Review

What is causing my arthritis, doctor? A glimpse beyond the usual suspects in the pathogenesis of rheumatoid arthritis

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Summary

Rheumatoid arthritis (RA) is a common, but heterogeneous, disease. Usually, when it comes to the pathogenesis of RA the physician faces a complex network of cytokines and cells of the immune system—the so-called effector level. However, is this network ‘the cause’ of the disease? Or is this rather the level most physicians are somewhat familiar with, as modern anti-rheumatic medications are having their targets there? In this review, we are looking beyond the usual culprits from the physician’s perspective and discuss how other factors, such as genes, epigenetics, environmental factors, local joint characteristics or processes of aging might influence the clinical phenomenon RA.

Introduction

Rheumatoid arthritis (RA) is a common inflammatory rheumatic disease. Its prevalence is estimated to be ~1%.1,2 Many physicians are familiar with signs and symptoms of this disorder as well as its treatment. However, despite its frequency many physicians are less familiar with the underlying mechanisms responsible for the development of RA. Physicians reading reviews about the pathogenesis of RA often face an endless list of cytokines, cells, proteins and many more biologic effectors, which undoubtedly have a role in RA. Likewise, many reviews tend to focus on one aspect of the disease and leaving out other relevant mechanisms. This makes it difficult to reach a holistic view on the mechanisms that are involved in the development of the disease. Furthermore, in daily clinical practice one often has to recognize that the disease behaves differently from patient to patient. To provide an overview of the different principal mechanisms involved in the pathogenesis of RA, we summarized relevant aspects in this review, which was written from the physician’s perspective.

Doctors are aware of RA as a disease that affects the joints, as this is the main clinical manifestation. At second sight it becomes obvious that RA is a systemic disease. RA can cause systemic manifestations, such as nodules, serositis, vasculitis, interstitial lung disease, Felty’s syndrome, Sjögren’s syndrome and many more.3,4 Despite that, systemic involvement is also reflected by the fact that systemic ‘abnormalities’ can be found in blood samples even before the onset of synovitis, including rheumatoid factor (RF), anti-citrullinated proteins/peptides antibodies (ACPA)5,6 and a number of cytokines.7

As a starting point it appears appropriate to look at present biologic treatment strategies to see what...
unquestionably plays a role in RA. Today, there are a number of biologic therapies available in RA, including tumour necrosis factor (TNF) alpha inhibitors, interleukin (IL) 1 antagonist, inhibitors of co-stimulation, anti-B-cell therapy and IL 6 receptor antagonist. All these biologic drugs have proved to be effective in the treatment of RA. In addition, a number of other anti-rheumatic treatment options will be available within the coming future. Despite their different mode of action, all these mentioned treatment strategies have one thing in common: they all act on the effector level. But even if cytokines and cells are important players on the effector level, they are not necessarily the cause of the disease. In the following, we want to give some insights into the different mechanism which seem to be involved in the development of RA. One of the problems in presenting these mechanisms in an order is that it may be interpreted as a form of hierarchy. In RA there does not appear to exist a ‘hierarchy’ or an ‘order’ of mechanisms. More likely, there are a number of possible defects in the biologic system which finally result in a clinical entity which is known as RA.

Despite effective treatment options there are a number of patients who do not adequately respond to therapy. At that point, we want to introduce two short cases: (A) E.K., female 34 years old, RF and ACPA positive, previously failed treatment: methotrexate, sulfasalazine, leflunomide, etanercept, chloroquine, cyclosporine A, rituximab, abatacept and tocilizumab. None of these agents could sufficiently control the patient’s arthritis. (B) S.S., female 53 years old, RF and ACPA positive, previously failed treatment: methotrexate, leflunomide, adalimumab, infliximab, rituximab, abatacept and tocilizumab. None of these agents could sufficiently control the patient’s arthritis.

