Review

Association of MHC and rheumatoid arthritis HLA-DR4 and rheumatoid arthritis: studies in mice and men

Lars Fugger and Arne Svejgaard*

Aarhus University Hospital, Skejby Sygehus, Denmark, and *Rigshospitalet, Copenhagen University Hospital, Denmark

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Abstract

Inherited susceptibility to rheumatoid arthritis (RA) is asociated with the DRB1 genes encoding the human leukocyte antigen (HLA)-DR4 and HLA-DR1 molecules. Transgenic mice expressing these major histocompatibility complex (MHC) class II molecules have been developed to generate humanized models for RA. The relevance of these models for understanding RA will be discussed.

Keywords: human leukocyte antigen, rheumatoid arthritis, transgenic mice

Introduction

More than 20 years ago, Stastny [1] reported that HLA-DR4 is associated with RA. Nine years later, Gregersen *et al* [2] proposed the shared epitope (SE) hypothesis based on the observation that the RA-associated DRB1 alleles encode a common sequence of amino acids corresponding to residues 67–74. Several SE-positive (SE+) DRB1 alleles have since been reported to be associated with RA and include the DR4 subtypes DRB1*0401, *0404, *0405 and *0408 as well as the DRB1*0101, *1402, and *1001 alleles. Nepom [3] has summarized the relative risk estimates for Caucasians for three of the most frequent SE+ DRB1 alleles in the Caucasian population. The relative risk is 6 for the DRB1*0401 allele, 5 for the DRB1*0404 allele and 1 for the DRB1*0101 allele. Thus, while the relative risk for individuals carrying the DRB1*0401 allele or the *0404 allele is approximately five times higher compared with that for individuals not carrying these alleles, the DRB1*0101 allele does not confer risk on its own. It has been debated whether the RA-associated DRB1 alleles are disease risk genes or prognostic markers for a more progressive disease course, and whether individuals carrying two SE+ DRB1 alleles either have a higher risk of developing disease or develop more severe disease compared with individuals with only one SE+ DRB1 allele (for recent reviews, see for example [3,4]). So far, no consensus has been reached in answering these questions, which have been addressed most recently in an unselected population based study of 680 new-onset cases with inflammatory polyarthritis, of whom 404 fulfilled the American College of Rheumatology (ARC) criteria for RA [5]. Such a study seems optimal to answer these questions. The study confirmed the association between RA and the presence of any SE allele, and thus demonstrated that the SE allele does in fact confer susceptibility to RA even though the relative risk was modest (RR = 2.3). Both of the two most frequent DRB1*04 alleles, *0401 and

CIA = collagen-induced arthritis; CII = collagen type II; HLA = human leukocyte antigen; MHC = major histocompatibility complex; RA = rheumatoid arthritis; SE = shared epitope.

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*0404, were significantly associated with RA, and it was noted that the *0404 allele apparently had the strongest effect, but this point was not formally proven because the relative risk values for these two alleles had overlapping confidence limits. This study also provided evidence that the risk conferred by carrying two SE+ DRB1 alleles was only slightly greater than the risk conferred by carrying one SE+ DRB1 allele, with the exception of SE+ combinations that included the DRB1*0404 allele. However, the latter interpretation was based on relatively few patients and needs confirmation. Future follow-up studies of the Norfolk cohort [5] will most likely provide important information about the possible role of SE+ DRB1 alleles in the clinical course of RA.

