Ankylosing spondylitis and HLA-B27: restriction fragment length polymorphism and sequencing of an HLA-B27 allele from a patient with ankylosing spondylitis

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Abstract
Two groups of patients with ankylosing spondylitis (AS) from England and Poland were examined for restriction fragment length polymorphisms (RFLPs) associated with the disease. No preferential association was found between the 9·2 kb *Pvu*II fragment in HLA-B27 positive patients with AS compared with HLA-B27 healthy subjects as had been previously reported. In the English group, however, a 14 kb *Pvu*II fragment was more common in HLA-B27 positive subjects with AS than in normal controls. Also 4·6 and 3·7 kb *Pvu*II fragments were more prevalent in subjects without AS than in the group with AS, but these results were confined to the English group. Furthermore, the sequence of an HLA-B*2705 gene isolated from a patient with AS was examined, and no significant differences were found compared with the sequence isolated from a healthy subject. There do not seem to be significant genetic differences in the coding or in the regulatory region in HLA-B27 alleles, in subjects with or without AS.

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Ankylosing spondylitis (AS) is an inflammatory disease affecting the sacroiliac joints, spine, and peripheral joints. Patients with reactive arthritis associated with HLA-B27 often have a history of antecedent infection with microorganisms such as salmonella, shigella, and yersinia,[1] whereas in patients with AS klebsiella has been implicated as a possible cause.[2 3] A strong association exists between AS and the major histocompatibility complex (MHC) class I antigen HLA-B27,[4 5] with over 90% of patients with AS carrying this HLA antigen compared with a prevalence of HLA-B27 of less than 8% in the white population. The relative risk for subjects carrying HLA-B27 has been shown to be greater than 70.[6] HLA-B27 is present in 50% of American black subjects with AS, whereas only 2% of the American black population have the HLA-B27 allele.[7]

Despite the close association between AS and HLA-B27 the mechanism underlying it remains unclear. It has been suggested that HLA-B27 is linked directly to the pathology of AS through the mechanism of molecular mimicry by means of immunological cross reactivity between antigens on the associated bacteria and the HLA-B27 molecule.[8] Alternatively, it may not be HLA-B27 itself which plays a part in the disease development but a gene in linkage disequilibrium with HLA-B27. There is reported to be no difference between the amino acid sequence of the extracellular domains of HLA-B27 antigen in patients with AS and in non-diseased subjects.[9]

Restriction fragment length polymorphism (RFLP) analysis has been used to examine the possibility of linking a particular allelic form of HLA-B27 with AS. Using an HLA-B7 cDNA probe, McDaniel *et al* showed that a polymorphic 9·2 kb *Pvu*II DNA fragment was present in 72·9% of HLA-B27 positive patients with AS but in only 22% of HLA-B27 positive normal white subjects from Alabama, USA.[10] Segregation studies in families showed that the 9·2 kb *Pvu*II polymorphic DNA fragment is not a polymorphism of the HLA-B27 allele. A number of more recent RFLP studies have failed to confirm an association between the 9·2 kb *Pvu*II fragment and AS.[11–13]

We report here our studies of two groups of European patients with AS, usually genetically more homogeneous than American patients, who were investigated to determine a possible association between RFLPs and AS.

Materials and methods

SUBJECTS

Thirty four unrelated English patients with AS (seven women, 27 men), all with classical AS according to the New York criteria,[14] were studied. Thirty two of the 34 patients were white. Thirty two patients had peripheral joint disease and 14 had had uveitis. Only one HLA-B27 negative patient was included, who had peripheral joint disease but not uveitis. Thirty two healthy English subjects with no previous history of AS in the family, including seven who were positive for HLA-B27, served as controls.

Twenty Polish white patients with AS were also studied, of whom 10 had peripheral joint disease. The incidence of uveitis among these patients was not known. Thirteen healthy, white Polish subjects, of whom seven were HLA-B27 positive without any history of arthritis or spondylitis, served as controls.

PROBES

Full length HLA-B27 cDNA probe pB1[15] was kindly provided by Dr E Weiss. HLA-B27 5′

and 3′ cDNA probes were derived from pB1. The 5′ cDNA probe was a 415 bp *Pst*I-*Pvu*II fragment containing the sequences encoded in the leader sequence, the presequence, the α1 domain, and 194 bp of the α2 domain. The 3′ cDNA probe consisted of the 550 bp *Pvu*II-*Pst*I fragment containing the 3′ untranslated region of pB1 cDNA. The HLA-B27 3′ flanking probe was a 1650 bp *Pst*I-*Bam*HI fragment from an HLA-B27 gene isolated from a subject without AS[16] and was also kindly provided by Dr E Weiss. The probe contains sequences immediately downstream from the poly-A addition signal.