How is it possible that some patients show an excellent response to treatment, whereas others fail to improve? Most likely disease mechanisms differ from patient to patient (Figure 1). Probably this also reflects a different distribution of underlying mechanisms which finally lead to arthritis. In the following, we will discuss such mechanisms that are beyond the effector level of the immune system.

Are genes the culprits?

Genes significantly contribute to the risk of developing RA and, thus, RA may be seen as a complex genetic disease. Twin studies found a disease concordance of 15.4% in monozygotic twins and 3.6% in dizygotic twins. Using quantitative genetic analysis, the heredibility of RA was estimated to be ~60%, meaning that genes account for ~60% of...
the ‘RA burden’ in the population. However, given this number it needs to be emphasized that a single gene or mutation adds only little to the total risk.

Shared epitope

The genetic locus that shows the strongest association with RA is the major histocompatibility complex (MHC) locus. The human leukocyte antigen (HLA)-DRB1 locus is estimated to account for about one-third of the genetic burden of RA. It codes for the third hypervariable region of the DR-beta-chain of the MHC complex. Some alleles code for a unique sequence of amino acids in the position 70–74 resulting in a similar 3D configuration of the encoded proteins. Those alleles are named the ‘shared epitope’ (SE). Basically, carrying the SE increases the risk of developing RA. However, the risk differs depending on the particular allele. For instance, carrying DRB1*0401 or *0405 poses a relative risk (RR) of ~3 of developing RA, the alleles DRB1*0101, *0404, *1001 or *0901 of ~1.5. Of interest, some alleles are common in one ethnic group, while they may be found infrequent in another population. However, it could be demonstrated that in different ethnic groups the SE is associated with RA, regardless of the particular alleles that are present in a given population.

PtPn22

The PtPn22 gene codes for protein tyrosine phosphatase N22, a protein that reduces T-cell receptor (TCR) activity. A single nucleotide polymorphism (SNP), R620W, is associated with a number of autoimmune diseases, including RA. In Northern America, for instance, the PtPn22 polymorphism is present in ~10% of the population. Of interest, the mentioned SNP is a gain-of-function variant.

PAd4

Peptidyl-arginine-deiminase 4 (PAd4) is an enzyme which leads to posttranslational modification of peptidylarginine to peptidylcitrulline. Because of the importance of antibodies to such proteins in RA this enzyme may be linked to the development of ACPA. In Japanese and Korean RA patients, an association with PAd4 could be demonstrated. However, in several RA cohorts of European ancestry no such association could be found. This again underlines the differences in the genetic background in RA patients, which depend on the ethnic ancestry.

Other genes

Several other RA risk loci in the human genome are associated with the disease. Today, in Europeans 31 such genes are known. These genes and their proteins are of interest rather from a pathogenetic point of view, as many of them have a particular role in immune function (e.g. TCR, B-cell receptor, etc.). However, ‘new’ RA risk loci will add only little to the RR of developing RA (RR somewhat >1), as SE and PtPn22—at least in RA patients of European ancestry—already explain a large part of the genetic burden.

Summing up this section, there is a strong and complex genetic background which predisposes to the development of RA. However, the influence of genes always needs to be interpreted in conjunction with the ancestry, as particular genes may be of relevance in one group, but not in the other.

Is there more than genes? Yes, epigenetics!

Despite the fact that each cell with a nucleus contains the whole genome the phenotype of cells may vary considerably. Epigenetic is responsible that
phenotypic cell characteristics are transmitted from mother to daughter cells. It defines heritable changes in the expression of genes without being directly encoded on the deoxyribonucleic acid (DNA) and may be seen as a non-genetic memory of function. Epigenetic modifications are able to ‘switch on’ or ‘switch off’ genes.

