

Genetics of B27-associated diseases—2

E. D. ALBERT, S. SCHOLZ, AND U. CHRIST

From the Histocompatibility and Human Genetic Laboratory, Munich, W. Germany

The association between the HLA-antigen B27 and Reiter's syndrome (RS) has been firmly established³⁰⁹ and has confirmed that the long-known relationship between RS and ankylosing spondylitis (AS) is due to a common genetic denominator. We discuss a number of questions concerning the underlying genetic mechanisms.

Relationship between AS and RS

The association of B27 with RS is slightly less strong than that of B27 with AS. Nicholls²³⁷ showed that sacroiliitis—the common denominator of AS and RS—is strongly associated with B27. A similar situation is found in psoriatic arthritis, where B27 is quite clearly associated with the presence of sacroiliitis (Table 1). But sacroiliitis is not so common in psoriatic arthritis as it is in RS.

The fact that RS and AS segregate with HLA in the same family suggests that the underlying HLA-linked gene coding for susceptibility to AS is probably the same as that for susceptibility to RS. It is, however, unclear which circumstances, genetic and/or environmental, influence the expression of disease in RS. The frequent occurrence of RS as a forerunner of AS suggests that it could be a certain group of micro-organisms causing urethritis that are—in genetically susceptible individuals—setting off a misled or uncontrolled immune response, which then causes arthritis, spondylitis, sacroiliitis, and other related conditions.

Table 1 Correlation between sacroiliitis and HLA-B27 in patients with psoriatic arthritis (Schattenkirchner and Albert 1975, unpublished)

	Sacroiliitis		Total
	Positive	Negative	
HLA-B27			
Positive	8	5	13
Negative	4	33	37
Total	12	38	50

$\chi^2 = 10.95$, $P = 0.025$ (corrected for number of comparisons¹⁸³).

Is B27 itself part of the pathogenesis of RS or AS?

The very high association with B27 could indeed favour an assumption that it is itself involved in the pathogenesis of RS or AS. If the antigen plays a direct role the degree of association between B27 and AS should be about the same in different ethnic groups. This, however, is clearly not the case. In some American Indian populations with a high incidence of AS and of B27 the association is weaker than in Caucasians. Also in the American Negro population the association is weaker than in Caucasians. In Japan, where the incidence of B27 is normally very low, the association of AS with B27 is very strong, perhaps even stronger than in Caucasians.

These findings in different ethnic groups suggest that it is *not* the antigen B27 itself which plays the role but a closely linked gene which is in strong linkage disequilibrium with B27. We may speculate that the AS gene could have arisen by mutation in an individual who happened to be B27-positive, forming a B27-ankylosing spondylitis haplotype which may have increased in frequency by local inbreeding. Later this haplotype could have spread over the world.

Degos and Dausset⁷⁸ showed that in the process of population migration and mixture the degree of linkage disequilibrium (that is, the degree of association) increases with the distance that such a haplotype has travelled. Thus one could speculate that the AS-B27 haplotype may have arisen in some North American Indian tribe or their ancestors and may have spread from there throughout the world. Then the Caucasians and the Japanese ethnic group would be the most distant ethnic groups and therefore have the strongest linkage disequilibrium for the AS-B27 haplotype.

One might reason differently and assume that the biochemical similarity of the antigen B27 with certain micro-organisms (such as, for instance, *Klebsiella*) could be why a B27-positive individual would be unable to mount an appropriate immune response against a micro-organism responsible for the lesions in RS or AS. B27 is a member of a group

of strongly cross-reacting, and therefore biochemically very similar, alloantigens which includes B7, B13, B40, and BW22. If cross-reactivity of B27 with a micro-organism was the reason for the B27-AS association it is conceivable that other very similar antigens could also convey some susceptibility for AS. We investigated this in a study of 220 patients with AS (Table 2). None of the antigens tested showed any particular prevalence in AS, making a B27 cross-reactivity with micro-organisms less likely.

A direct involvement of the antigen B27 is by no means ruled out, but it would require a number of additional factors to explain, for example, the different B27-AS associations in different ethnic groups. From all these considerations we conclude that the assumption of a susceptibility gene closely linked with HLA-B and highly associated with B27 remains the most appealing hypothesis.

Localisation of the AS susceptibility gene on chromosome 6 within the HLA-complex

From the strong association between AS and B27 we may conclude that the AS susceptibility gene must be located close to HLA-B. The question now arises whether direct or indirect clues to the localisation of the AS susceptibility gene can be got from the association with alleles of other closely linked loci such as HLA-A or HLA-C. Such information can best be derived from genotyped individuals. In a population of 220 HLA-genotyped patients with AS we determined the gene frequencies by haplotype counting. With the possible exception of A1, none of the antigens of the HLA-A locus showed a deviation which was significant after correction for the number of comparisons (Table 3).

The deviation observed for A1 just reached the borderline of statistical significance, but until confirmed in other studies we do not attribute any biological significance to it. Therefore there was no positive evidence that the AS susceptibility gene is

Table 2 Gene frequency* of B27 cross-reacting antigens in 220 patients with ankylosing spondylitis

HLA-	Patients (n=220)	Controls (n=3142)
B7	0.082	0.144
B13	0.028	0.029
B40	0.035	0.064
BW22	0.009	0.015

*Gene frequencies were determined by a method of maximum likelihood estimation of haplotype frequencies developed for the analysis of patients with HLA-associated disease.⁷

Table 3 Gene frequencies of 220 genotyped patients with ankylosing spondylitis compared with controls

HLA-	Patients	Controls	P
A1	0.090	0.154	0.015
A2	0.367	0.293	NS
A3	0.139	0.166	NS
A9	0.139	0.107	NS
A10	0.061	0.060	NS
A11	0.049	0.046	NS
A28	0.025	0.035	NS
A29	0.019	0.025	NS
AW30/31	0.020	0.013	NS
AW32	0.038	0.023	NS

NS = non-significant.

associated with A locus antigens and there is no need to localise this gene to a place between HLA-A and B, although this possibility is, of course, not excluded.

