

Rheumatoid arthritis and the concept of autoimmune disease

We critically review the concept of autoimmunity in Rheumatoid Arthritis (RA). To classify a disease as autoimmune, it is necessary to demonstrate that the immune response to a self-antigen causes the observed pathology. In RA, neither autoantigens nor autoreactive T-cell clones were found. Molecular mimicry exists in some cases, e.g. after *Proteus mirabilis* infection. In some patients, cross-reactivity between microbial DnaJ and human HSP40 can occur. However, none of the autoantibodies described in RA, with perhaps exception of Anti-Citrullinated Peptides Antibodies (ACPAs), are responsible for the onset of disease and tissue damage. ACPAs appeared early in the disease process and could activate macrophages. In turn, this leads to the release of pro-inflammatory cytokines that stimulate synovial fibroblasts. In this disease, neither autoantibodies, nor leucocytes destroy bone and cartilage. This is caused by aggressive epigenetically modified synovial fibroblasts. Current treatments did not directly target those cells. The term “autoimmune disease” for RA should be used with caution until the link between immune phenomena and joint damage has been elucidated. However, we have to be open and to accept that RA is a heterogeneous disease. This is reflected by the large variability of responses to immunosuppressive drugs or biologicals.

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Introduction

Rheumatoid Arthritis (RA) is the most common autoimmune disease with 0.5 to 1% prevalence worldwide. In many textbooks of medicine, RA is presented as a typical systemic autoimmune disease. This is no more so obvious in the latest edition of Harrison's principles of internal medicine [1,2]. We will review here the reasons. Hallmarks of RA include immune dysfunctions, inflammation, synovial hyperplasia and joint destruction. Arguments in favour of the autoimmune disease concept are production of autoantibodies, genetic association with HLA-DRβ1 polymorphism, release of pro-inflammatory cytokines and chemokines, infiltration of leucocytes into the inflamed synovial tissue and beneficial effects of anti-inflammatory and of some immunosuppressive therapies.

Autoimmunity refers to the presence of autoantibodies or T-cells that react with self-antigens. This doesn't necessarily imply that the self-reactivity has pathogenic consequences [1]. Physiological autoimmunity is present in all individuals and increases with age. Polyreactive autoantibodies are essential e.g. to remove apoptotic debris. When autoimmunity is induced by an inciting event, e.g. tissue damage, it is in general limited.

Backed on Koch's postulates of infection, clear criteria were developed to distinguish

“autoimmune diseases” from physiological autoimmunity [3-5]. Thus, to classify a disease as autoimmune, it is necessary to demonstrate that the immune response to a self-antigen causes the observed pathology [1]. Classically, autoimmune diseases are attributed to a breakdown of tolerance when lymphocyte clones fail to discriminate between self and nonself [6]. This has necessarily to do with interactions of T-cells and antigen-presenting cells, recognition of one or more defined autoantigens and production of specific pathogenic autoantibodies [1].

Literature Review

Humoral immunity

To establish the role of an autoantibody in disease pathogenesis, it must be [3,4]:

- Detectable in the blood or injured tissue
- Responsible for the onset of disease and tissue damage
- Shown to correlate with the clinical manifestations
- Able to replicate the disease in an experimental model and/or to passively transfer the disease

In RA, prominent autoantibodies are IgM-rheumatoid factors (IgM-RF), anti-type II collagen antibodies and Anti-Citrullinated Protein Antibodies (ACPA); none of them fulfil the last 3 criteria. RA is not alone with this

problem, for example an important subset of patients with systemic sclerosis are positive for Scl-70 antibodies; in spite of a high specificity, the pathogenic role of those autoantibodies is hypothetical only [7]. This does not keep most authors from calling it an autoimmune disease.

IgM-RF and IgA-RF are diagnostic and prognostic markers, respectively. However, IgM-RF can also appear during various infection, e.g. with influenza A virus [8]. ACPA are more specific and useful diagnostic tool to detect early RA [9]; however, ACPA also have been observed in lung diseases and in normal smokers. The naturally occurring epitopes recognised by these autoantibodies are not precisely characterised [10]. In addition, ACPA are very heterogeneous in terms of fine specificity in an individual patient and among different patients.

A natural “experiment” of passive transfer is the pregnant RA patient; however, a successful pregnancy, normal delivery, and a healthy infant is the usual and expected course [4]. Moreover, pregnancy has a known beneficial effect on the course of the disease.