These processes include modification of histones. Acetylation, for instance, increases gene transcription, whereas deacetylation (mediated by histone deacetylases) leads to silencing of genes. In addition, DNA itself may be modified: methylation of cytosine reduces gene transcription. In RA, for instance, it could be demonstrated that DNA methylation is involved in the development of apoptosis resistant fibroblast-like synoviocytes (FLS). A further mechanism of epigenetic modification is guided by short RNA strands that can bind to messenger RNA (mRNA), thereby leading to deactivation of the affected mRNA strands. For instance, this mechanism is of relevance that proteins encoded by the affected mRNA do not accumulate. MicroRNAs have a detrimental role in central biologic mechanisms. In oncology, for example, they may function as oncogenes or tumour suppressors and are, therefore, under intense investigation as new targets for treatment. In the immune system, microRNA-155 is essential for the function of B and T lymphocytes as well as dendritic cells. Finally, there is evidence that microRNAs have a role in the development of aggressive FLS and arthritis in animal models.

The recognition of epigenetic modification of DNA is especially important, as it offers a plausible explanation of how environmental factors may lead to disease in a genetic predisposed subject and why genes alone do not explain the total risk of developing RA. Smoking is associated with epigenetic changes and development of cardiovascular disease. Even if not yet demonstrated in RA, smoking—a significant risk factor for RA—may also cause epigenetic modifications that result in autoimmune disease. Going further, as discussed later, aging and autoimmunity are two related processes and epigenetic mechanisms may link these two together.

Are environmental influences of relevance?

The risk of developing RA can be significantly influenced by a number of effectors. Environmental factors may be seen as external (e.g. smoking) or—in a broader definition—internal factors (e.g. hormones or stress).

The probably strongest and best investigated environmental factor is ‘smoking’. It could be demonstrated that smokers have a RR of ~1.4 to develop RA when compared with non-smokers. It has been hypothesized that smoking in the presence of a SE is linked to increased protein citrullination, which in turn may lead to the development of ACPA. However, the interaction between SE and smoking is complex and not all studies could find an association. As smoking is an important and modifiable risk factor, its relevance needs to be emphasized.

‘Infections’ may be involved in the pathogenesis of RA as those may act as an unspecific stimulus of the immune system. For instance, periodontitis, caused by porphyromonas gingivalis, is associated with the development of RA. However, it is not finally clear whether one is causing the other or both reflect an underlying common defect. Also viruses, such as the Epstein-Barr-Virus (EBV), have been associated with RA. Previously, it could be demonstrated that EBV DNA/RNA was significantly more often detected in synovial tissue from RA patients than from controls. Of note, the presence of the SE in RA patients further enhanced the risk of a positive test on EBV DNA/RNA. Research has led to the hypothesis that molecular mimicry between particular EBV proteins and components of the SE as well as other proteins in RA patients are involved in the pathogenesis of RA. Further studies indicated that RA patients have a decreased T-cell response to a specific EBV associated glycoprotein (EBV gp110), which might result in poor control of EBV infection, chronic exposure to other EBV antigens, and thus to a chronic inflammatory response in patients with RA. In that study, SE positive and SE negative RA patients did not differ with regard to their response. However, to date it is not clear whether the association of EBV and RA is causative or not. While EBV may trigger the immune system or cause molecular mimicry, the presence of EBV and RA both may be a consequence of a common defect in the immune system. Parovirus B19 is a further virus that has been linked to RA and other autoimmune diseases. While parovirus B19 can cause arthritis, it was also suggested that the virus can trigger the development of RA. Besides local viral replication further proposed mechanisms are autoantibody production (e.g. against collagen II), cytokine production (e.g. IL 6) and persistence of the virus in human tissues (e.g. the synovium). Of interest, patients with acute parovirus B 19 infection were found to be more frequently carriers of the SE as compared with controls and HLA-DR4 was
reported to be associated with a prolonged course of arthritis. However, as for EBV it is not evident whether the association of the virus and RA is causal or not.