SOUTHERN BLOTTING

The peripheral lymphocytes from 10 ml of whole EDTA blood were lysed in 90 ml of lysis buffer containing 0·32 M sucrose (BDH), 10 mM TRIS-HCl pH 7·5 (Sigma), 5 mM $MgCl_2$ (BDH), and 1% (v/v) Triton-X100 (Sigma). The nuclei were pelleted by centrifugation at 2000 *g* for 20 minutes and then resuspended in 5 ml 75 mM NaCl (BDH), 24 mM EDTA (BDH) pH 8·0. The nuclear membranes were lysed by addition of sodium dodecyl sulphate (Sigma) to a final concentration of 0·5% and 0·2 mg/ml proteinase K (Boehringer Mannheim) and incubated at 37°C for two to 12 hours. The DNA was then purified by extraction with an equal volume of phenol (BDH) followed by two extractions with chloroform (BDH). The DNA was further purified by addition of 11 ml ethanol (BDH) after addition of sodium acetate pH 5·2 (BDH) to a final concentration of 0·3 mol/l. The precipitated DNA was dissolved in 0·5 ml TE buffer.

DNA (10 μg) was digested to completion with 25–50 units of the restriction enzymes *Eco*RI, *Hind*III, or *Pvu*II (BRL/Gibco) as recommended by the manufacturers. The DNA fragments were separated through a 0·8% agarose (Sigma) gel at 60 V for 17 to 20 hours in 40 mM TRIS (Sigma), 20 mM acetic acid (BDH), 10 mM EDTA (BDH). The DNA fragments were denatured, neutralised, and transferred to Hybond-N membranes (Amersham, UK) followed by cross linking of the DNA to the nylon membrane by ultraviolet irradiation (305 nm) for three minutes.

Blots were prehybridised for a minimum of four hours at 65°C in 6×SSC (900 mM NaCl, 90 mM sodium citrate pH 6·15), 0·01% BSA (ICN Immunobiochemicals), 0·1% (w/v) polyvinylpyrrolidone (Sigma), 0·1% Ficoll 600 (Pharmacia), 10% dextran sulphate (Pharmacia), 0·1% sodium dodecyl sulphate, and 0·1 mg/ml denatured sonicated herring sperm DNA (Sigma). Fresh hybridisation buffer containing 0·1 M EDTA was used for overnight hybridisation at 65°C. Denatured probe labelled with [^{32}P]dCTP (NEN) to a specific activity of at least 10^8 cpm/μg according to Feinberg and Vogelstein[17] was also added to the hybridisation buffer at a final concentration of 1–2×10^6 cpm/ml. After hybridisation the membranes were washed twice in 2×SSC/0·5% sodium dodecyl sulphate at 65°C for 30 minutes followed by two 30 minute washes in 0·1×SSC/0·5% sodium dodecyl sulphate at 65°C. Autoradiography was carried out using screened x ray cassettes at −70°C with Kodak XAR–5 film.

CLONING OF THE HLA-B27 GENE FROM A PATIENT WITH AS

Lymphocytic DNA isolated from a patient with AS heterozygous for the HLA-B27 gene was digested to completion with *Eco*RI and selected according to size by agarose gel electrophoresis. DNA fragments of about 6·5 kb containing the HLA-B27 gene were electroeluted and cloned into λ gt10 phages (Amersham, UK). Recombinant phages (7×10^4) were screened with the 3′ flanking probe, and one strongly cross hybridising clone was purified. The 6·5 kb genomic insert was subcloned into pBluescript SK+ (Stratagene) for further characterisation. The DNA sequence was determined with Sequenase after subcloning of suitable DNA fragments into M13 mp18 or M13 mp19 bacteriophages.

Results

RFLP ANALYSIS

Genomic DNA from English and Polish patients with AS and healthy normal subjects was analysed for RFLPs by hybridisation with

