Viewpoint

HLA association with autoimmune disease: a failure to protect?

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Abstract

That certain HLA specificities are associated with predisposition to autoimmune disease does not necessarily imply that self-reactive T cells restricted to particular HLA alleles are eliciting the disease. In the present essay, we argue that HLA can be a major genetic factor in the development of autoimmune diseases without T cells being primarily involved in its initiation or perpetuation. There is now ample evidence that self-reactive, regulatory T cells can protect against pernicious autoimmunity. Hereafter, we propose that extended HLA haplotypes, such as DQ3-DR4, DQ3-DR9, DQ5-DR1 and DQ5-DR10 in the case of rheumatoid arthritis, predispose to impaired T-cell-mediated immune regulation. The haplotypes associated with impaired regulation are the combination of certain class II alleles and a yet unknown ‘amplifier’. In this model, products of the HLA class II region are not involved in the presentation of particular organ-specific autoantigens. Therefore, HLA does not predispose to autoimmune disease \textit{per se}, but rather fails to provide efficient protection.

A central dogma in immunology is that T and B cells provide the specificity of an immune response. Immunologists have built upon this idea a model of autoimmunity that is also centred on the crucial role of autoantigen-specific T and B cells. The association of certain HLA specificities or alleles with autoimmune diseases has been regarded as evidence that autoreactive T lymphocytes should be major histocompatibility complex (MHC)-restricted and recognize particular self-determinants. Disappointingly, more than 20 yr of intensive research has yielded little evidence for this concept.

An example: rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease that mostly affects the joints, although systemic manifestations are frequent. It starts with inflammation of the synovial membrane and often leads to the erosive destruction of adjacent cartilage and bone. It is a highly heterogeneous disease of unpredictable prognosis. The cause of RA remains unknown and its pathophysiology is controversial. Although it is undoubtedly characterized by autoaggression, the belief that it is autoimmune in nature is based mainly on the large proportion of lymphocytes in the joint infiltrates, the production of the so-called rheumatoid factor (RF) by B cells, and the association of certain HLA specificities with disease susceptibility. However, attempts to identify T-cell-specific determinants associated with the disease have been fruitless. Therefore, questions about the role of T cells in the immunopathogenesis of RA have been raised regularly [1]. Synovial T cells are hypoactive, and this chronic dysfunction is due to the accumulation of oxidative agents in the synovium [2]. As a result, these T lymphocytes produce low amounts of cytokines such as interleukin (IL)-2, IL-4, IL-5 and interferon (IFN)-\gamma when cultured in vitro. It is difficult to imagine that such hypoactive cells could play an active role in the perpetuation of RA, and the disappointing results observed with anti-T cell monoclonal antibody therapies are consistent with this idea [3, 4].

Synoviocytes and RA

As a consequence, there has been renewed interest in the role of synoviocytes in the pathogenesis of RA. There are two types of synoviocytes, referred to as types A and B. Type A synoviocytes are macrophage-like whereas type B are fibroblast-like [5]. They normally form a lining one or two cells deep at the interface between the synovium and the intra-articular space. In RA patients, the synovial membrane is hyperplastic and infiltrated with inflammatory cells. Synovioma-invading synoviocytes and macrophages produce large quantities...
of proteases, which are responsible for the destruction of the underlying cartilage and bone [6]. The hyperplasia is often regarded as a consequence of the production of cytokines by inflammatory cells, which is itself sustained by activated T cells. It is also not considered to be RA-specific as it has been observed in reactive arthritis and osteoarthritis. However, synoviocytes in RA patients are autonomous, to a great extent because of their autocrine regulation by IL-6 [7], a target of interest in the treatment of RA. Since synoviocytes are probably derived from monocyte precursors in the bone marrow, it has been suggested that RA is a bone marrow disorder [8].

The transformed phenotype of RA synoviocytes has led to the concept that these fibroblast-like cells could be ‘transformed aggressors’ rather than merely ‘passive responders’ [5]. Retroviruses and up-regulated proto-oncogenes have been implicated in the causation of RA [9]; the fact that the HTLV-I virus can induce an RA-like disease supports this [10]. Interestingly, highly differentiated synovial T cells are surprisingly resistant to apoptosis [11], and RA fibroblasts have been implicated in this rescue from apoptosis [11]. In this scheme, and in sharp contrast to common belief, T cells would become ‘passive bystanders’ at the chronically inflamed RA synovium.