In addition, microbial superantigens, which are capable to stimulate 1 of 10 T cells instead of ~1 of 10,000 cells by specific antigens, may act as a driver in the development of RA. Besides the adaptive immune system the innate immune system plays a role in the pathogenesis of RA. One principal way to activate cells of the innate immune system is via pattern recognition receptors (PRRs), which constitute one of the primary host defence mechanisms. A large number of different PRRs, such as Toll-like receptors, are known. These PRRs can be stimulated not only by pathogen-associated molecular patterns but also by endogenous danger-associated molecular patterns (DAMPs). Activation of PRRs results in the induction of a pro-inflammatory cascade. In the following, pro-inflammatory signals can trigger further tissue damage leading to increasing DAMPs levels and, thereby, perpetuating a vicious cycle of damage and inflammation. The role of inflammasomes, which play a central role for instance in gout (due to their ability to produce IL-1beta), still needs to be defined in RA.

In conclusion, there are some clues that infections may play a role in the development of RA, but a final proof to support this concept is still lacking. Some further environmental factors are under discussion: ‘Silica’ was found to be associated with RA by some. ‘Stress’ and ‘oestrogens’ may increase the risk of developing RA. ‘Alcohol’ consumption and ‘breast feeding’ appear to reduce the risk, whereas higher ‘birth weight’ and lower ‘socioeconomic status’ increase the RA risk.

At this point it may be appropriate to have an interim analysis. That far, several different factors have been discussed which are of relevance in the development of RA. In an interesting study, Kallberg et al. could demonstrate that interaction between genes (in their cohorts the SE and PTPN22) and an environmental risk factor (smoking) significantly act together and potentiate the risk of developing ACPA positive RA. Notably, such interactions could not be found in ACPA negative RA, emphasizing that there appear to be substantial differences in these two subgroups of RA patients.

Is there an arthritogenic antigen in RA?

At first sight, it would be reasonable that an arthritogenic antigen within the joint stimulates the immune system and finally leads to synovitis and joint destruction. However, despite considerable effort no such specific antigen could be clearly associated with RA. Given the importance of ACPA, citrullinated peptides/proteins could be a causative target in RA. However, PADI dependent processes, which convert arginine to citrulline, are ubiquitous and are neither restricted to the joint nor to disease. Therefore, it is not straightforward to classify these proteins as arthritogenic. Today, it is not definitely clear whether antibodies to citrullinated proteins do have a causal role or are more or less bystanders of disease. Of interest, PADI is also involved in the citrullination of histones. In theory, this could also provide a link to epigenetic modification. A further possible arthritogenic antigen, human cartilage antigen glycoprotein-39, seems only to be of relevance in animal models.

Are the joints themselves responsible for the development of arthritis?

Lipsky raised the fundamental question ‘Why does RA involve the joints?’ Under normal condition, the synovium contains a lining layer which harbours macrophages and FLS. The organization of the synovium is dependent on the presence of cadherin-11, which facilitates cell–cell interactions. In the absence of cadherin-11, the synovium is disorganized and the extracellular matrix is reduced. In the case of arthritis, absence of cadherin-11 leads to an unorganized synovium. Although bone erosions still develop, pannus tissue does not form and the cartilage is preserved. The synovium harbours a number of T cells, some B cells and cells of the innate immune system, which may also form germinal centers. However, the synovium is not only populated by cells from other origins (as lymphocytes) but also contains FLS. These cells have a detrimental role in the perpetuation of inflammation and are capable of excessive destructive properties. FLS of RA patients are less likely to undergo apoptosis, show local destructive behaviour and have signs of oligoclonality—factors which give them a tumour-like behaviour. Besides their aggressive appearance RA FLS produce proinflammatory cytokines, giving them an important role in sustaining inflammation. Thus, one may argue that local joint factors are involved in the pathogenesis of RA. Classifying the joints just as ‘battlefield’ of foreign forces (as the immune system) would be an inappropriate simplification.
Which role has aging in RA?