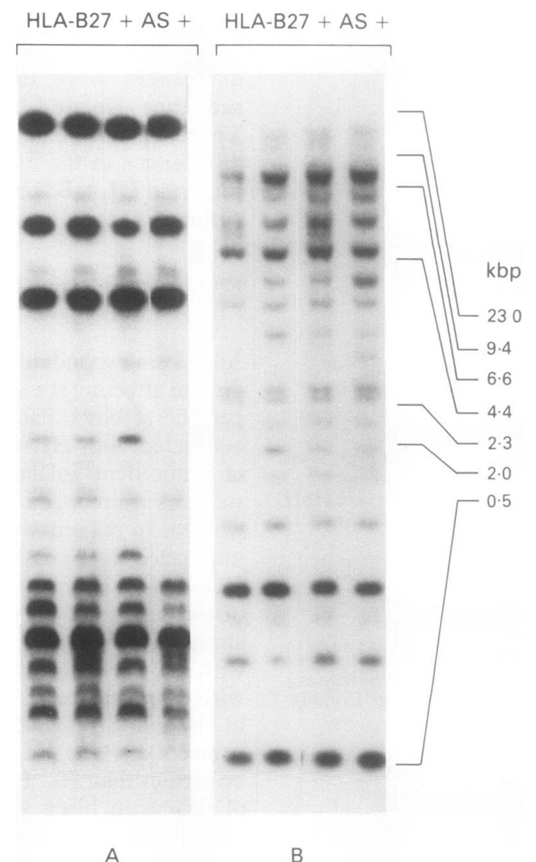

Figure 1 Autoradiograph of PvuII digested genomic DNA from four HLA-B27 positive Polish subjects with AS hybridised with full length HLA-cDNA probe. After hybridisation the blot was washed with 2×SSC, 0·1% sodium dodecyl sulphate (SDS) at 65°C and exposed against x ray film for three days (A) after which the stringency of the wash was increased to 0·2×SSC, 0·1% SDS and the Southern blot was re-exposed to x ray films for seven days (B).

a number of probes derived from different regions of the HLA-B27 gene or the corresponding cDNA. Hybridisation to Southern blots of *Eco*RI and *Hind*III digested DNA showed that the HLA-B27 cDNA probe detected several DNA fragments. Some of these were polymorphic, but none showed any association with the disease (data not shown).

In the light of this a more detailed analysis was performed on Southern blots of *Pvu*II digested DNA. Hybridisation at low stringency of a full length cDNA (fig 1) as well as a 5′ cDNA probe (fig 2) showed about 20 DNA fragments. Most of these are derived from other MHC class I genes which have DNA sequence homology with the HLA-B27 gene. The two probes detect virtually the same fragments, except that the 5′ probe detects no fragments below 2·6 kb. The table lists the frequencies of all fragments detected with the 5′ cDNA probe. A 14 kb *Pvu*II fragment was found in 82% (28/34) of English HLA-B27 positive patients with AS, whereas it appeared in only 29% (2/7) of the English control subjects with HLA-B27. The 14 kb *Pvu*II fragment, however, occurred with the same frequency in the HLA-B27 negative control group as seen among HLA-B27 positive healthy subjects. The 14 kb *Pvu*II fragment was not an AS susceptibility polymorphism among HLA-B27 positive Polish patients.

The prevalence of the 9·2 kb *Pvu*II fragment, described by McDaniel and coworkers,[10] was low among HLA-B27 positive English and Polish patients with AS (28% and 15% respectively). In contrast, this 9·2 kb fragment was significantly more prevalent in both English and Polish HLA-B27 positive controls (86% and 50% respectively). This suggests a negative association between the disease and the 9·2 kb fragment among HLA-B27 positive subjects.

The 4·6 and 3·7 kb fragments were more prevalent in HLA-B27 negative healthy subjects (70% and 67% respectively) than in HLA-B27 positive English subjects (35% and 23% respectively). The 3·7 kb fragment was also seen more commonly in healthy subjects (64%) than in patients with AS (16%), irrespective of the presence of the HLA-B27 allele. Among the English patients it was found that the 3·7 kb

