/Th1 cells was observed (29).

Treg cells normally exert their function by suppressing auto-reactive lymphocytes and prevent autoimmunity. Treg cells isolated from RA patients display lower suppressive activity associated to abnormally low expression of CTLA-4, caused by epigenetic modifications like the inability to activate the indoleamine-2,3-dioxygenase pathway induced by the methylation of a binding site within the CTLA-4 gene promoter (32). Reduced Treg control on B-cells in RA may be explained by both the inability of RA-Treg cells to suppress B-cell proliferation and the activation and resistance of RA-B cells to Treg-mediated suppression (33). In addition, Treg cells appear also to be reduced in number since from the very beginning of the disease (34).

As far as other T cells are concerned, natural killer (NK)-T cells, mainly Th1, directed against citrullinated self-proteins have been identified in RA patients, particularly in the first 5 years after diagnosis (35). However, the postulated role of NK T-cells in RA pathogenesis was in striking contrast with the evidence that PB/SF-invariant NKT-cells of RA patients show lower proliferative capacity and, therefore, may not be able in controlling the emergence of autoreactive T-cells (36). CD4-CD8 double positive (DP)-T-cells were also found more frequently in RA patients, mainly in ACPA positive than in ACPA negative, with respect to normal subjects. DP-T-cells were also found in rheumatoid synovium and showed an increased production of IL-21, IL-4 and IFNγ. These observations may underscore a possible pathogenic role also of DP-T cells in the pathogenesis of RA (37).

Similarly to T cells, B-cells contribute to RA pathogenesis both in a disease promoting role, autoantibody production, antigen-presenting functions, pro-inflammatory cytokines and chemokine secretion, and in a negative regulatory role via the release of IL-10 and transforming growth factor (TGF)-β secretion and the expression of FasL and TNF-related apoptosis-inducing ligand (TRAIL) expression.

Potentially autoreactive B-cell and plasma-cell dominant clones enriched for the IGHV4-34 gene and characterised by longer CDR3 sequences, reside in synovial tissue of multiple joints but not in PB, especially in the earliest phase of disease (38).

Synovial B cell may be supported by a proliferation inducing-ligand (APRIL), which is expressed at a higher level in the PB of seropositive RA patients compared to seronegative patients, and in SF of seropositive patients compared to other inflammatory arthritis/OA patients (39). In addition, IL7R+ B-cells play a pro-inflammatory role in arthritis, in fact Pongratz *et al*. observed that following the adoptive transfer of these cells in mice with experimental arthritis, a significant increase in clinical score and greater levels of antibodies compared to untreated mice was observed. The concurrent administration of the sympathetic neurotransmitter norepinephrine leading to the inhibition of IL-7R signalling was able to neutralise this effect (40).

The last data on IL-10 producing B-cells (Breg) showed that the proportion of Breg-precursors are similar in RA patients and normal subjects, while Breg percentages are lower in RA patients and are inversely correlated with DAS28, rheumatoid factor (RF) and ACPA levels (41). Similar results were found also in patients with new-onset RA (34). Furthermore, in newly-onset RA, the number of Breg cells positively correlated with that of Treg in RA patients, supporting the hypothesis that Breg cells support the induction of Treg cells via IL-10 secretion (34).

Beyond the joint: the burden of cardiovascular risk in RA

The medical literature assessing the prevalence and pathogenesis of the extra-articular manifestations in RA, namely cardiovascular, pulmonary, gastrointestinal, peripheral neurological, cutaneous, ocular involvement, highlighted in particular the burden of the increased cardiovascular risk (CVR) in patients with chronic erosive arthritis.

Cardiovascular disease is the leading cause of death in patients with RA. A number of epidemiological studies have shown that these patients have a risk of mortality up to 50% higher than the general population and that myocardial infarction risk (MI), as well as ischaemic heart disease in evolutionary silent heart failure and sudden death, adjusted for the classical cardiovascular risk factors, increased up to 2-fold compared with healthy control groups. Two recent studies have also shown that the increased risk of cardiovascular diseases (CVD) in RA is comparable to that observed in patients with type 2 diabetes (T2D). In view of this, recent EULAR recommendations for the proper management of patients with RA highlighted the importance of adequate control of the disease and periodic cardiovascular follow-up.

