Major histocompatibility complex class II genes and systemic sclerosis

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Systemic sclerosis is not primarily a genetic disorder, but it is generally agreed that the disease does have a genetic component. There are reports of familial occurrence, but these are few. For example, in a British study¹ of 60 families only one was multicase. In 1953 Rees and Bennett described localised scleroderma in father and daughter,² and this was followed by the publication of around 30 interesting reports of familial scleroderma covering different populations, disease manifestations, and intrafamilial relationships.³⁻⁵ These include the incidence of systemic sclerosis in three sisters in a single generation.⁶ There is a report of monozygotic twins who were concordant for the disease,⁷ but a further case of discordant identical twins.⁸ In 1984 Christie and Rodnan reported a husband and wife who both developed systemic sclerosis within a three year period,⁹ and Arnett described an 8-10-fold increase in the prevalence of systemic sclerosis in related and non-related inhabitants of a small American rural community.¹⁰ Between first degree relatives there are cases of both parent and child, and of sibs sharing the disease, though the latter may be more common. Interestingly, the age of disease onset between sibs is typically within 10 years, and the differences in age of onset between affected parents and children indicate that shared environment is the dominant predisposing factor within families.

It is apparent that certain chemicals and metabolic or hormonal factors trigger the disease in genetically susceptible people. To explain why subjects without susceptibility markers get the disease it is necessary to postulate that when the inducing agent is of sufficient magnitude the restriction may be overcome. Alternatively, there may be two forms of the disease, one of which is dependent on exposure to an environmental toxin. An increasingly higher proportion of cases of systemic sclerosis are being recorded which coincide with occupational, chemical or toxin exposure, and susceptibility in these patients seems not to be linked to gender (reviewed in ref 11). On the other hand, the idiopathic disease may be exclusively a female disorder.

The argument for genetic predisposition in systemic sclerosis is based on the following observations. The prevalence of autoantibodies characteristic of systemic sclerosis is high in the blood relatives of patients with this disease.¹ An increased incidence of antinuclear antibodies found in the spouses implicates an environmental component, but 36% of the relatives with antinuclear antibodies had clinical features of connective tissue disorders. Familial

clustering of clinical features of systemic sclerosis and related diseases, particularly Raynaud's phenomenon (which occurs in about 95% of patients with systemic sclerosis), has been reported.¹² Finally, many centres, world wide, have noted abnormal prevalences of major histocompatibility complex (MHC) alleles associated with systemic sclerosis, and this is discussed below.

Recent interest in the genes of the MHC is a result of: (a) the sequencing of most of the class II alleles, and some of the class I alleles, resulting in a more comprehensive understanding of the extent of MHC polymorphisms in man¹³; (b) a detailed understanding of the structure of these cell-surface glycoproteins derived from crystallographic analysis^{14 15}; (c) a functional analysis of antigen binding and presentation of particular alleles.¹⁶

It has been appreciated for many years that MHC molecules are involved in the presentation of antigen to specific T cells, and that the specificity of the interaction is restricted to the allotype of the MHC molecule.¹⁷ A recent advance of great significance was the determination of the three dimensional structure of a human class I molecule and its antigen binding site by x ray crystallography.¹³ It seems that the membrane distal domains of the molecule form two parallel α helices sitting on a platform of β sheets. A groove between the two helices was found to contain electron dense material considered to be peptide (processed antigen). Sequence comparisons of class II with class I gene products show that they probably have the same structural features.¹⁵ For both class I and II molecules the polymorphic residues tend to cluster on the floor of the peptide binding cleft and on the inner surfaces of the two helices. These are most likely to influence the affinity of peptide binding. Residues on the outer surfaces of the helices, some of which are polymorphic, are likely to interact directly with the T cell receptor, and represent the specificities recognised by alloantisera and monoclonal antibodies.¹⁸ Certain MHC allotypes bind particular peptides preferentially, allowing determinant selection to contribute to immune responsiveness. The binding of peptide in itself may not always be sufficient to induce a T cell response. Although certain MHC-peptide combinations can be shown to have high affinity, the combined residues may not be recognised by a subject's T cells, thus representing a T cell repertoire hole.