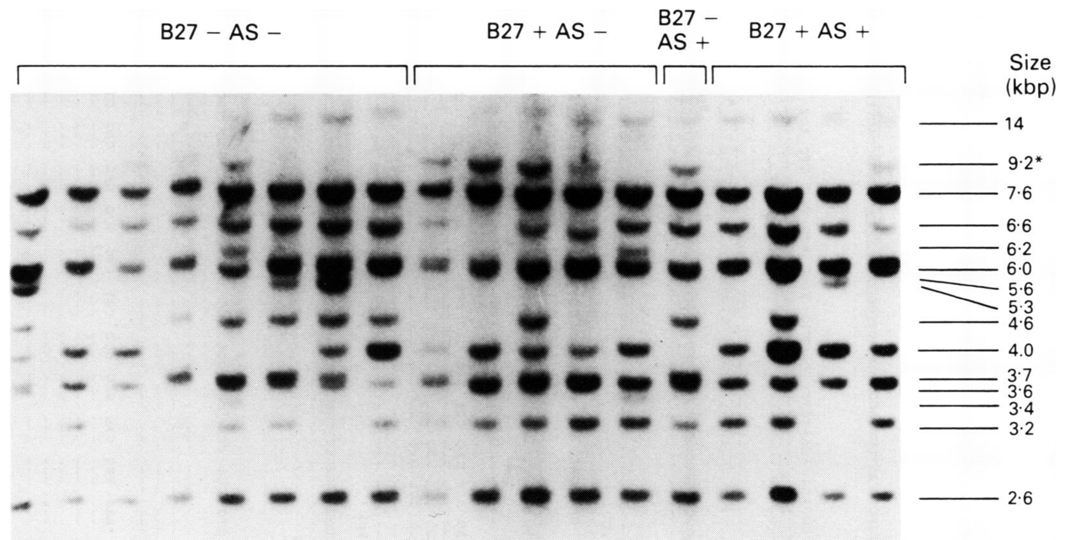

Figure 2 Autoradiograph of PvuII digested genomic DNA from eight HLA-B27 negative healthy subjects, five HLA-B27 positive healthy subjects, one HLA-B27 negative English patient with AS, and four HLA-B27 positive English patients with AS hybridised with the pB27-5′ cDNA probe. The 9·2 kb PvuII fragment is indicated with an asterisk.

Prevalence of PvuII fragments hybridising to the pB27-5′ cDNA probe in each subset of the English and Polish groups. The 9·2 kb PvuII fragment is indicated with an asterisk. The percentage of patients with each fragment is shown

Size (kbp)	English			Polish		
	B27+AS+ (n=34)	B27+AS− (n=7)	B27−AS− (n=25)	B27+AS+ (n=20)	B27+AS− (n=4)	B27−AS− (n=9)
14	82	29	72	70	75	78
9·2*	28	86	35	15	50	44
7·6	100	100	100	100	100	100
6·6	100	100	100	100	100	100
6·2	12	43	8	10	0	0
6·0	36	29	68	15	25	22
5·6	76	71	76	85	100	100
5·3	30	0	39	30	25	44
4·6	38	14	70	35	50	44
4·0	94	100	76	95	75	78
3·7	16	57	67	55	50	33
3·6	94	86	76	75	100	67
3·4	9	14	14	0	0	0
3·2	91	86	81	95	100	89
2·6	100	100	100	100	100	100