The traditional cardiovascular risk factors, such as hypertension, smoking and T2Ds, chronic use of steroidal and non steroidal antinflammatory drugs and new biologic drugs with consequent alteration of the lipid profile certainly contribute to accelerated atherosclerosis (42-47). All these factors predispose RA patients to an increased incidence of premature cardiovascular events (CVE) with respect to the general population. However, it is also well recognised that such traditional risk factors do not fully explain the close association between the systemic inflammatory disease and cardiovascular mortality and morbidity in RA. We will focus in particular on two key aspects in the pathogenesis of CVD in course of RA: the chronic inflammatory state and the apparent paradoxical alteration of lipid profile.

It is widely recognised that chronic systemic inflammation represents one of the main cardiovascular risk factors; the underlying pathogenetic mechanisms seem to be summarised in accelerated atherosclerosis supported by multiple and complex aetiological factors; CRP and some pro-inflamma-

tory cytokines, IL-1, IL-6 and TNF-α not only directly promote endothelial dysfunction and structural abnormalities of the micro and macro vessels but induce alteration of further potential cardiovascular risk factors such as dyslipidaemia, insulin resistance and oxidative stress. Inflammation is involved in all stages of atherosclerosis, from training, instability and possible rupture of the plaque; it is in fact shown how some synovial inflammation pathway are common to those of vascular inflammation in atherosclerosis.

Below, a selection of the major studies conducted in 2014 on the mechanisms underlying the high cardiovascular risk in RA is reported.

In a recent study conducted by the Italian group of Lo Gullo *et al*. blood levels and biochemical characteristics of EPC, in particular $CD34^+$ inflammatory status, intracellular ox-redox balance parameters and cardiovascular risk were evaluated in 33 patients with RA comparing to 33 healthy controls. The results showed a significant reduction in number of EPC in RA patients, indirect index of reduced capacity for endothelial regeneration, and an imbalance in sense oxidation in red-ox status within those cells (like ROS/CAT, MnSOD, GPX1). These conditions are correlated in a statistically significant higher risk of a cardiovascular parameters such as carotid intima-media thickness (cIMT) and arterial stiffness (AS) (48). More recently, the same group investigated the association between inflammation and $CD34^+$ cell number, intracellular levels of reactive oxygen species and expression of toll-like receptor 3 (TLR3) and interleukin 1β (IL-1β), showing that the inflammatory status in RA is associated with an increased expression of TLR3 and of IL-1β in $CD34^+$ cells (49).

Similar results were obtained by Javier Rodriguez-Carrio *et al*. evaluating, in patients with RA and SLE, levels of IFN-alpha and some EPCs as potential markers of indirect endothelial damage; this work has shown that patients with long duration RA have high levels of IFN-α compared to SLE control patients associated to high levels of pro-inflammatory cytokines; IFN-α also seems to be directly related to blood levels of EPC suggesting an increased rate of endothelial damage and reduced ability of cell resetting by progenitor cells exposing patients to increased CVE (16).