In an interesting paper, Crowson et al. analyzed the mortality rate in a large cohort of RA patients. Using calculation models, the authors demonstrated that from a mathematical perspective, at the time of diagnosis, seropositive RA patients were 2 years older than controls and that in 10 years, RA patients aged 11.4 years. The authors raised the question whether accelerated aging might explain the increased mortality found in their RA patients. Indeed, RA patients show a number of changes compatible with premature aging. These include premature atherosclerosis, osteoporosis, muscle weakness, degeneration of elastic tissue or sleep disorders. While in normal aging these processes take place over decades, they are significantly accelerated in RA. The biologic compartment most affected by accelerated aging is the immune system. The immune system of RA patients undergoes changes which result in an immune system with characteristics of a 20-year older host. These changes include lymphopenia, T-cell oligoclonality, loss of CD 28 on T cells, a reduced number of T-cell receptor excision circle, increased peripheral proliferation of T cells, shortened telomers in T cells, and hematopoietic stem cells and failures of DNA repair systems. While aging might be a consequence of chronic inflammatory stimulation, it does not sufficiently explain why some tissues, first of all the immune system, do show such rapid changes.

What is the link of such changes in aging with RA? Aging is associated with an increasing loss of tolerance to self- or neo-antigens (as for instance citrullinated proteins). Finally, all these changes lead to immunosenescence with an overaged and auto-reactive T-cell repertoire. Following this theory, it is not necessary that an arthritogenic antigen is the cause of RA. Much more likely autoimmunity is a result of the breakdown of immunotolerance to self- or neo-antigens which finally leads to an over-reactive and auto-aggressive immune system.

Thinking these thoughts to the end results in a provocative hypothesis: RA—and its ‘complication’ coronary artery disease—are both a consequence of immune aging. RA and coronary artery disease co-occur in a host whose immune system is prematurely aged. Despite its provocative nature and final lack of proof, this hypothesis is supported by a number of observations. HLA-DRB1*04 is associated with RA, but it is also associated with loss of telomers in non-RA patients (HLA type as a ‘risk factor’ of aging). Coronary plaques in non-RA patients with instable angina show changes which resemble characteristics of RA, including the presence of numerous T cells with loss of CD28 and monoclonality. In addition, also in unstable angina a specific antigen is unlikely: it appears that a lowered TCR threshold leads to stimulation of the immune system.

Many culprits in ‘one’ disease

Going through the different mechanisms that may be involved in the pathogenesis of RA, it is obvious that RA is not ‘one’ disease. Probably seeing RA as a phenotypic malady would be more appropriate. Different disturbances may prevail in one patient, while others may dominate in another. And probably these mechanisms are also dependent on time in one and the same individual. The complex background may explain why the phenotypic appearance may vary between RA patients and, importantly, patients do respond very differently to anti-rheumatic treatment.

Further research will be necessary to elucidate which components in the biologic system—apart from the cytokines of the effector level—would be worthwhile to target in RA. While some factors (e.g., genes) will be more or less given, others (such as epigenetic mechanisms) will be modifiable and appear to be a very promising field for therapeutic interventions. Also cells besides lymphocytes (e.g., FLS or cells and pathways of the innate immune system) could become interesting targets. Having the opportunity to tackle the disease on different levels will improve the chance of achieving remission in a substantial proportion of RA patients.
Concluding remarks

In conclusion, the basis of RA is complex and heterogeneous. The ‘one’ cause does not appear to exist. RA has to be seen as interplay of many different ‘causes’. In this review, we present different mechanisms that appear to play a role in the pathogenesis of RA, but it is very likely that this list by far will not be complete. It appears to be appropriate to see RA as a phenotypic group with substantial differences from one to another patient. Especially, the distinction between ACPA positive and ACPA negative RA patients seems to be of importance. A number of factors are involved in the development of RA, such as genes, epigenetics, environmental influences, local joint characteristics and—very likely—mechanisms of aging. Finally, the existing evidence argues against the presence of an arthritic antigen. More likely the breakdown of self-tolerance of the immune system appears to have a central role. Finally, it needs to be underlined that a single cause for the development of RA is unlikely to exist. The interplay of numerous disturbances in the biologic system results in the phenotypic disease RA.

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