Two-dimensional gel analysis demonstrates no structural alteration of HLA-B27 polypeptides between patients with ankylosing spondylitis and healthy individuals

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SUMMARY After precipitation of the HLA-B27 antigen from the surface of peripheral blood lymphocytes (PBL) by means of an anti-HLA-B27 allospecific monoclonal antibody 2-dimensional gel electrophoresis was used to compare the structure of the B27 antigens derived from 5 patients with ankylosing spondylitis with that of healthy HLA-B27 positive counterparts. No significant difference in polypeptide structure was noted, which suggests that the pathogenesis of ankylosing spondylitis does not involve a structural alteration in cell surface HLA-B27 molecules.

Despite the demonstration a decade ago of the strong statistical association of ankylosing spondylitis (AS) with the HLA-B27 antigen¹ the mechanism responsible for this phenomenon has remained unclear. Theories which implicate the HLA-B27 molecule itself hold that it may act as a binding site for a foreign micro-organism, providing transport into the cell for such an invader, and this view is supported by the frequent isolation of certain *Klebsiella* species from the faeces of patients during acute exacerbations of the disease.² Alternatively, a structural similarity between the HLA-B27 determinant and the micro-organism may protect it against immunological detection. Other theories suggest that HLA-B27 is simply a detectable marker for hitherto serologically undetectable ('immune response') gene(s) with which it is in linkage disequilibrium and which is responsible for disease susceptibility.³

Any valid theory must accommodate the fact that, while the great majority of patients with AS possess the B27 marker, the converse is certainly not true, as 80% of B27 carriers are never afflicted.⁴ One such explanation would hold that the micro-organism can alter the structure of B27 molecules after infection has occurred (in those exposed to the organism) or, indeed, that only a subtype of HLA-B27 molecules is

susceptible to microbial binding. This would suggest a heterogeneity within the HLA-B27 specificity that has never been demonstrated with alloantisera. One monoclonal antibody, B27m2, does split the specificity, as it does not detect 12% of conventionally typed HLA-B27 positive individuals, but B27m2 status does not appear to correlate with disease susceptibility.⁵ More recently cytotoxic T-lymphocyte (CTL) subtyping of HLA-B27 has been described,⁶ but again neither subtype appears to be associated with disease.

The nonserological techniques of peptide mapping and gel electrophoresis have recently been used by Schwartz and colleagues to compare the structures of HLA-B27 molecules in diseased and healthy individuals.⁷ These studies, which used an alloantiserum for immunoprecipitation, revealed apparent structural identity between the 2 groups of B27 molecules, allowing for minor (and inconsistent) charge heterogeneity believed to be related to differences in the carbohydrate moieties of the molecules. Recognising the importance of clearly elucidating any significant structural alteration of the HLA-B27 molecule in disease and noting the failure of previously described anti-HLA-B27 monoclonal antibodies to precipitate the B27 molecule,⁷ we have performed further 2-dimensional gel analyses using a new allospecific anti-HLA-B27 monoclonal reagent for immunoprecipitation.

Patients and methods

Study subjects. Five HLA-B27 positive patients (4 male and 1 female) with classical AS by the New York criteria⁸ were studied, as were 4 HLA-B27 positive healthy controls (3 male and 1 female). The latter group all denied symptoms of inflammatory arthritis, had no past history of psoriasis, inflammatory bowel disease, or uveitis, and no family history of spondyloarthropathy.

Sera used. HLA-ABC m3, a murine monoclonal antibody which reacts specifically with the HLA-B27 alloantigen, and HLA-ABC m1 (also a murine monoclonal reagent) detecting a monomorphic HLA-ABC (class I) determinant have been described elsewhere.⁹

Iodination and immunoprecipitation. Peripheral blood lymphocytes (5×10^7) derived from heparinised blood by centrifugation through an Isopaque-Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient were radiolabelled with 500 μ Ci of Na^{125}I by the lactoperoxidase method and lysed with Nonidet P-40 (BDH, Poole, England).¹⁰ 500 μ Ci of ^{125}I lysate was incubated for 2 hours with 10 μ l of monoclonal mouse serum and immune complexes were precipitated with staphylococcal protein A.¹¹

Gel electrophoresis. Immune complexes were recovered in 8 M urea containing buffer and analysed as previously described.¹² The isoelectric focusing (IEF) separating gel contained 2% total ampholines, pH 3–10:pH 5–7 (1:4) (LKB, Wallac, Finland). The sodium dodecyl sulphate gel dimension was 12.5% in acrylamide. Autoradiography of dried gel slabs was carried out with preflashed XRP-5 film (Kodak) and Cronex intensifying screens (DuPont, Wilmington, Delaware, USA). Autoradiography was carried out at -80°C for between 4 and 20 days.

Results

Surface-labelled antigens, immunoprecipitated by either monomorphic anti-HLA-ABC or by the B27-specific monoclonal antibody, were examined by 2-dimensional (2-D) gel electrophoresis. Fig. 1(a) shows a typical gel obtained from the cells of a healthy HLA-B27 positive control precipitated with the monomorphic monoclonal. The spot representing β_2 -microglobulin appears as a distinct entity on the dye front and has a basic pI. Two linear clusters of spots appear at about 45 Kd and correspond to the HLA class I heavy chains. When gels obtained with the monomorphic and polymorphic antibodies were compared, the position of the B27 alloantigen (in each of the 9 individuals) always corresponded to the slightly smaller, more acidic clusters of spots.