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                                                                                                                                                   200
             GAATTCTAATCATTCAGGGATTACCAATATTGTGCTACCTACTGTATTAATAAACAAAAAGGAAACTGGTCTCTATGAGAATCTCTGTGTGGTTGGCTTCAGACAAAACTTCGCCAGGTTTAGAGAGAAAACCCCTGTCTCTACACCTCCATTCCCAGGGCGAGCTCACTCTCTGCCATCAAGTTCTCCGTGATCAGTTT
HLA-B*2705-W                                                                                                                                  -------------------------------------
                                                                                                                                                   400
             CCCTACACAAGATCCAAGAGGAGAGGTAAGGAGTGAGAGGCAGGGAGTCCAGTTCAGGGACAGGGATTCCAGGAGGAGAAGTGAAGGGGAAGCGGGTGGGCGCCACTGGGGGTCTCTCCCTGGTTTCCACAGACAGATCCTTGTGCCGGACTCAGGCAGACAGTGTGACAAAGAGGCTGGTGTAGGAGAAGAGGGATCAG
HLA-B*2705-W ----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
                                                                CRE        IRS
                                                                                                                                                   600
             GACGAACGTCCAAGGCCCCGGGCGCGGTCTCAGGGTCTCAGGCTCCGAGAGCCTTGTCTGCATTGGGGAGGCGCACAGTTGGGGATTCCCCACTCCCACGAGTTTCACTTCTTCTCCCAACCTATGTCGGGTCCTTCTTCCAGGATACTCGTGACGCGTCCCCATTTCCCACTCCCATTGGTGTCGGGTGTCTAGAGAA
HLA-B*2705-W ----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2703                                                                                                                                                                                                     ------
             NF1
                                                                                                                                                   756
             GCCAATCAGTGTCGCCGGGGTCCCAGTTCTAAAGTCCCCACGCACCCACCCGGACTCAGAATCTCCTCAGACGCCGAG  ATG CGG GTC ACG GCG CCC CGA ACC CTC CTC CTG CTG CTC TGG GGG GCA GTG GCC CTG ACC GAG ACC TGG GCT G GTGAG
HLA-B*2705-W --------------------------------------------------------------------------------  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- - -----
HLA-B*2705-S --------------------------------------------------------------------------------  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- - -----
HLA-B*2705-C                                                                                   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
HLA-B*2702   --------------------------------------------------------------------------------  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- - -----
HLA-B*2703   --------------------------------------------------------------------------------  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- - -----
                                                                                   Sp1                     AP2
                                                                                                                                                   927
             TGCGGGGTC/GGCAGGGAAATGGCCTCTGTGGGGAGGAGCGAGGGGACCGCAGGCGGGGGCGCAGG/ACCCGGGGAGCCGCGCCGGGAGGAGGGTCGGGCGGGTCTCAGCCCCTCCTCGCCCCCAG GC TCC CAC TCC ATG AGG TAT TTC CAC ACC TCC GTG TCC CGG CCC GGC
HLA-B*2705-W ---------A------------------------------/-----------/----------------------------------------------------------------------  -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-S --------G/------------------------------/---------A---------------------------------C--A------------/------  -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-C                                                                                                                            -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2702   --------G/------------------------------/---------A---------------------------------C----T----------/------  -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2703   ---------/----------------------------/CG/----------/-------------------------------------------------------  -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                                                                                                                   1056
             CGC GGG GAG CCC CGC TTC ATC ACC GTG GGC TAC GTG GAC GAC ACG CTG TTC GTG AGG TTC GAC AGC GAC GCC GCG AGT CCG AGA GAG GAG CCG CGG GCG CCG TGG ATA GAG CAG GAG GGG CCG GAG TAT
HLA-B*2705-W --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-S --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2702   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2703   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- C--
                                                                                                                                                   1202
             TGG GAC CGG GAG ACA CAG ATC TGC AAG GCC AAG GCA CAG ACT GAC CGA GAG GAC CTG CGG ACC CTG CTC CGC TAC TAC AAC CAG AGC GAG GCC G  GTGAGTGACCCCGGCCC/GGGCGCAGGTCACGACTCCCCATCCCCCACGTACG
HLA-B*2705-W --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -  -----------------/-----------------------------------
HLA-B*2705-S --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -  -----------------G-------------/---------------------
HLA-B*2705-C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
HLA-B*2702   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- A-- --- --- -T- GC- --- --- --- --- --- --- --- --- --- -  -----------------G-------------/---------------------
HLA-B*2703   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -  -----------------G-----------------------------------
                                        Sp1                  Sp1                       AP2                                                 Sp1 Sp1
                                                                                                                                                   1394
             GCCCGGGTCGCCCCGAGTCTCCGGGTCCGAGATCCGCCCCCGAGGCCGCGGGACCCGCCCAGACCCTCGACCGGCGAGAGCCCCAGGCGCGTTTACCCGGTTTCATTTTCAGTTGAGGCCAAAATCCCCGCGGGTTGGTCGGGGCGGGGCGGGGCTCGGGGGGACGGGGCTGACCGCGGGGG//CGGGGTCCAG
HLA-B*2705-W ------------------------------------------------------------------------------/-------------------------------------------/------------------------------------------------------//----------
HLA-B*2705-S -------------------------------------------------------G-T-------/------/----------T----A----------------------------------------------------------------------------------------GA----------
HLA-B*2702   -------------------------------------------------------G-T-------/------/----------T----A----------------------------------------------------------------------------------------GA----------
HLA-B*2703   ----------------------------------------------------------------------------C/G---------------------------------------------------------------------------------/------///-----G----
                                                                                                                                                   1524
             CG TCT CAC ACC CTC CAG AAT ATG TAT GGC TGC GAC GTG GGG CCG GAC GGG CGC CTC CTC CGC GGG TAC CAC CAG GAC GCC TAC GAC GGC AAG GAT TAC ATC GCC CTG AAC GAG GAC CTG AGC TCC TGG