A unique feature of the RA synovium is the formation of germinal centres. In normal lymphoid follicles these germinal centres contain follicular dendritic cells (FDCs), which can induce immunoglobulin isotype switching in B cells and the maturation of plasma cells. Interestingly, it has been shown recently that type B synoviocytes from RA patients have the intrinsic characteristics of FDCs [12]. This finding could explain the polyclonal activation of memory B cells, producing large amounts of non-joint-specific autoantibodies such as RF and antibodies against citrulline-containing determinants [13]. In this scheme, B-cell activation would be secondary to the abnormal transformation of type B synoviocytes into FDCs.

Lessons from experimental models of autoimmune diseases

Experimental models in animals have been instrumental in leading us to think that T and B cells are major initiators and effectors in autoimmunity. It is to be expected that we should find antigen-specific T and B cells as a driving force in these models in the light of the fact that immunization protocols have been designed to recruit them in the first place. It might not be surprising, therefore, that anti-CD3 and anti-CD4 treatments are therapeutically efficient in mice. In collagen-induced arthritis (CIA), an experimental model with similarities to RA, immunization with foreign type II collagens (CII) induces the production of anti-mouse CII antibodies only in mice with a susceptible MHC haplotype, i.e. H-2k or H-2q. Presentation of arthritogenic T-cell determinants by the corresponding MHC class II alleles provides help in the humoral response. However, without mycobacterial extract in the adjuvant, these mice rarely develop disease, suggesting that joint inflammation is first induced by mycobacterial components and then exacerbated by CII-specific T cells.

T cells are not always sufficient or necessary to elicit autoimmunity. In mice expressing a liver-specific MHC class I allele, autoreactive T cells against this antigen do not induce autoimmune disease without infection with Listeria monocytogenes [14]. T-cell receptor-deficient MRL mice develop a lupus-like disease with hypergammaglobulinaemia and autoantibodies against DNA and ribonucleoproteins [15]. Human type B synoviocytes engrafted into SCID mice induce pannus formation with cartilage invasion in the absence of T and B lymphocytes [16]. Some strains of mice can develop spontaneous, severe arthritic lesions without lymphocytes in the joints [17].

Autoreactive T cells protect against autoimmune disease

Ehrlich’s concept of ‘horror autotoxicus’ has been seriously challenged in recent years. The two major points relevant to this essay are (i) that self-reactive T cells are not necessarily deleted during thymic education, and (ii) that these T cells can protect against autoimmunity. The general concept emerging from recent observations in experimental models is that ‘anergic, naive’ T cells in the periphery are regulatory T cells that are constantly stimulated by their specific autoantigen [18, 19]. These T cells can protect against autoimmune disease efficiently, by producing either transforming growth factor (TGF)-β or IL-10. They are therefore similar or identical to the IFN-γ- and IL-10-producing CD4+ Tr1 cells generated in vitro in the presence of IL-10 [20].

What makes an autoantigen a potential target for regulatory T cells remains to be clarified. Candidates of interest are peptides derived from ubiquitous proteins that are over-expressed during inflammation and highly conserved during evolution, such as heat-shock proteins (Hsps) and the human cartilage glycoprotein (gp)-39 [21, 22]. Hsp peptides have been shown to be involved in immunoregulation and protection against autoimmunity [21]. Human cartilage gp-39 is over-expressed by macrophages during inflammation [22], and induced tolerance to gp-39 by nasal treatment can alter the course of CIA in mice [M. Boots, personal communication].

Other candidates are peptides derived from MHC molecules themselves. MHC peptides constitute a major fraction of the natural ligands eluted from MHC molecules [23]. We and others have shown that the immune response towards MHC peptides or MHC peptide expression correlates with protection against arthritis [24], lupus-like disease [25], Th1er’s virus-induced multiple sclerosis-like disease [26] and type 1 diabetes [27]. We believe that this protection involves MHC peptide-specific, regulatory T cells.

In RA, T cells are continuously recruited in the joints, although only a minority seem to recognize an
arthritogenic determinant [28]. A significant proportion of them produce both IFN-γ and IL-10, similarly to the regulatory Tr1 cells [20, 29]. It is tempting to speculate that such T cells, directed towards non-joint-specific determinants, infiltrate the synovium in an attempt to damp the autogressive process. There is indeed evidence that T cells, such as IL-16-producing CD8+ T cells, are involved more in the regulatory aspect of the synovial inflammation than in its initiation and perpetuation [30].

HLA class II and RA

We have shown previously that some MHC class II alleles can protect susceptible mice against CIA. A correlation exists between this protection and the immunogenicity of peptides derived from the third hypervariable region of the protective class II alleles. On the basis of these observations [21], we have re-evaluated the contributions of both HLA-DQ and HLA-DR in RA. Our analysis of the distribution of HLA-DQ-DR haplotypes in RA patients followed for 1 yr in an early arthritis clinic is summarized in Table 1 [K. Vos et al., submitted for publication]. Similar results were obtained in a cross-sectional study using Dutch and Swiss RA patients [31].