HLA-DR or HLA-DQ as the primary risk factor for RA

On the basis of observations of experimental mouse models of collagen-induced arthritis, Zanelli *et al* [6] advanced the provocative hypothesis; that it is HLA-DQ molecules that predispose to RA, whereas DR molecules are either permissive or protective. In the first variant of this hypothesis, it was proposed that it was the DQB1 locus which was responsible for the DQ association. However, a comprehensive review of the literature demonstrated that the DQB1 association with RA is secondary to the HLA-DRB1 association [7]. Zanelli *et al* [8] subsequently introduced a revised version of the DQ-association hypothesis. One of the cornerstones in this hypothesis was the observation that individuals carrying certain HLA-DQA1 alleles (DQA-RA) are highly susceptible to developing RA. Interestingly, these DQA alleles were not investigated in the reported patients or controls, but their presence or absence was deduced from the presence of certain DRB1 and DQB1 alleles. On the basis of these data we have tested the RA-association of their DQA-RA alleles against the RA-association of the SE alleles (see supplementary data below) using our previously described method [9]. It was found that the SE association is still significant when stratified for the DQA1-RA allele combination, while the DQA1-RA combination is not significant when stratified for SE, indicating that the association with SE is stronger than that for the DQA1-RA allele combination. In a subsequent report, Zanelli and coworkers [10] claim to find support for their hypothesis by introducing a new variable: homozygosity for some but not all of their DQA-RA markers. But because these data are to some extent at variance with their earlier ones, support for their hypothesis is not evident and difficult to accept. Taken together, our analyses do not support the idea that HLA-DQ molecules play a major role in the general susceptibility to RA, and demonstrate that the strongest association in RA is with DRB1 genes rather than DQB1 or DQA1 genes. This conclusion is further substantiated by three studies that also found no support for HLA-DQ encoded susceptibility in RA patients from Germany [11], Holland [12] and Australia [13].

HLA class II transgenic mice and RA

The molecular basis for the HLA-DRB1 association with RA is still unclear. One prevailing hypothesis is that the RA-associated HLA-DR molecules present self-antigens to autoaggressive T cells, which subsequently induce an inflammatory response that leads to the development of arthritis. This hypothesis is based partly on the biological role of MHC class II molecules in T cell dependent immunity and the presence of T cells in the synovial compartment, and partly on extrapolated data from other human HLA class II associated autoimmune disorders such as insulin-dependent diabetes mellitus and multiple sclerosis, and the animal models for these diseases. However, the sporadic evidence for the involvement of autoreactive T cells in the pathogenesis of RA [14], probably reflects several competing factors, some of which are related to difficulties in sampling T cells from RA patients. First, autoreactive T cells do not need to be present in large numbers; second, sampling generally occurs considerably after the inflammatory process has started, which excludes the analysis of T cells that are short-lived and/or play a role only in the initiation of the disease process; and third, patients are often on multiple immunomodulatory medications that further complicate sampling and subsequent analysis of T cell reactivity. Furthermore, it is likely that several autoantigens are targeted by inflammatory attacks, and that the relative involvement of these autoantigens may change from patient to patient and within the individual patient as disease progresses [15].

To delineate the role of RA-associated DR molecules in immune responses possibly related to RA in a less complex biological setting than RA patients, transgenic mice expressing DR4 (DRB1*0401) and DR1 (DRB1*0101) were generated [16,17]. It was initially shown that the human class II molecules in the thymus contributed to the selection of the mouse T cell repertoire, and in the peripheral lymphoid compartment mediated T cell responses to different antigens upon immunization. These studies demonstrated, therefore, for the first time, that it is possible to generate functional human MHC class II transgenic mice. Of more direct relevance to RA, it was subsequently shown that these mice were useful for identifying T cell epitopes in proteins such as collagen type II (CII), which is a candidate autoantigen in RA. The dominant DRB1*0401 and DRB1*0101 restricted T cell epitope in CII was shown to correspond to residues 261–273 [18,19], which is interesting because this epitope overlaps with the dominant CII T cell epitope presented by the mouse MHC class II molecule, I-A^q, associated with collagen-induced arthritis (CIA) [20,21]. Moreover, by defining MHC and T cell receptor contacts in CII 261–273 peptide [22] and by

generating a molecular model of the DRB1*0401 molecule in complex with this peptide [23], it was directly demonstrated that the CII 261–273 peptide matches the peptide binding specificity of RA-associated DR molecules [24]. The important question then was whether the CII 261–273 peptide is recognized by T cells from RA patients. Two recent studies have investigated this, and came to different conclusions [25,26].