HLA-B*2705-W -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-S -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-C -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2702   -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2703   -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                                                                                                                   1651
             ACC GCC GCG GAC ACG GCG GCT CAG ATC ACC CAG CGC AAG TGG GAG GCG GCC CGT GTG GCG GAG CAG CTG AGA GCC TAC CTG GAG GGC GAG TGC GTG GAG TGG CTC CGC AGA TAC CTG GAG AAC GGG AAG
HLA-B*2705-W --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-S --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T --- --- --- ---
HLA-B*2705-C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2702   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T --- --- --- ---
HLA-B*2703   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                                                                                                                   1858
             GAG ACG CTG CAG CGC GCG G AGCCTTCCCCATCTCCTATAGGTCGCCGGGGATGGCCTCCCACGAGAAGAGGAGGAAAATGGGATCAGCGCTAGAATGTCGCCCTCCCTTGAATGGAGAATGGCATGAGTTTTCCTGAGTTTCCTCTGAGGGCCCCCTCTTCTCTCTAGGACAATTAAGGGATGACGTCT
HLA-B*2705-W --- --- --- --- --- --- - ------------------------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2705-S --- --- --- --- --- --- - -------------------------------------------///-----------G--------/--------------------------------------------------------------------/---------
HLA-B*2705-C --- --- --- --- --- --- -
HLA-B*2702   --- --- --- --- --- --- - -------------------------------------------///-----------G--------/--------------------------------------------------------------------/---------
HLA-B*2703   --- --- --- --- --- --- - ------------------------------------------------------------------------------------------------------------------------------------------------------------------
                                                                                                                                                   2059
             CTGAGGAAATGGAGGGGAAGACAGTCCCTAGAATACTGATCAGGGGTCCCCTTTGACCCCTGCAGCAGCCTTGGGAACCGTGACTTTTCCTCTCAGGCCTTGTTCTCTGCCTCACACTCAGTGTGTTTGGGGCTCTGATTCCAGCACTTCTGAGTCACTTTACCTCCACTCAGATCAGGAGCAGAAGTCCCTGTTCCCCGC
HLA-B*2705-W ----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2703   ----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
                                                                                                                                                   2253
             TCAGAGACTCGAACTTTCCAATGAATAGGAGATTATCCCAGGTGCCTGCGTCCAGGCTGGTGTCTGGGTTCTGTGCCCCTTCCCCACCCCAGGTGTCCTGTCCATTCTCAGGCTGGTCACATGGGTGGTCCTAGGGTGTCCCATGAGAGATGCAAAGCGCCTGAATTTTCTGACTCTTCCCATCAG AC CCC CCA
HLA-B*2705-W ------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------  -- --- ---
HLA-B*2705-S                                                                                                                                                                                   -----  -- --- ---
HLA-B*2705-C                                                                                                                                                                                          -- --- ---
HLA-B*2702                                                                                                                                                                                     -----  -- --- ---
HLA-B*2703   ------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------  -- --- ---
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                                                                                                                                2382
               AAG ACA CAC GTG ACC CAC CAC CCC ATC TCT GAC CAT GAG GCC ACC CTG AGG TGC TGG GCC CTG GGC TTC TAC CCT GCG GAG ATC ACA CTG ACC TGG CAG CGG GAT GGC GAG GAC CAA ACT CAG GAC ACT
HLA-B*2705-W   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-S   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-C   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2702     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2703     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                                                                                                2511
               GAG CTT GTG GAG ACC AGA CCA GCA GGA GAT AGA ACC TTC CAG AAG TGG GCA GCT GTG GTG GTG CCT TCT GGA GAA GAG CAG AGA TAC ACA TGC CAT GTA CAG CAT GAG GGG CTG CCG AAG CCC CTC ACC
HLA-B*2705-W   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-S   --- --- --- --- --- --- --- --C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-C   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2702     --- --- --- --- --- --- --- --C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2703     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                                                                                                2672
               CTG AGA TGG G GTAAGGAGGGGGATGAGGGGTCATATCTCTTCTCAGGGAAAGCAGGAGCCCTTCAGCAGG/TCAGGGCCCCTCATCTTCCCTTCCTTTCCCAG AG CCG TCT TCC CAG TCC ACC GTC CCC ATC GTG GGC ATT GTT GCT GGC CTG GCT GTC CTA
HLA-B*2705-W   --- --- --- - ------------------------------------------------/--------------------------------- -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-S   --- --- --- - ---------------------------------/-------------/--------G------------------------- -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2705-C   --- --- --- -                                                                                    -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2702     --- --- --- - ---------------------------------/-------------/--------G------------------------- -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2703     --- --- --- - -----------------------------------------------------------G------------------------- -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                                                                                                2838
               GCA GTT GTG GTC ATC GGA GCT GTG GTC GCT GCT GTG ATG TGT AGG AGG AAG AGC TCA G GTAGGGAAGGGGTGAGGGGTGGGGTCTGAGTTTTCTTGTCCCACTGGGGGTTTCAAGCCCCAGGTAGAAGTGTTCCCTGCCTCATTACTGCGGAAGCAGCATCCACACAG
HLA-B*2705-W   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- - ---------------------------------------------------------------------------------------------------------------------
HLA-B*2705-S   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- - ------------------T--------------------------------------------------------------------------------------------------/-
HLA-B*2705-C   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
HLA-B*2702     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- - ------------------T--------------------------------------------------------------------------------------------------/-
HLA-B*2703     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
                                                                                                                                3038
               GGGCTAACGCAGCCTGGGACCCTGTGTGCCAGCACTTACTCTTTTGTGCAGCACATGTGACAATGAAGGACGGATGTATCACCTTGGTGGTTGTGGTGTTGGGGTCCTGATTCCAGCATTCATGAGTCAGGGGAAGGTCCCTGCTAAGGACAGACCTTAGGAGGGCAGTTGGTCCAGGACCCACACTTGCTTTCCTCGTG