Cellular immunity

Autoimmune diseases occur in those individuals in whom the breakdown of immune tolerance results in self-reactivity causing tissue damage [6]. The HLA-DR β 1 “shared epitope” genetic association is an important argument in favor of an autoimmune disease. However, it explains only 15-20% of the cases. To establish the role of autoreactive T-cells in disease pathogenesis, it must be [1]:

- Shown that T-cells specific for defined autoantigens are found in patients
- Shown that monoclonal expansions of T-cells occurs, as well as a restricted V β T-Cell Receptor (TCR) gene usage
- Able to attenuate the disease by an anti-T-cell therapy
- Able to transfer the disease.

Of course, these criteria are extremely dogmatic and can be discussed.

T-cell clones able to recognize type II collagen have been isolated from blood in both healthy subjects and RA patients [3]. Clearly, they belong to physiological autoimmunity, i.e., in humans are not pathogenic and unable to provoke cartilage damage. Hypotheses about antigens recognized by autoreactive T cells have stemmed

from data on low affinity autoantibodies found in RA sera or from animal models. In recent years, the discovery of antibodies against peptides that have been modified post-translationally has opened new perspectives. Importantly, an array of citrullinated peptides fit avidly into the HLA-DR binding groove and activates T cells much more efficiently than the native protein [11]. The production of ACPA, however, probably represents a normal adaptive immune response against altered antigens rather than true autoimmunity [12]. Again, it's a question of definition.

Lymphocytes and monocytes infiltrate RA synovial tissues. However, a small minority of T cells only express activation markers that denote local stimulation, a fact consistent with a polyclonal origin of the T-cell infiltrate. The diversity of autoreactivity in RA suggests that it results from a general activation of the immune system [2]. Studies of the T-cell repertoire showed preferential use of the V β TCR families but also found that overexpressed TCRs varied across patients [13]. In contrast to the findings in animals, most studies in RA did not support the role of specific V β TCR genes [14]. Recently, however, the question reappeared. Thus, in synovial tissues of ACPA-positive RA patients, the T cell repertoire is specifically restricted [15]. However, the origin and role of these clonal alterations remain to be determined.

Adoptive transfers have been performed with RA mononuclear cells into SCID mice. High IgM-RF production occurred. However, IgM-RF levels gradually decreased with time and synovial hyperplasia or joint injury were absent. In RA, IgM-RF immune complexes and complement activation are involved in vascular endothelial injury in rheumatoid nodules, but not in joint damage.

Despite the intensive efforts put into studying the effect of depleting and nondepleting anti-CD4 antibodies, no sufficient benefit was observed that would justify pursuing this option in patients with RA [16]. In a specific genetic background (HLA-B27+), asymmetric polyarthritis can even occur in HIV-positive patients [17]. Animal models suggested that a biased differentiation of pro-inflammatory Th1 and Th17 cells might occur. Unfortunately, in humans, an IL-17 receptor antibody, failed to show clinical benefit [2]. A provocative question is whether animal models of arthritis can represent human RA. Of course, It is unrealistic

to expect a single model of arthritis to fully recapitulate human disease; however, the severe inflammatory component necessary to induce or to maintain arthritis in certain of those models can be criticized, e.g. the LPS boost in type II collagen-induced arthritis. Moreover, it has been claimed that mouse models may poorly reflect human genomic inflammatory responses [18]. However, this is a matter of debate [19].

Molecular mimicry

In animals, various joint-related molecules, among them type II collagen, glycoprotein gp39 and aggrecans/proteoglycans showed arthritogenic potentials [13]. In addition, in both animals and human, various infectious agents are associated with self-limited reactive arthritides. Molecular mimicry or cross-reactivity between a joint-related molecule or a microorganism and a self-antigen may lead to autoreactive T-cell clones, as e.g. in streptococcus-induced rheumatic fever. Animal models of arthritis have strongly suggested an association of autoimmunity with the human disease. This has caused investigators to study humans to validate an idea generated in mice, rather than vice-versa [5]. Molecular mimicry exists in some cases resembling RA, e.g. after infection with *Proteus mirabilis* [20]. A hypothesis also exists involving cross-reactivity between human Hsp40 and bacterial DnaJ [21]. At least, animal models showed that different ways can lead to arthritis. Similarly, in humans, the fact that various combinations of genes and types of environmental stress lead to arthritis suggests that we are not looking at a single disease, but at a process with multiple pathways [12]. Human RA should therefore be regarded as a syndrome, rather than a disease.