It is now clear that MHC class I and II molecules are similar in structure and function. In contrast, although both require processed

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antigen for presentation, the pathways leading to the combining of peptide and MHC molecules are distinct for the two classes. Class I products seem to associate with endogenous antigens, which will include peptide fragments of self proteins as well as peptides of viral origin in infected cells. Co-expression of peptide and class I molecules requires *de novo* protein synthesis, but this is not a requirement for class II mediated peptide presentation.¹⁹ Thus class II molecules have been shown to present exogenously derived peptides. As class I restricted T cells (CD8+) tend to have cytotoxic effector function, and class II restricted T cells (CD4+) appear to have helper or regulatory functions, it seems that the two compartments of antigen presentation have important and divergent functional consequences. The mechanisms of the MHC involvement in diseases associated with class I and class II alleles must therefore be considered to be different. Thus we speculate that a class II association is due to activity of a regulatory as opposed to an effector mechanism.

Until about 1984 genetic analysis of HLA molecules was by serological, biochemical, or cellular methods. Of these, only the serological method offers a practical and rapid analysis, but allows only a limited identification of DR and DQ (but not DP) alleles. Cellular typing techniques have been used to define most of the known class II alleles (Dw specificities), but these are essentially cumbersome methods. In contrast, the full extent of the polymorphisms of MHC class II loci (DR, DQ, and DP) can be defined by DNA analysis. Restriction fragment length analysis provided the first method of subdividing class II alleles at the DNA level suitable for routine use.²⁰ Although genomic sequencing is probably the most precise approach

to allele assignment, the most reproducible, rapid, and cost effective method uses a combination of polymerase chain reaction²¹ amplified target DNA with sequence specific oligonucleotides, with a resolving power of single base-pair differences. The first application of polymerase chain reaction technology to immunology was in the characterisation of MHC class II alleles, and strategies have now been published for the discrimination of DR,²² DQ,²³ and DP²⁴ alleles. The advance made by this typing method is clearly seen if the number of alleles, which can be reproducibly identified by the following methods, is compared. By serology there seem to be about 12 DR specificities, whereas with restriction fragment length analysis this is extended to about 20 alleles. Nucleotide sequencing has disclosed over 40 DRB alleles to date,¹³ and these can be identified by polymerase chain reaction sequence specific oligonucleotides.

There is as yet no clear pattern of association between specific MHC antigens or alleles and systemic sclerosis, particularly when data from North American and European centres are compared. There is agreement, however, in that where an association has been noted class II rather than class I alleles are involved. In the US early negative studies²⁵⁻²⁶ were followed up by weak positive associations with the class I MHC antigen HLA-Bw35 and the class II MHC antigen DR1.²⁷ Further north in Canada, Gladman *et al* showed a stronger association with the HLA-DR5 antigen.²⁸ Early reports from the European mainland showed an increase in the common 'autoimmune disease associated' haplotype, HLA A1-B8-DR3.²⁹ In the United Kingdom, however, weak associations were found with HLA A1-B8-DR3, HLA-Bw35, and HLA-DR1, but a strong association with DR5 was shown for the overall study group.³⁰ Thus the United Kingdom seemed to be a cross between Canada, the USA, and mainland Europe. The picture was further complicated by Japanese studies which associated the disease with HLA-DR2 and with HLA-DR4.³¹ In two reports from Australia DR5 was shown to be associated with the disease.³²⁻³³ Particular attention should be attached to one of these studies³³ as the class II alleles were defined by restriction fragment length analysis.