A comparison of the 2-D gel patterns of HLA-B27

Fig. 1 2-D gel electrophoresis of ^{125}I -labelled HLA class I molecules precipitated from the surface of HLA-B27 positive PBLs. The autoradiograph resulting from precipitation with a monomorphic anti-HLA class I reagent (a) shows β_2 -microglobulin as a distinct spot toward the basic end of the dye front, while 2 clusters of spots representing the HLA heavy chains appear at about 45 Kd. Precipitation with the anti-B27 reagent (b) reveals only the slightly lighter and more acidic of these clusters, while normal mouse serum (c) precipitates neither cluster. Actin was not a significant contaminant. MW=molecular weight. K denotes kilodalton, and — represents position on gel of molecular weight marker.

molecules precipitated from the peripheral blood lymphocytes (PBLs) of 2 AS patients and 2 healthy controls is shown in Fig. 2. In each case the pattern can be resolved into 3 or 4 distinct spots as well as a short 'smear' appearing at the acidic end of the spots. Small differences occur from gel to gel, and these probably result from difficulties in reproducing exactly the same pH gradient in multiple IEF gels. However, no consistent variation in electrophoretic pattern was observed between AS patients and normal controls. The multiplicity of spots characteristic of the B27 antigen is likely to be due to nonuniform sialic acid addition to a single polypeptide backbone, as Karr *et al.* demonstrated⁷ that this charge heterogeneity could be largely eliminated by treating both groups of molecules with neuraminidase. Thus within the limits of our 2-D gel technique we are unable to demonstrate any consistent structural dif-

ference between the B27 molecules derived from spondylarthritic patients and healthy counterparts.

Discussion

Our study examined the immunochemical structure of the HLA-B27 specificity in health and disease and demonstrates no significant structural alteration in the HLA-B27 molecules derived from healthy individuals and patients with AS. Our findings corroborate and extend those of Schwartz and colleagues, who used an anti-B27 alloantiserum for immunoprecipitation, analysed B27 molecules by the 2-D gel technique and tryptic and chymotryptic peptide mapping, and similarly found no significant structural difference.⁷ It should be noted that they studied biosynthetically labelled B27 molecules, while we have precipitated ¹²⁵I-labelled HLA-B27 from the cell surface. Taken together the 2 sets of results suggest, within the limits of resolution of the 2-D gel technique, that no modification of structure of the HLA-B27 specificity occurs in diseased persons either intracellularly or once the molecule is expressed on the lymphocyte surface. Our study also utilised the precipitating properties of a monoclonal anti-HLA-B27 antibody (HLA-ABC m3) to examine the polypeptide structure of the HLA-B27 molecule and serves to illustrate the usefulness of such highly specific xenogeneic monoclonal reagents in investigating human disease.

Both our study and that of Karr *et al.*⁷ failed to detect the apparent polymorphism indicated by Grumet's 2-D gels.¹³ In this study 2 HLA-B27 positive lymphoblastoid cell lines, one bearing and the other lacking the determinant detected by the anti-HLA-B27 monoclonal antibody B27m2,⁵ were biosynthetically radiolabelled and the B27 antigens solubilised and analysed by 2-D gel electrophoresis. The HLA-B27 molecule derived from the B27m2 positive variant had a more basic pI¹³ than that of the B27m2 minus variant. As B27m2 fails to detect only 12% of conventionally typed HLA-B27 positive PBLs, the likely explanation for our failure (and that of Karr *et al.*⁷) to detect this polymorphism is the chance omission of B27m2 minus subjects from our study. It is planned to determine the B27m2 status of our study population when this serum is obtained. The polymorphism defined by B27m2 has not thus far been associated with a predisposition to any form of B27-associated spondylarthropathy, though a possible association between the B27m2 subtypes and those defined by CTL⁶ is under investigation (F. C. Grumet, personal communication).

Geczy and his coworkers have demonstrated by serological means an apparent structural alteration on the surface of lymphocytes derived from patients

Fig. 2 2-D gel patterns of ¹²⁵I-labelled HLA class I heavy chains precipitated from PBLs of patients with AS (a and b) and healthy controls (c and d). The monomorphic anti-HLA reagent was used for precipitation in (b), while the anti-B27 reagent was used in (a, c, and d). A comparison of the corresponding HLA-B27 bands shows only minor, inconsistent differences in structure which do not correlate with disease susceptibility.

with AS in that such cells are susceptible to lysis by sera raised in rabbits against certain strains of klebsiella, while B27 positive lymphocytes of healthy controls are not.¹⁴ Further, this alteration can be reproduced in vitro as B27 positive PBLs of healthy individuals are rendered susceptible to lysis following incubation with a cell-free culture filtrate of certain strains of klebsiella.¹⁵ It is tempting to postulate that the molecule altered by this process is indeed HLA-B27 itself, but 2 studies now exist which have failed to detect such a structural heterogeneity. Further, none of the 4 anti-HLA-B27 monoclonal antibodies which have been described⁹ can detect an epitope defining such an alteration in structure. Our studies suggest that some other molecule may be responsible for these changes in reactivity. Indeed there is no strong evidence that such a molecule need even be in close physical apposition with HLA-B27. It is clear that more specific definition of the molecular target of anti-klebsiella sera which can lyse AS positive B27 positive PBLs could be of crucial interest and importance in clarifying the aetiopathogenesis of HLA-B27 related spondyarthropathy.

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