Neopeptides

A new concept is the emergence of neopeptides. Relationships of ACPA with smoking and *Porphyromonas gingivalis* were suggested by epidemiological studies. *P. gingivalis* is able to citrullinate arginine residues, thereby producing neopeptides. The causal question remains at least for non-smoker and patients with proper dental hygiene.

Innate immunity

Alternatively, recent observations suggest that ACPAs directly influence monocytes/macrophages, inducing M1 polarisation [22] and stimulating the release of IL-1 β via phosphorylation of ERK1/2 and JNK pathways [23]. In addition, interferon-gamma released by

T-lymphocytes responding to citrullinated neo-epitopes can enhance the ability of macrophage to present antigens [24]. Monocyte-derived inflammatory macrophages, which have infiltrated the synovial tissue, are the main producers of pathogenic cytokines, in particular TNF β , IL-1 β and GM-CSF [25]. Recent findings suggest that specific microRNA is responsible for the maintenance of the inflammatory state [26]. An attractive hypothesis is that RA monocytes/macrophages are “trained” [27].

Clinical clues

RA is designated as an autoimmune disease based on clinical clues for example the chronic inflammation and the favourable response to antiproliferative and immunosuppressive drugs. The number need to treat for new generation biologicals ranged from four to six treated patients to achieve one ACR50 response [28], meaning that a large proportion of RA patients are non-responders. Most efficient are etanercept, tocilizumab, and rituximab [29], suggesting roles for TNF β , IL-6 and B-cells in subsets of patients. The most persuasive argument that RA has multiple pathways leading to the same phenotype is the diversity of responses to these highly specific immunotherapies [12].

Joint damage

Great efforts have been made in the last decade regarding early diagnosis and treatment of RA; this allows to substantially slow progression of joint damage, thereby preventing irreversible disability. However, it has to be noted that autoantibodies or leucocytes did not directly destroy bone and cartilage. The link between immune phenomena and joint damage has been called “a mystery” [1]. In RA, epigenetically modified synovial fibroblasts have an intrinsic aggressive behaviour and constitute the effector cells of joint destruction [30]. They release matrix-degrading enzymes, but also chemokines and survival signals attracting CD45RO⁺ memory T-cells and CD5⁺ IgM-RF-producing B-cells into the inflamed synovial tissue. Normally, synovial fibroblasts suppress T-cell proliferation, but in RA this regulatory mechanism is deficient [31]. This allows the appearance of lymphoid follicles and germinal centre-like structures. RA synovial fibroblasts are further activated by monocytes and macrophages releasing TNF β and IL-6. Interestingly, transgenic TNF mice (TNF ^{Δ ARE}) back-crossed to a RAG1^{-/-} background still develop erosive polyarthritis, indicating a key role for this cytokine downstream of T and B cell

responses [32,33]. Current treatments- mostly anti-inflammatory- did not directly target RA synovial fibroblasts.

Discussion and Conclusion

In RA, an infectious trigger or an autoantigen associated with T-cell monoclonal activation are unknown, neither autoantibodies nor leucocytes directly cause joint damage, a polyclonal rather than a monoclonal T-cell activation occurs, immunotherapy fails in many cases to cure the disease, and synovial fibroblasts are in fact the key effectors of cartilage and bone destruction. Probably neglected is the contribution of the innate immune system, i.e., epigenetically modified inflammatory macrophages. Taken together, regarding RA, this call for greater caution in the use of the term “autoimmune disease” until the link between immune phenomena and joint damage has been elucidated. This debate will require that we discuss the possible alternative to classify RA if the term “autoimmune” is to be abolished. A term like “autoinflammatory” would underline the contribution of innate immunity to the pathogenesis. It has been suggested that abnormal activation of pattern recognition receptors, like Toll-like receptors and receptors for advanced glycation end products by endogenous danger signals (alarmins/DAMPs) are crucial for perpetuating a vicious inflammatory cycle. However, the term “autoinflammatory” is already used for familial Mediterranean fever, TNF-related periodic fever syndrome, cryopyrin-associated periodic syndrome, adult-onset Still disease, Behcet's disease and gout. Again, it would reflect only a part of the RA pathogenesis. We have to be open to any suggestion.

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