

Review

Association of MHC and rheumatoid arthritis Regulatory role of HLA class II molecules in animal models of RA: studies on transgenic/knockout mice

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Received: 23 December 1999

Accepted: 29 February 2000

Published: 12 April 2000

Arthritis Res 2000, 2:205–207

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Abstract

Human leucocyte antigen (HLA) class II molecules have been shown to be associated with predisposition to rheumatoid arthritis (RA). We generated HLA-DR and DQ transgenic mice that lacked endogenous class II molecules to study the interaction between the DR and DQ molecules and define the immunologic mechanisms in rheumatoid arthritis. Using collagen-induced arthritis (CIA) as an experimental model for inflammatory polyarthritis, we show that both DQ and DR are involved in predisposition or resistance to arthritis. Our studies suggest that polymorphism in DQB1 genes may determine predisposition to RA while the DRB1 polymorphism may dictate severity/protection of the disease. These mice provide powerful tools to develop immunotherapeutic protocols.

Keywords: animal model, collagen induced arthritis, human leucocyte antigen class II, rheumatoid arthritis, transgenic mice

Introduction

Over a period of nearly two decades, several studies have shown the association of various HLA class II molecules to autoimmune diseases. Since the antigens are presented as a MHC-peptide complex by antigen presenting cells, a crucial role of HLA molecules is indicated in distinguishing self and nonself. However, the pathophysiological role of the HLA associations is poorly understood, although a break in peripheral tolerance to distinguish self from nonself has been suggested to be a critical step for development of autoimmune disease. RA is an autoimmune disorder, leading to pathological damage to joints. Although etiology and candidate antigen for RA is unknown, type II collagen is a potential candidate since it is a major component of joint articular cartilage, and both anti-type II collagen antibodies and T cells specific for type II collagen have also been detected in patients. Extensive documen-

tation exists linking HLA-DR molecules that share the HV3 region with the DRB1*0401 'shared epitope' to susceptibility to RA, although the mechanism is not clear. Recently, it has been proposed that shared epitope might function by determining the charge in peptide binding pockets [1]. Alternatively, this epitope might be responsible for shaping the T cell repertoire in thymus, thereby determining the outcome of disease when the relevant antigen is presented in periphery [2]. However, since DRB1 genes occur in linkage with DQB1, it is difficult to rule out the role of DQ molecule in disease development. Studies in certain ethnic populations do suggest that DQ genes play an important role in the disease process. Recent insights into structure and function of MHC has enabled us to better understand the interaction between MHC-peptide complexes and T cell receptor along with other T cell surface molecules. Since immune response to a particular

antigen depends on the specificity and affinity of binding to MHC and T cell recognition, it is important to define the causative antigen presented by various DR and DQ molecules. In humans, most of the studies have been carried out *in vitro* using T cell lines and clones. Animal models of autoimmunity are useful to study the immunopathogenetic mechanisms in experimentally controlled conditions and also to define the causative autoantigen, thereby helping to develop therapeutic strategies for the disease.

CIA is an experimental model for RA that has been extensively studied to define the pathogenic mechanisms. Immunization of mice with heterologous type II collagen (CII) leads to development of severe arthritis in susceptible strains of mice [3]. Susceptibility to CIA is controlled by polymorphism in H2A loci (homolog of DQ) and protection by H2E loci (homolog of DR). To study the functions of HLA class II molecules in disease induction and predisposition, transgenic mice expressing HLA-DR and HLA-DQ molecules have been developed. This decade has seen a spate of research on these transgenic animals to explain immunogenetic and immunological basis of the disease [4].

HLA-DR and HLA-DQ transgenic mice for arthritis

In early studies, transgenic mice expressing an alpha or beta chain from DR or DQ molecules were generated, which showed that human class II chain could pair with mouse class II chains and interact efficiently with mouse CD4, although an antigen specific response could not be generated. Since antigen specific T cell response is important to initiate disease, it was recovered in some studies by co-expressing huCD4 along with DR transgene [5]. However, a lot of studies showed that requirement for species-matched CD4 may not be absolute. The major hurdle for deciphering the role of HLA in these transgenic mice was the presence of endogenous mouse molecules. Introduction of HLA transgenes in mice lacking endogenous mouse class II molecules [6] led to expression of HLA molecules on the cell surface, which were functional. The potential value of HLA transgenic mice as a model for RA was first illustrated in the A β o.HLA-DQ8 transgenic mice. These mice expressed functional DQ8 molecule (DQA1*0301, DQB1*0302), and also restored maturation of CD4⁺ T cells [7**]. Since DQ8 occurs in linkage with DR4, it provided an attractive model for defining the role of DQ molecules in arthritis. Immunization of A β o.DQ8 with bovine type II collagen led to a severe RA-like polyarthritis, thus establishing a novel approach to studying pathogenic autoimmune response in mice expressing human MHC molecules. Also, these mice mount a strong CD4-mediated and DQ-restricted T cell response against immunizing antigen. This study clearly showed an important role of DQ genes in predisposition to RA.