In the first study [25], T cell proliferative responses to native CII and a CII 255–275 peptide (including the 261–273 peptide) were examined in RA patients, osteoarthritis (OA) patients and healthy controls. All medications were stopped 48 h before study entry. Even though the peripheral blood T cell responses to native CII were modest, the stimulation indices and the fraction of individuals with positive T cell responses were significantly higher in the RA group than in the OA patients and the healthy controls. Comparison of synovial fluid and peripheral blood samples from RA patients showed that T cell responses to native CII and the CII peptide in general were higher in synovial fluid than in peripheral blood, and that there was a good correlation between the T cell responses to CII and the CII peptide. Interestingly, those RA patients with a positive T cell response to native CII often had a shorter disease duration than those with negative responses, and positive T cells responses to CII were significantly enhanced in early disease (<3 years) compared with late disease (>3 years). Whether this correlation also extends to the CII peptide was not investigated, but is likely to be the case because of the observed correlation between T cell reactivity to native CII and the CII peptide.

In the second study, fluorescent, soluble CII 261–273 peptide–DRB1*0401 complexes (tetramers) were used to search for $CD4^+$ T cells in synovial fluid from RA patients [26]. The tetramers were shown to stain DRB1*0401-restricted and CII 261–273-specific T cell hybridomas in a specific manner, but did not stain a detectable fraction of synovial $CD4^+$ T cells. This suggests that the major oligoclonal $CD4^+$ T cell expansion set in joints from this group of RA patients does not recognize the dominant CII epitope. However, this may be partly due to the fact that nearly all patients were on multiple immunomodulatory medications on study entry, and partly due to a rather long disease duration in this RA group (mean duration of disease, 13.8 years; range, 5–28 years). It will be interesting to see whether tetramer stainings of T cells from RA patients with shorter disease duration give another result, and also interesting to compare tetramer staining and functional T cell assays directly.

Development of humanized animal models for RA

One of the goals in generating transgenic mice expressing RA-associated DR molecules was to develop humanized animal models for RA. Neither DRB1*0401 nor DRB1*0101 transgenic mice develop spontaneous arthritis [16,17], which, however, was expected as RA is a polygenic disease with genetic factors other than HLA class II. In addition, undefined nongenetic factors are thought to play an important role in the development of disease [4]. When DRB1*0401 [22,27] and DRB1*0101 transgenic mice [19] are immunized with native CII emulsified in complete Freund's adjuvant, the majority of the animals develop inflammatory arthritis, which has interesting similarities with RA and is more or less indistinguishable from classical CIA as seen in, for example, H-2q mice [28]. Somewhat surprisingly, DRB1*0401 and DRB1*0101 transgenic mice seem to be equally susceptible to CIA, which clearly is in contrast to the situation in humans, where DR4 is a stronger RA-risk gene than DR1. Furthermore, both strains develop severe arthritis, which is also at variance with the risk factor situation for RA, where DRB1*0401 is associated with more severe disease than DRB1*0101 [3]. A trivial explanation for these discrepancies arises from the obvious fact that CIA is a disease provoked by immunization with CII in complete Freund's adjuvant, and thus differs from RA. This powerful arthritis induction scheme may override the differential risks and severities conferred by DRB1*0401 and DRB1*0101 in humans, which may also depend on complex interactions with proteins encoded by other (non-HLA) loci. Such epistatic interactions are most likely difficult to reproduce in transgenic mice expressing a single human disease-risk gene. These comparisons across the species barrier demonstrate that one should be cautious in extrapolating from humanized animal models of disease to actual human diseases.

Another example of how cautious one should be in the interpretation of results from humanized animal models comes from a study on HLA-DQ8 transgenic mice [29]. These mice also develop a severe inflammatory arthritis upon immunization with CII and complete Freund's adjuvant, which merely demonstrates that the DQ8 molecule is permissive for CIA in mice. This observation, together with additional data from nonhumanized animal models of CIA, was taken as evidence for the hypothesis that DQ8 rather than DR4 confers the strongest susceptibility to RA in the DR4-DQ8 haplotype. As already discussed, this hypothesis has very short roots in human genetics, and illustrates that the development of humanized animal models should be based on careful analyses of human genetics.