DQ3 (DQB1*03/DQA1*03 and DQB1*04/DQA1*03 in Asians) is in linkage disequilibrium with the DR4 and DR9 alleles and DQ5 (DQB1*0501/DQA1*0101(4)) with the DR1 and DR10 alleles. Table 1 shows that DQ3- and DQ5-related haplotypes are both associated with RA, but in a distinct fashion. DQ3/3 homozygotes, but not DQ3/5 heterozygotes, are significantly more at risk of developing RA than DQ3/x individuals. More DQ5 homozygotes tend to be more predisposed to RA than DQ5/x individuals. Protective DRB1 alleles carrying the motif DERAA in their third hypervariable region can overcome the DQ5-related predisposition (DQ5/DERAA versus DQ5/x; Table 1) [32], but interfere with early disease progression only in DQ3-positive patients [K. Vos et al., in preparation]. Along with this weaker susceptibility to RA, DQ5-but not DQ3-related haplotypes are also associated with an unclassified form of arthritis. Moreover, on their first visit to hospital, DQ5/x patients have a significantly less active disease than DQ3/x patients [K. Vos et al., submitted for publication].

There is a clear gene dosage effect in the association of HLA class II alleles with RA (Table 1) [33, 34]. Individuals with two doses of the DQ3-DR4 haplotypes are more predisposed to RA than individuals with a single dose, who are themselves more predisposed than individuals with no dose. The same gene dosage effect can be observed with DQ2-DR3 in systemic lupus erythematosus, Sjögren’s syndrome and Graves disease, and with DQ6-DR2 in multiple sclerosis. These observations are in stark contrast with the dominant mode of inheritance of HLA-B27-related predisposition to spondyloarthropathies, in which a B27 homodimer may present neo-autoantigens to eliciting T cells [35]. Recently, we have also observed a dominant contribution of HLA-DQ2-DR3 to sarcoid arthritis [36], a self-limiting disorder of probable infection-related aetiology.

Table 1. HLA-DQ-DR phenotypes and risk of developing RA

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Patients (n = 198)b</th>
<th>Controls (n = 306)c</th>
<th>Odds ratiod</th>
<th>95% confidence interval</th>
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<tr>
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<td>26</td>
<td>13</td>
<td>4</td>
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</tr>
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<td>18</td>
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</tr>
<tr>
<td>DQx/x</td>
<td>50</td>
<td>25</td>
<td>160</td>
<td>52</td>
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*DQ3/3, DQ5/5 and DQ3/5 represent individuals with two doses of predisposing DQ alleles, DQ3/x and DQ5/x represent individuals with a single dose, and DQ5/DERAA represents individuals with one dose of the predisposing DQ5 allele along with a protective DERAA allele. DQ3 alleles are the combinations of DQB1*03 (DQB1*04 in Asians) with DQA1*03 (but not DQA1*0501), DQ5 alleles are the combinations of DQB1*0501 with DQA1*0101(4), and DERAA-positive DRB1 alleles are *0103, *0402, *1102, *1103, *1301 and *1302.

*bDiagnosis at 1 yr follow-up in an early arthritis clinic.

*cCadaveric organ donors.

*dDQx/x individuals are used as reference.
DQB1-DQA1-DRB1 complex, encoding antigen-presenting molecules (including self-antigen-presenting molecules) and playing an essential role in immunoregulation, constitutes one such subregion. In this context, DQ3 alleles are strongly associated with impaired T-cell-mediated dysregulation. This genetic defect is transmitted as a recessive trait and is therefore overcome by most HLA class II haplotypes. In this model, DQ3 molecules have impaired presentation of self-peptides, either because of the particular instability of the HLA-peptide complexes [40] or because of an abnormality in the regulation of gene expression [41]. On the other hand, DQ5 is either neutral or only moderately associated with this dysregulation.

The actual locus (or loci) involved in the second subregion remains to be identified, but the TNF-α gene is a possible candidate in view of the beneficial effect of therapies against this cytokine [42]. An ‘amplifier’ factor in this region predisposes to abnormal and limited inflammation, independently of HLA class II. However, it is found in linkage disequilibrium with both DQ3-DR4 and DQ5-DR1/10-related haplotypes and therefore contributes strongly to the association of these haplotypes with RA. In particular, it has an important confounder effect in the observed association of DQ5 with both RA and unclassified arthritis. Because DQ5 by itself is not or is only weakly associated with impaired immunoregulation, DERAA-bearing DRB1 alleles can provide dominant and efficient protection in DQ5/DERAA individuals (Table 1). It should be noted that the same additive effect of the DQ5-DR1 haplotypes in susceptibility to autoimmunity is also observed in type I diabetes and sarcoid arthritis [36].