In two studies carried out by Ikonomidis *et al* and Yoshida *et al*., respectively, it has been shown how new biologic drugs directed against pro-inflammatory interleukins have a significant role in reducing cardiovascular risk factors. The Greek study was conducted in 80 RA patients, 60 with known ischaemic heart disease and 20 without, receiveing anakinra (r-metHuIL-1ra). The results showed significant improvement of cardiovascular parameters in terms of endothelial stress, coronary and myocardial efficiency in both patient groups (50). The results of the Japanese study were very original and interesting. The role of TNF-α in the pathogenesis of RA is well established, but this cytokine is also able to induce an increase in blood pressure. In this study, conducted on 16 patients in infusion therapy with anti-TNF-α agents, it emerged that the blood pressure reduction could be attributed to the reduction in plasma levels of norepinephrine and plasma renin activity, but not dopamine and epinephrine (51). In conclusion, interesting results come from the new genomic studies, particularly in patients with chronic polyarthritis, led by Ibrahim *et al*.; analysing gene polymorphisms of IL-6 receptor they showed a significant correlation between *rs2228145* polymorphism and higher CVR (52). Altered lipid profile in RA is one of the causes of accelerated and increased rate of cardiovascular accidents. However, recent studies have confirmed that chronic inflammation is associated with a paradoxical reversal of the usual relationship between cardiovascular risk and lipid blood levels. A similar inverse relationship has also been demonstrated in the course of chronic inflammatory diseases other than RA such as sepsis, cancer and in the context of immediate post-ischaemic heart diseases, where higher CRP is associated with low levels of circulating lipids; similarly, an inverse association between IL-6 elevation and cholesterol levels was observed in patients after surgical procedures. Of great interest, several studies have shown a correlation between increased lipid levels and the reduced activity of RA following anti-inflammatory treatment. The mechanisms that explain how the inflammatory process can lead to these lipid changes are not fully understood, but may include the suppression of the reticulo-endothelial system and reduced synthesis of low-density lipoprotein (LDL) particles. Furthermore, CRP average absorption of LDL and oxidised LDL by macrophages induces LDL deposition and increases LDL uptake by hepatocytes. A recent study by Johnsson *et al*. evaluated the relationship between systemic inflammatory illness and lipid levels using CRP as the prototypical marker of inflammation. A total of 11437 blood samples were evaluated and a significant ($p<0.001$) biphasic relationship between total cholesterol level (TC) and CRP was found: TC increased within the CRP range of less than 5 mg/l, but decreased with CRP levels above 10 mg/l. There was also an inverse relationship between high density-lipoprotein (HDL) and CRP. This study confirmed that lipid levels change significantly during inflammatory illness in a population with both acute and chronic conditions like RA (53). Similar results were obtained in the study by Shang *et al*. In their work the association of serum inflammatory markers, (erythrocyte sedimentation rate (ESR) and CRP), serum lipid measures (LDL and HDL-cholesterol) with risk of MI and ischaemic stroke (IS) among RA patients was investigated. All these parameters were evaluated in 44,418 eligible RA patients; the results confirmed the non-linear association between LDL and MI, the lowest risk was observed among patients with LDL between 70 mg/L (1.8 mmol/L) and 100 mg/L (2.6 mmol/L). The association observed between LDL levels and IS was not significant. This study provides evidence supporting the hypothesis that RA-related systemic inflammation plays a role in determining cardiovascular risk and a complex relationship between LDL and CVR (54).

The other side of the coin is represented by the potential pathogenetic mechanisms underlying the apparent paradoxical correlation between inflammation and lipid levels, but particularly of certain substances secreted by adipose tissue, namely adipokines, and closely related to inflammation with protective or inductive skills on atherosclerosis process.

The role of adipokines, cytokines with endocrine-metabolic functions but that act also on the immune system in particular on the secretion of substances such as IL-1, TNF-α and IFN-γ, has been demonstrated in several studies attempting to evaluate possible correlations between altered lipid profile in obese compared to normal weight RA patients.

In different studies published by Dessein *et al*. the effect on the CV risk profile of three adipokines, leptin, retinol binding protein 4 (RBP4) and adiponectin were described.