Conclusion

The development of humanized animal models for RA has so far been shown to be a feasible approach, but, even taken together, the models have added very little to our understanding of this disease. The development of a new generation of humanized models in which the RA-associated HLA class II transgenes are expressed more physiologically, and where additional RA susceptibility genes are incorporated, combined with a better understanding of the

nongenetic component of the disease will provide a more optimal setting for mechanistic studies of the disease process and, ultimately, the development of new drugs.

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Author's affiliation: Lars Fugger (Department of Clinical Immunology, Aarhus University Hospital, Skejby Sygehus, Denmark) and Arne Svejgaard (Department of Clinical Immunology, Rigshospitalet, Copenhagen University Hospital, Denmark)

Correspondence: Lars Fugger, Department of Clinical Immunology, Aarhus University Hospital, Skejby Sygehus, DK-8200 N, Aarhus, Denmark. Tel: +45 8949 5300; fax: +45 8949 5333; e-mail: fugger@inet.uni2.dk

Supplementary data

On the basis of Tables 2 and 3 in Zanelli *et al* [8], the RA-association of the DQA-RA marker was tested against that of the SE marker using a 2 × 4 table [9]. This procedure is illustrated here in Tables 1 and 2 on Dutch patient and control data from Zanelli *et al* [8]. It appears that both the DQA-RA and SE markers are quite strongly associated with RA. Stratification of the two markers shows that the DQA-RA marker cannot be tested in SE-positive patients and controls because all SE-positive individuals were also DQA-RA-positive, leaving no information. When testing the DQA-RA association in SE-negative patients and controls, the association is no longer significant. Conversely, when testing the SE association in DQA-RA-positive patients and controls, it appears that this association is still significant, indicating a stronger association with SE than with DQA-RA. Unfortunately, the absolute inclusion of SE in DQA-RA patients prohibits testing of SE in DQA-RA-negative individuals. When combining the data on Dutch people with those on Swiss individuals in [8] in Table 3, the results in Table 1 are further supported: the stratification procedure gives only evidence for a stronger SE than a DQA-RA association, and it may be noted that there is no significant heterogeneity between the two data sets.

Table 1

A 2 × 4 table of DQA-RA and SE markers in Dutch patients with RA and Dutch controls

DQA-RA	SE	Patients	Controls
+	+	172	127
+	–	7	19
–	+	0	0
–	–	58	160
Total		237	306

Data from Zannelli *et al* [8]. DQA-RA, RA-associated DQA markers as defined by Zanelli *et al* [8]; SE, shared epitope for RA-associated HLA-DR markers.

Table 2

The 2 × 2 analyses [9] of the data in Table 1

Test	Comparison	a	b	c	d	Odds ratio	Fisher's *P* value
DQA-RA association		179	58	146	160	3.4	3E-11
SE association		172	65	127	179	3.7	3E-13
DQA-RA association in SE-positive	++ vs –+	172	0	127	0	ND	ND
DQA-RA association in SE-negative	+– vs ––	7	58	19	160	1.1	NS
SE association in DQ-RA-positive	++ vs +–	172	7	127	19	3.5	0.0024; *P*c = 0.029
SE association in DQ-RA-negative	–+ vs ––	0	58	0	160	ND	ND

ND, Not defined; NS, not significant; *P*c, corrected *P* value; a and b, numbers of patients with and without marker in question; c and d, numbers of controls with and without marker in question.

Table 3

Combined analyses in 2 × 2 analyses of data in Table 1 and data on Swiss patients and controls given in Zanussi *et al* [8]

Test	Odds ratio	Significance*	*P*	Heterogeneity†	*P*‡
DQA-RA association	3.4	90.5	2E-21	1.35	0.51
SE association	4.1	102.0	5E-24	0.62	0.74
DQA-RA association in SE-positive	ND				
DQA-RA association in SE-negative	1.5	1.7	0.19	1.2	0.54
SE association in DQ-RA-positive	2.9	12.7	0.0004	0.35	0.84
SE association in DQ-RA-negative	ND				

ND, Not defined. * Significance for the deviation of odds ratio from unity. † Heteroengeneity between the two sets of data. ‡ Significance for the heterogeneity.