The distribution of the four genetic markers (DQ3, DQ5, DERAA and the amplifier) on three loci in linkage disequilibrium and on two chromosomes accounts for the complex association of HLA with RA (Fig. 1). HLA fails to protect rather than predisposes

Hereafter, we will describe a model in which self-reactive T cells regulate RA susceptibility but do not play an active role in the disease process (Fig. 2).

The initial event in RA is the activation of synoviocytes after trauma or infection. In most cases, this local inflammation subsides within days. However, one or more genetic factors, inside and outside the HLA region, are associated with aberrant activation of synoviocytes, leading to their hyperplasia and a more chronic disease. The resulting production of large amounts of cytokines, such as IL-15 [43], can recruit inflammatory cells in the joint. As extensively discussed by Thomas and Lipsky [44], the chemotraction of dendritic cells (DCs) from the periphery into the synovium and draining lymph nodes, or the recently proposed transdifferentiation of synoviocytes into FDCs, is a key event in the mutation of a local inflammation into a more chronic disease. We agree that endogenous self-peptides presented by DCs play an important role in RA [44]. However, we propose that their function is not to promote disease but rather to interfere with its progression. The amplifier would be an important genetic factor in this attraction of DCs to the joints or, more generally speaking, to the exacerbation of the inflammation.

Along with DCs, self-reactive regulatory T cells recognizing determinants such as MHC peptides (DERAA) are recruited (Fig. 2). This interaction between DCs and regulatory T cells constitutes an essential checkpoint in the control of the ongoing autoagression. DCs indeed express high levels of self-peptides, including self-MHC peptides [45], and play an active role in the maintenance of peripheral tolerance [46]. They are the major antigen-presenting cells in the autologous mixed lymphocyte reaction, and a deficiency in this reaction is associated with disease activity in autoimmune diseases such as

![Fig. 1. Distributions of the four RA-predisposing factors (DQ3, DQ5, DERAA and the amplifier) in extended HLA haplotypes and representative genotypes. Transcomplementation between the four factors in diploid individuals accounts for the complexity of the association of HLA with RA.](image-url)
Loss of immunoregulation by self-peptide-specific regulatory T-cells leads to autoimmune disease. The initial hyperplasia of the synovial membrane amplified in an autocrine fashion leads to the attraction of DCs, which in turn recruit regulatory T cells. In most cases, the resulting interaction prevents further inflammation. Abnormal T-cell regulation associated with certain HLA extended haplotypes, i.e. DQ3-DR4, DQ3-DR9, DQ5-DR1 and DQ5-DR10, leads to the loss of self-tolerance followed by polyclonal activation of T and B cells.

RA [47]. The concomitant exacerbation of the inflammatory process under the control of the amplifier and the failure of DQ3- and DQ5-restricted regulatory T cells to provide protection lead to at least local loss of tolerance. This is in turn can provide help in the polyclonal activation of B-cells, leading to the production of autoantibodies such as RF. Joint-specific T cells might be generated in the process. However, they are not a prerequisite and they are not necessarily restricted by a particular HLA allele. DRB1*0401 might present a particular determinant(s), which would explain its association with the most severe form of RA, although a confounder effect of a gene in linkage disequilibrium cannot be excluded. This constant cross-activation of B- and T-lymphocytes, monocytes, DCs and synoviocytes generates an amplification loop that leads to the chronicity of RA and its severity (Fig. 2).

Implications and predictions
Our model has several important implications.
• The search for a common autoantigen in RA is irrelevant.
• Any attempt to interfere exclusively with T-cells in the pathogenesis of RA has no chance of success, and might even worsen the disease.
• Future therapies should concentrate either on re-establishing a normal phenotype in synoviocytes or on restoring a functional immunoregulatory loop.
• More than 50% of the genetic predisposition to RA is not HLA-linked, and a significant part is probably represented by genes involved in the early phase of disease. Attempts to identify these genes should include patients with remitting undifferentiated arthritis, osteoarthritis or reactive arthritis.
• The analysis of extended MHC haplotype conformation in relation to RA should have a high priority.

Finally, we emphasize that our model is also relevant to other autoimmune disorders. As studies in both mice and rats have shown [48], common genes are probably involved in the pathology of many autoimmune diseases.

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