Is any HLA-A, B-haplotype particularly associated with AS?

The eleven different B27-containing haplotypes were counted in 220 genotyped patients with AS. Table 4 shows that all haplotypes containing B27 are strongly increased by an average factor of 10. To test whether any one of these haplotypes has a particularly high association with AS we investigated how the distribution of the different B27 haplotypes in the patients compared with that in the normal population (Table 5). It was very similar.

From these findings we conclude that there is—at least in our material—no evidence of an association of an HLA-A, B-haplotype combination with AS. Therefore we find no evidence for a possible compound nature of the AS susceptibility gene in which one gene would be associated with HLA-A and the other with HLA-B and both genes would together determine disease susceptibility.

Table 4 Frequencies of B27 haplotypes in 220 patients and 3000 controls

	Patients	Controls
A1, B27	0.0325	0.0040
A2, B27	0.2087	0.0172
A3, B27	0.0652	0.0054
A9, B27	0.0656	0.0041
A10, B27	0.0263	0.0018
A11, B27	0.0225	0.0016
A28, B27	0.0104	0.0019
A29, B27	0.0034	0.0010
AW30/31, B27	0.0103	0.0005
AW32, B27	0.0166	0.0021
A blank, B27	0.0267	0.0016

Table 5 *Frequency distribution of B27-positive haplotypes in patients with ankylosing spondylitis and apparently healthy B27-positive controls*

Haplotype	Per cent of all B27-positive haplotypes		
	B27-positive patients with AS	B27-positive 'normal' controls	P
A1, B27	6.7	9.7	NS
A2, B27	42.7	41.7	NS
A3, B27	13.4	13.1	NS
A9, B27	13.4	10.0	NS
A10, B27	5.4	4.4	NS
A11, B27	4.5	4.0	NS
A28, B27	2.1	4.6	NS
A29, B27	0.7	2.4	NS
AW30/31, B27	2.1	1.2	NS
AW32, B27	3.4	5.1	NS
A blank, B27	5.5	3.8	NS

Are there allelic forms of the disease-susceptibility gene (or 'protective' alleles)?

In the absence of any significant negative association compensatory decreases should be distributed evenly among the alleles depending on the gene frequency. If one allele is decreased beyond this measure this particular allele could be assumed to convey a particular protection against disease, presumably by virtue of linkage disequilibrium of a protective gene with the marker allele. This has been observed in coeliac disease, where a decrease for the antigen B7 goes beyond the degree expected to compensate for the increase of another antigen (in this case B8).⁸ In Table 6 the expected compensatory decrease in frequency of HLA-B alleles is compared to the observed decrease. There is a very good

Table 6 *Observed and expected frequency of HLA-B alleles in 220 patients with ankylosing spondylitis*

HLA-B allele	Observed	Expected*	P
B5	0.056	0.039	NS
B7	0.082	0.077	NS
B8	0.044	0.054	NS
B12	0.045	0.065	NS
B13	0.028	0.015	NS
B14	0.018	0.014	NS
B15	0.020	0.037	NS
BW16	0.009	0.009	NS
B17	0.030	0.022	NS
B18	0.020	0.023	NS
BW21	0.011	0.012	NS
BW22	0.009	0.008	NS
BW35	0.085	0.050	0.01
B40	0.035	0.034	NS
B-blank	0.019	0.051	0.04

*Expected on the basis of an even distribution of compensatory decrease.

agreement between them. By this analysis, therefore there is no evidence for the existence of any protective alleles of the AS susceptibility gene.

Mode of inheritance of susceptibility for AS and RS.

Since the ankylosing spondylitis susceptibility gene is assumed to be closely linked with an HLA-B gene we should be able to determine the mode of inheritance by studying the distribution of HLA-B homozygotes and heterozygotes. A high number of homozygotes would strongly favour a recessive mode of inheritance, and an excess of heterozygotes a dominant one. Based on a gene frequency (determined by gene counting) of 0.493 for B27—in cases of recessive inheritance—one would expect 56.4 individuals to be homozygous. Among the 200 B27-positive AS patients only 17 were B27 homozygous. This difference is highly significant ($\chi^2 = 27.6$, $P < 0.001$). We may therefore conclude that susceptibility for AS is inherited in a dominant fashion. Similar conclusions were drawn by Kidd *et al.*¹⁸² and by Thompson and Bodmer³¹² from more complicated calculations.

A dominant mode of inheritance indicates that in the pathogenesis of AS and RS there is 'too much' or wrongly directed or regulated reactivity rather than lack of it.

Conclusions

The following working hypothesis seems to be reasonably well supported. The expression of AS or RS depends on a genetically determined susceptibility coinciding with environmental realisation factors. Among the genes coding for susceptibility is one of paramount importance that is very closely linked to the HLA-B locus on the short arm of human chromosome C6. This gene is located in a chromosomal region which encodes immune responses and immune regulation. It is therefore appealing to speculate that the HLA-linked susceptibility gene represents a pathological immune response gene which codes an over-shooting or falsely directed reaction. Probably the environmental realisation factor is an infection with a virus or certain types of bacteria. The interaction between infection and immune reaction is under the modulating influence of sex and sex-dependent factors. There is no clear evidence that the HLA-linked susceptibility gene is of a compound nature or that there are allelic variants.