These findings led to a new hypothesis to explain the role of shared epitope, suggesting that shared epitope shapes

the T cell repertoire in the thymus by serving as self-peptide to the DQ molecule [2]. Thus, a high binding peptide should negatively select autoreactive T cells, while a low-affinity DR peptide would positively select potential self-reactive T cells. This hypothesis was explored in A β o.DQ8 mice, and the data showed that peptides derived from RA-associated DRB1 alleles fail to induce a DQ restricted response, while those from non-RA-associated alleles are highly immunogenic [8]. This again indicated that DQ genes play a dominant role in predisposition. The importance of polymorphism within the DQ loci in determining susceptibility to arthritis was further confirmed by studies in A β o transgenic mice expressing DQ6 (DQA1*0103, DQB1*0601), an allele associated with resistance to RA, which were also resistant to CIA.

To investigate the role of DRB1 molecules in RA, transgenic mice expressing functional DR molecules were generated. Introduction of H2E in A β o.DQ8 mice as well as DR2 transgene in CIA susceptible H2A^q mice protected them against arthritis. To address whether DRB1 polymorphism could modulate the response and outcome of disease in A β o.DQ8 mice, transgenic mice expressing DR3/DQ8/A β o (DRB1*0301, DQB1*0302) and DR2/DQ8/A β o (DRB1*1501, DQB1*0302) were generated. DR2 has been shown to be associated with protection in some ethnic groups, while DR3 is a neutral allele in human RA. The data on DQ/DR transgenic mice showed that DRB1 polymorphism could modulate the disease in A β o.DQ8 mice [9**]. Mice expressing DR2 were clearly protected, while the presence of DR3 did not alter the response and outcome of disease. Interestingly, the protected DR2\DQ8 mice respond to CII but secrete primarily T helper 2 cytokines, while susceptible DR3\DQ8 mice secrete higher amounts of T helper 1 and low amounts of T helper 2 cytokines similar to DQ8 mice. These data clearly showed that DRB1 polymorphism could modulate the DQ restricted CIA. Thus, presence of DR2 could change the cytokine profile from T helper 1 to T helper 2 type in DR2\DQ8 mice. Since cytokines are important mediators for inflammation, this change in profile might have been important for protection. This does indicate that DRB1 can influence the T cell repertoire, and further emphasizes the role of extended haplotypes in determining susceptibility to disease. Transgenic mice expressing only A β o.DR2 or A β o.DR3 are resistant to disease, indicating that the DR molecules alone were not sufficient for initiation of disease. This again confirmed our notion that both DR and DQ molecules are important in pathogenesis, where DQ genes may be the predisposing allele while DR molecules may be modulating the disease by being protective, neutral or exacerbative.

Studies using DR alleles associated with disease have shown that DR1 and DR4 transgenic mice get disease when mouse class II molecules are also expressed [10].

Mice expressing DR4 (DRB1*0401, DRA1*0101) suffer only mild disease in those deficient in endogenous class II molecules, even though they can mount DR4 restricted response to DR4 binding proteins. However, introduction of DRB1*0401 in H2A^q mice, resistant to porcine type II collagen (PII), makes them susceptible to severe arthritis induced by PII [11]. This showed that there is a specific gene complementation between A^q and DR4 molecules, leading to development of disease. This could involve presentation of antigens by autoreactive T cells, thus resulting in production of cytokines, which is different to resistant mice. This is supported by the fact that although many nonsusceptible mice also respond to CII and produce IFN γ , the regulatory cytokines produced are different. These data suggest that certain DR molecules can present some antigens and cause mild arthritis, but require the DQ molecule for severe disease. It has been shown recently that RA-associated DQ molecules bind many peptides derived from CII, while nonassociated DQ molecules bind few peptides [12*]. Computer modeling of the DQ6 molecule shows that it is a very compact molecule with restrictive binding [13]. The P1 pocket of all RA-associated DQ molecules is also similar [8]. Binding and presentation of immunodominant CII peptide to CIA susceptible A^q and resistant A^p has been found to differ [14*], which suggests that DQ polymorphism determines susceptibility. On the other hand, DR molecules can bind few peptides derived from CII, irrespective of their association with RA [15].

From the studies on DQ and DR transgenic mice, it can be extrapolated that gene complementation or interaction between DQ and DR molecules mediates susceptibility to RA in the human. Depending on the haplotypes carried by an individual, they could be susceptible to severe or mild disease. A homozygous haplotype for predisposing DQ and permissive DR will lead to severe disease. Also, heterozygous RA-susceptible haplotypes will result in very severe disease since there will be two predisposing DQ molecules. However, one predisposing and one protective haplotype should show less severity and low incidence. These HLA transgenic mice have the potential to further our insight into the function of HLA molecules in disease pathology and development of therapeutic protocols.

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