Viewpoint

HLA-B27 and the causes of arthritis: Does molecular biology help?

J R ARCHER AND V R WINROW

From the Bone and Joint Research Unit, The London Hospital Medical College, London

DNA and protein sequences make dull reading, and the recent publications of structures for the gene and molecule of HLA-B27 and a number of its relatives^{1–7} have met with less enthusiasm from rheumatologists than perhaps they deserve. Nucleic acid sequences provide us with a primary structure for the protein. What can we learn?

Three theories are current to explain the association of B27 with arthritis.

(1) Arthritis may be associated with an adjacent gene too close to the B locus to have been separated through many generations of breeding. This must have appeared before the half dozen recently described subtypes, all of which seem to be associated with ankylosing spondylitis.⁸

Little space now remains for such a gene,⁴ and for the moment it seems pointless to cling to the hope that discovery of a new sequence for an unknown molecule will suddenly throw light on an old problem.

(2) B27 may be the most important of a cross reactive group of HLA antigens centred on B7 (the B7 CREG)⁹ which also cross react with certain bacterial antigens. This immunological reactivity, or its failure, somehow causes arthritis.¹⁰ There is a mass of evidence for this, but much of it is indecisive or contradictory.¹¹ Recent publications, however, confirm at least that some monoclonal antibodies against B27 are also able to react with certain bacterial products.^{12 13}

If we hope to find sites in the B27 molecule which cross react with bacteria we should be looking for sequences common to B7 CREG genes but absent from other human class I molecules.

(3) B27 could act as a receptor for the product of a gene found in enteric bacteria present in all B27 positive patients with ankylosing spondylitis, but

only very occasionally in people without the disease: patients' cells are infected with a similar gene.¹⁴ Unfortunately, only one laboratory in Sydney is able to repeat consistently experiments favouring this idea.

To find additional clues relevant to the receptor hypothesis we need to identify sites unique to the B27 molecule which are likely to act as receptors.

Class I HLA molecules (HLA-A, B, and C) share a basic protein structure (Fig. 1). The genes are divided into eight translated sections, thought to be expressed as six or seven domains in the final molecule, a glycoprotein of molecular weight 43 kd consisting of some 340 amino acids and a single oligosaccharide chain.¹⁵ Antigenic determinants may be expressed only in the three N-terminal domains (α_1 , α_2 , and α_3), each of about 90 amino acids, which remain external to the cell membrane. In practice, variability seems restricted almost exclusively to α_1 and α_2 . Sequences which can be related to serological specificities must lie on outside 'available' parts of these domains, as do the sequences recognised by cytotoxic T cells used to define many of the B27 subtype specificities. Another molecule, β_2 microglobulin, is needed to retain class I molecules on the surface¹⁶ and is in dynamic equilibrium with β_2 microglobulin in the plasma.¹⁷

In the search for amino acid differences which might explain the cross reactive hypothesis (2 above) four HLA-B molecules have been sequenced. Of these, B7, B27, and B40 belong to the B7 CREG; B27 and Bw58 carry the Bw4 specificity, while B7 and B40 carry the latter's allele Bw6.

The primary amino acid sequences on which the following discussion is based are summarised by Ways and coworkers.⁵

B27 differs from B7 in 18 positions and from B40 in 22, all in the external domains. Three amino acid differences in α_2 , in the region 177–180, represent the Bw4 sequence,⁵ which is not associated with

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Correspondence to Dr J R Archer, Bone and Joint Research Unit, London Hospital Medical College, 25–29 Ashfield Street, London E1 2AD.

Fig. 1 General structure of HLA-B27 on a cell membrane. α_1 , α_2 , α_3 =external antigenic domains; tm=transmembrane domain; Cyt 1,2=cytoplasmic domains. (Some specificities have a third cytoplasmic domain.); $\beta_2 M$ = β_2 microglobulin. Figures in parentheses represent amino acid positions.

arthritis. There are nine locations in the external domains where the three B7 CREG molecules are identical but differ from Bw58. Bw58 and A2, however, are identical at one of these locations, which is probably involved in a known immunological reaction,⁵ and a further five show similarities with sequences in a number of A locus antigens, indicating a basic 'standard' structure for HLA class I molecules. Thus only three sites show differences likely to define the B7 CREG. These are at positions 65-66, 163, and 94-95. Of these, amino acids 65-66 are very close to residues defining the serological specificities of B antigens and seem most likely to be the B7 CREG specific sequence. The monoclonal antibody B27.M2,¹⁸ although it cross reacts with some bacterial products,¹² recognises subtypes specified in the α_1 domain by the sequence 77-81,³ and is therefore unlikely to identify the crucial antigenic site.

The receptor hypothesis (3 above) requires a unique site on B27 likely to react with external factors. Targets for most HLA typing sera to class I molecules are thought to be coded in the α_1 and α_2 domains. Although B27 differs from B7, B40, and Bw58 in respectively 18, 22, and 28 positions, it differs from *all* class I molecules in only five positions. Of these sites, only two are different in every HLA-B molecule that has been sequenced.

These are positions 67 and 70 in the α_1 domain. If any segment codes for the serologically defined epitope of HLA-B, this is the most likely. It is difficult to imagine the precise effect that Lys₇₀ has on the reactions of B27, though presumably it causes a local alteration in charge. The same cannot be said for the Cys residue in position 67. Here is a free reactive sulphhydryl group at the most exposed antigenic spot on the molecule. Could proponents of the receptor hypothesis have asked for more?

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