In the first study, the authors investigated the potential impact of demographic characteristics on the independent leptin-metabolic cardiovascular risk factor and leptin-endothelial activation relationship in black and white patients with RA. In this work 217 patients were enrolled and the blood levels of leptin, soluble E-selectin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and monocyte chemoattractant protein were assessed. The results confirmed that patients with RA aged <50 years experienced an independent adiposity-driven leptin-endothelial activation relationship in the absence of leptin-metabolic risk factor associations. Young but not older patients with RA may sustain obesity-induced endothelial activation that is directly mediated by leptin (55). In the second study RBP4 levels in PB of RA patients were evaluated. This protein belongs to the lipocalin family and is the specific carrier for retinol, vitamin A alcohol, in the blood delivering retinol from the liver stores to the peripheral tissues; recent studies included RBP4 in adipokine family. RBP4 concentrations are also associated with inflammatory markers and successful lifestyle intervention in obese subjects results in reduced RBP4 concentrations that are closely related not only to decreased insulin resistance, lower triacylglycerol levels and blood pressure but also reduced systemic inflammation. However this study shows a paradoxical adipokine – CVD risk association, in particular between RBP4 concentrations, metabolic risk and endothelial activation. Such a relationship seems to be a compensatory change in adipokine production in the presence of chronic vascular disease and aimed at reducing metabolic risk. However, the authors found that the presence of plaques does not influence the impact of RBP4 concentrations on CVR. By contrast, the inverse RBP4 - CVD risk associations were mostly reproduced only in patients with adverse traditional or non-traditional cardiovascular risk profiles (56).

In the third study, the authors examined the potential impact of adiponectin on carotid ultrasound in 210 (119 black and 91 white) RA patients. Total adiponectin concentrations were smaller in patients with metabolic syndrome compared to those without. Both total and high molecular weight (HMW) adiponectin concentrations were larger in patients with more severejoint deformities. Total and HMW adiponectin concentrations were associated with carotid artery plaque in patients with metabolic syndrome. The results confirmed that in RA patients with abdominal obesity or not clinically evident joint damage, adiponectin concentrations are reduced but also associated with decreased carotid atherosclerosis (57).

Another study, conducted by the same group, evaluated the role of osteprotegerin (OPG). OPG, also known as "osteoclastogenesis inhibitory factor"(OCIF) or "tumour necrosis factor receptor superfamily member 11B" (TNFRSF11B), is a cytokine receptor and a member of TNF receptor superfamily. OPG specifically acts on bone, increasing bone mineral density and bone volume, but recent study hypothesised that OPG may be the link between bone and cardiovascular disease, in particular vascular calcifications. OPG concentrations and those of other endothelial activation molecules were measured in 34 patients who were treated with infliximab (IFX), both immediately before and after an IFX infusion. cIMT and plaque were determined by ultrasound in 27 of the study participants. The results demonstrated that OPG levels are independently associated with endothelial activation and carotid atherosclerosis in RA. Reductions in OPG concentrations upon IFX administration are associated with decreased endothelial activation (58). In conclusion, the burden of increased CVR in patients with RA is well-established, and recent studies shed additional light on the inflammatory pathways involved in such a risk. Therefore, a thorough cardiovascular follow-up is mandatory in RA patients to ensure that traditional cardiovascular risk, as well as disease-related risk, are kept under control. The importance of a proactive approach in controlling the CVR in RA patients is confirmed by a recent nationwide study on patients with recent-onset disease who received consistent and early RA medication in the biologic drugs era; in this study no increased risk for CV mortality compared to the general population has been reported (59).

Conclusion

The pathogenesis of RA results from the interaction between genetic and environmental factors leading to innate and adaptive immune response and to systemic inflammation. Despite the recent advances in the knowledge of this field, several aspects still need to be fully elucidated and, although intriguing, the majority of observations require to be confirmed in additional studies. The identification of new loci associated with RA susceptibility, radiological findings, severity of joint destruction and inflammatory cytokines expression further supports the need to tailor patient follow up and therapeutic strategies. In addition, some genetic abnormalities are strongly associated with the characteristics and the extent of the immune response hence it is reasonable to speculate the possible employment of such data in a preclinical phase as screening tools to predict disease onset. Furthermore, the better

characterisation of aberrant immune responses occurring in the pathogenesis of RA and, possibly, the identification of pathways that characterise early stages of the disease or identify subgroup of patients with peculiar clinical features, would facilitate the development of both predictive tools and new therapeutic strategies. Finally, the clear association between RA and CVD underscores the importance to identify patients at higher risk and perform a careful cardiovascular follow up.

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