HLA-B*2705-W   -----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2705-S   --------------------------------------------------------------G------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2702     --------------------------------------------------------------G------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2703     ----------------------------------------------------------------------------------T--------------------------------------------------------------------------------------------------------------------------------
                                                                                                                                3217
               TTTCCTGATCCTGCCTTGGGTCTGTAGTCATACTTCTGGAAATTCCTTTTGGGTCCAAGACGAGGAGGTTCCTCTAAGATCTCATGGCCCTGCTTCCTCCCAGTCCCCTCACAGGGAAATTTTCTTCCCACAG GT GGA AAA GGA GGG AGC TAC TCT CAG GCT GCG T GTAAGTGATGGGG
HLA-B*2705-W   --------------------------------------------------------------------------------------------------------------------------------------- -- --- --- --- --- --- --- --- --- --- --- - -------------
HLA-B*2705-S   ----------------------------------------------------------------------------------------------------/C--------------------------------- -- --- --- --- --- --- --- --- --- --- --- - -------------
HLA-B*2705-C                                                                                                                                           -- --- --- --- --- --- --- --- --- --- --- - -------------
HLA-B*2702     ----------------------------------------------------------------------------------------------------/C--------------------------------- -- --- --- --- --- --- --- --- --- --- --- - -------------
HLA-B*2703     --------------------------------------------------------------------------------------------------------------------------------------- -- --- --- --- --- --- --- --- --- --- --- - -------------
                                                                                                                                3391
               GTGGGAGTGTGGAGGAGCTCACCCACCCCCTAATTCCTCCTGTCCCACGTCTCCTGCGGGCTCTGACCAGGTCCTGTTTTTGTTCTACTCCAG GC AGC GAC AGT GCC CAG GGC TCT GAT GTG TCT CTC ACA GCT TGA AAAGGTGAGATTCTTGGGGTCTAGAGTGGGTGGGGTG
HLA-B*2705-W   --------------------------------------------------------------------------------------------- -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -------------------------------------
HLA-B*2705-S   --------------------------------------------------------------------------------------------- -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -------------------------------------
HLA-B*2705-C                                                                                                 -- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
HLA-B*2702     --------------------------------------------------------------------------------------------- -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -------------------------------------
HLA-B*2703     --------------------------------------------------------------------------------------------- -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -----------------------------------
                                                                                                                                3591
               GCAGGTCTGGGGGTGGGTGGGGCAGTGGGGAAAGGCCTGGGTAATGGAGATTCTTTGATTGGGATGTTTCGCGTGTGTGGTGGGCTGTTTAGACTGTCATCACTTACCATGACTAACCAGAATTTGTTCATGACTGTTGTTTTCTGTAGCCTGAGACAGCTGTCTTGTGAGGGACTGAGATGCAGGATTTCTTCACGCCT
HLA-B*2705-W   --------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2705-S   ---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------G----
HLA-B*2702     ---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------G----
HLA-B*2703     --------------------------------------------------------------------------------------//--------------------------------------------------------------------------------------------------------------------------
                                                                                                                                3791
               CCCCTTTGTGACTTCAAGAGCCTCTGGCATCTCTTTCTGCAAAGGCACCTGAATGTGTCTGCGTCCCTGTTAGCATAATGTGAGGAGGTGGAGAGACCAGCCCACCCCCGTGTCCACTGTGACCCCTGTTCCCATGCTGACCTGTGTTTCCTCCCCAGTCATCTTTCCTGTTCCAGAGAGGTGGGGCTGGATGTCTCCAT
HLA-B*2705-W   -------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2705-S   ------------------------------/-----------------------------------------------------------/---------------------------------------------------------------------------------------------AA-----------
HLA-B*2702     ------------------------------/-----------------------------------------------------------/---------------------------------------------------------------------------------------------AA-----------
HLA-B*2703     -------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
                                                                                                                                3990
               CTCTGTCTCAACTTTATGTGCACTGAGCTGCAACTTCTTACTTCCCTACTGAAAATAAGAATCTGAATATAAATTTGTTTTCTCAAATATTTGCTATGAGAGGTTGATGGATTAATTAAATAAGTCAATTCCTGGAATTTGAGAGAGCAAATAAAGACCTGAGAACCTTCCAGAATCTGCATGTTCGCTGTGCTGAGTC
HLA-B*2705-W   ---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2705-S   ---------------------------------------------------------C--------------------
HLA-B*2702     ---------------------------------------------------------C--------------------
HLA-B*2703     --------------------------------------------------------------------------------------------------------------------------------------------------------------G------------------------------------------
                                                                                                                                4189
               TGTTGCAGGTGGGGTGTGGAGAAGGCTGTGGGGGGCCGAGTGTGGAC/GGGCCTGTGCCCATTTGGTGTTGAGTCCATCATGGGCTTTATGTGGTTAGTCCTCAGCTGGGTCACCTTCACTGCTCCATTGTCCTTGTCCCTTCAGTGGAAACGTTGTCCAGCGGGAGCTGTGACCACAGAGGCTCACACATCGCCCATGG
HLA-B*2705-W   -----------------------------------------------/-------------------------------------------------------------------------------------------------------------------------------------------------
HLA-B*2703     ------------------------------------------------G--------------------------------------------------------------------------------------------/--------------------------------------/----/---
                                                     4259
               GCGGCCCCATGCACACGGGGGTCTCTGTGCATTCATAGAGAACATTTTACAGAGACACTTACACACTCT
HLA-B*2703     --------/--------------------------/-/----CA-----/-----/-/A------/----
```

*Figure 3 Nucleotide sequence of the HLA-B*2705 gene of diseased haplotype. Nucleotides are numbered from the EcoRI cloning site. The promoter (TCTAAA), CAAT box, the mRNA start site, and the polyadenylation signal (AAATAAA) are in bold face. The known regulatory regions and potential binding sites for transacting transcription factors are underlined and named as follows: CRE (class I regulatory element), IRE (interferon responsive sequence), NF1, Sp1, AP2. The sequence is compared with the sequence HLA-B*2705-W and HLA-B*2705-S of the normal haplotype isolated by Weiss et al[16] and Seeman et al.[20] respectively; B*2705-C is the exon sequence of the HLA-B27 gene of the diseased haplotype characterised by Coppin and McDevitt;[9] B*2702 is the sequence reported by Seeman et al[20] and the B*2703 sequence that isolated by Choo et al.[25] The nucleotide sequences are indicated and the symbol / represents a missing nucleotide.*