For Caucasoid patients it is apparent that by consensus, DR1, DR3, and DR5 each contribute to the aetiology of systemic sclerosis. Further analysis has shown that these alleles typically associate with particular disease subsets, but here there are complete contradictions between American and European studies. The table shows data for these alleles. In two studies from the USA DR1 associates with diffuse cutaneous systemic sclerosis, while in European studies the increased prevalence of DR1 is found in patients with limited cutaneous systemic sclerosis or CREST (references are given in the table). The pattern of DR5 is similar. DR3 in the USA associates with limited cutaneous systemic sclerosis or CREST, but in European patients the association is either with the overall disease or diffuse cutaneous systemic sclerosis. Data from a study of vinyl chloride disease³⁴ are

Major histocompatibility complex class II antigens, disease subsets, and autoantibodies: data from studies during the previous 10 years of caucasoid patients

Allele*	Ref	Year	Size†	Geo‡	Disease association	Autoantibody association
DR1	27	1982	237	USA	dSSc	ACA
	37	1983	125	USA	Weak dSSc	
	45	1985	44	USA	SSc	
	38	1987	35	USA	Weak SSc	
	49	1988	191	USA	ISSc§	ACA
	30	1984	54	Eur	ISSc§	ACA
	46	1987	136	Eur	CREST§	ACA
	50	1990	118	Eur	SSc	ACA
DR3	47	1981	14	USA	CREST	
	38	1987	35	USA	ISSc	
	29	1981	28	Eur	SSc	
	48	1981	21	Eur	SSc	
	34	1983	21	Eur	Severe VCD§	
	30	1984	54	Eur	Weak dSSc	
	35	1991	21	Eur	IPF§	
	46	1987	136	Eur	Male SSc	
DR5	28	1981	34	Can	Severe SSc	Scl-70
	45	1985	44	USA	SSc	
	38	1987	35	USA	Weak SSc	
	49	1988	206	USA	dSSc§	
	34	1983	44	Eur	VCD	
	30	1984	54	Eur	ISSc	
	46	1987	136	Eur	SSc	
	50	1990	118	Eur	SSc	
	32	1989	46	Aus	dSSc	
	33	1989	51	Aus	SSc	

*Only the alleles DR1, DR3, and DR5 are considered, as explained in the text.

†Size indicates the number of patients studied.

‡For each DR allele the data are arranged by the geographical (Geo) origin of the patients: USA, Canada (Can), Europe (Eur), and Australia (Aus). The European studies include data from Holland, Great Britain, Germany, and Spain.

§dSSc=diffuse cutaneous systemic sclerosis; ISSc=limited cutaneous systemic sclerosis; CREST=calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia; VCD=vinyl chloride disease; IPF=interstitial pulmonary fibrosis; ACA=anticentromere antibody.

included in the table and show that DR3 is a marker of severity. In a recent study analysing DR alleles at the DNA level we showed that DR3 in combination with DRw52a significantly associates with pulmonary fibrosis in systemic sclerosis.³⁵ DR3/DRw52a is characteristic of the MHC haplotype comprising HLA-A1, B8, and DR3. Thus DR3 may be a severity marker in the disease.

Included in the table are associations of DR1, DR3, and DR5 with autoantibodies specific for Scl-70, or topoisomerase I, and centromere. There is remarkable agreement, in view of the above, particularly with the association of DR1 with anticentromere antibodies, and to a lesser extent of DR5 with anti-Scl-70 expression. No study has shown a correlation between DR3 and the presence of these autoantibodies. The associations between anticentromere antibodies and anti-Scl-70 with limited cutaneous systemic sclerosis and diffuse cutaneous systemic sclerosis, respectively, is consistent with the MHC associations with these disease subsets found in the European studies.

The lack of correlation between study centres might in part be explained by three sources of variability:

1 Ethnic and genetic variability. This is important as it is known that in different ethnic groups different degrees of linkage disequilibrium occur between MHC alleles. For example, B7 and DR2 are found together more often than would be expected by chance in Caucasoid populations, but less often than would be expected by chance in Japanese populations. Knowledge of such associations is often useful in determining the primary disease marker.³⁶ For comparison of genetic markers between patients and controls these must be matched. Whiteside *et al* noticed that if those patients referred from more than 100 miles from the study centre were excluded then the strength of the DR1 association increased.³⁷ The DR5 prevalence in their patients is also interesting as it is remarkably similar to that found in studies which reported a significant association, but was related to a comparatively high control prevalence of DR5.³⁸

Both DR1 and DR5 have been shown to be heterogeneous at the DNA level when compared with their serological counterparts, and there are many well characterised haplotypes bearing DR3.³⁹ It is plausible that different forms of DR1, for example, will be shown to associate with the different forms of the disease shown in the table.

2 Geographical and environmental variability. The environmental contribution to this disease is unknown, except for the toxin and solvent associated diseases. The range of known agents that can induce systemic sclerosis like disease is wide.¹¹ The toxic oil related disease is characterised by a raised incidence of DR4,⁴⁰ while vinyl chloride disease is primarily associated with DR5 (DR3 being a marker of severity).³⁴ For idiopathic systemic sclerosis the hypothesis would be that different environmental agents are found in different global regions and that each might have a separate MHC association.

3 Clinical heterogeneity. There are two possibilities here. Firstly, there is the problem of uniformity in diagnosis, though a broad consensus on disease classification has been achieved.⁴¹ Secondly, within the disease the extent of skin involvement, severity, and of organ involvement varies greatly. Clearly, the different clinical subsets of scleroderma are characterised by different associated MHC alleles. There are also many cases of clinical overlap with related diseases, and certain of these diseases have a different HLA association, which might detract from the primary HLA type associated with systemic sclerosis. These are important points as we are still at the stage of determining the exact nature of the MHC association in this disease.

Summary and conclusions

1 In no ethnic group is the overall association between systemic sclerosis and the MHC strong enough for direct clinical use. MHC associations do support the classification of the disease into limited cutaneous systemic sclerosis and diffuse cutaneous systemic sclerosis.

2 Indications are that associations between specific subsets of patients with systemic sclerosis and genetic markers will assume greater importance both diagnostically and prognostically. The group with lung fibrosis look prime candidates, for example.

3 Genetic markers are useful means of relating chemically induced systemic sclerosis like disorders with the classical disease. Vinyl chloride disease provides an example.

4 Evidence is emerging of strong associations between certain genetic markers and autoantibody production; a similar story has emerged in systemic lupus erythematosus.⁴²

We believe that, eventually, genetic tests will be used to influence treatment in at least a subset of patients with systemic sclerosis but that a dramatic breakthrough will not be made until we know how the genetics of the disease relate to the primary biochemical disease characteristic—that is, the overproduction of collagen. In this respect it has been suggested that the 5' flanking DNA of dermal collagen genes is particularly susceptible to the action of Scl-70 (topoisomerase I).⁴³ A problem is how to tie this and the other observations discussed above together. The association of autoantibodies with topoisomerase I provides a tentative link between the MHC and collagen gene expression. Although the role and reason for anti-Scl-70 in systemic sclerosis is unknown, humoral autoimmunity, at least in systemic lupus erythematosus, seems to be strongly dependent on specific HLA genes.

With an understanding of the function of MHC products at the molecular level, HLA and disease associations can now be analysed on a mechanistic level. For insulin dependent diabetes mellitus it has been shown that the MHC determined susceptibility to the disease is conferred by neutral residues (Val, Ser, Ala) at position 57 of the DQ β chain, while Asp at this position correlates with resistance.⁴⁴ A similar

phenomenon has been described in rheumatoid arthritis. Although DR4 in general is associated with rheumatoid arthritis, it is heterogeneous, but a subtype of DR4 which is characterised by positively charged residues at positions 70 and 71 of the β chains is not found in patients with rheumatoid arthritis (Wordsworth B P *et al.*, unpublished data). A similar approach applied to the study of systemic sclerosis is likely to be similarly rewarding. The precise subtyping of the class II genes and the characterisation of their associated haplotypes is therefore required for a complete understanding of the contribution of the MHC to the disease. Additional genes linked to the MHC must not be overlooked, and are relevant to associations of haplotypes with the disease. Of particular interest are the recent reports of a new class of proteins, which are determined by genes in the MHC and which are considered to play a part in the assembly of the antigen peptide/MHC molecule complex.⁵¹⁻⁵³

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