```
α1 Domain
                            20                  40                  60                  80
          GSHSMRYFHTSVSRPGRGEPRFITVGYVDDTLFVRFDSDAASPREEPRAPWIEQEGPEYWDRETQICKALAQTDREDLRTLLRTTNQSEA
B*2701    -------------------------------------------------------------------------Y--N---A---------
B*2702    ----------------------------------------------------------------------------N--IA---------
B*2703    ----------------------------------------------------------H-------------------------------
B*2704    ----------------------------------------------------------------------------S-------------
B*2705    ------------------------------------------------------------------------------------------
B*2706    ----------------------------------------------------------------------------S-------------

α2 Domain
                            20                  40                  60                  80
          GSHTLQNMYGCDVGPDGRLLRGYHQDAYDGKDYITLNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGECVEWLRRYLENGKETLQRA
B*2701    --------------------------------------------------------------------------------------------
B*2702    --------------------------------------------------------------------------------------------
B*2703    --------------------------------------------------------------------------------------------
B*2704    -------------------------------------------------------------E------------------------------
B*2705    --------------------------------------------------------------------------------------------
B*2706    -----------------------D-Y-----------------------------------E------------------------------
```

*Figure 4 Comparison of the deduced amino acid sequence of the α1 and α2 domains of the HLA-B27 gene presented here with those of other HLA-B27 subtypes. The amino acid sequences were compiled from Rojo et al [26] (B*2701), Seeman et al [20] (B*2702), Choo et al,[25] (B*2703), Rojo et al [27] (B*2704), Weiss et al [16] (B*2705), and Vega et al [28] (B*2706).*

*Pvu*II fragment was seen more commonly among patients with uveitis (36%) than in those without (5%).

Hybridisation of the Southern blots of *Pvu*II digested DNA with the 3′ cDNA and the 3′ flanking probe showed a more simple hybridisation fragment pattern detecting few polymorphic fragments. None of these, however, showed any particular association with the disease in either of the two groups studied.

DNA SEQUENCE OF AN HLA-B27 ALLELE

As the RFLP analysis did not show any striking disease association the HLA-B27 allele from a patient with AS was cloned and sequenced to identify possible differences between it and that of a healthy subject. To ensure that the HLA-B27 allele isolated indeed would be associated with the disease, a partial genomic library of DNA isolated from a patient with AS carrying only one copy of the HLA-B27 allele was prepared. As characterisation of cloned HLA-B genes had previously shown that the HLA-B gene was contained on a 6·5 kb *Eco*RI fragment,[16] DNA fragments of about 6·5 kb were cloned into λ gt10 phages. Screening with 3′ flanking probe identified one HLA-B27 clone. Figure 3 shows the DNA sequence of 4259 bp of this clone. DNA sequence comparison between the HLA-B27 gene from normal subjects and the one isolated here from a patient with AS showed no striking differences either in the promoter and regulatory region or in the region containing the structural gene. In particular exons V, VI, and VII were identical. Indeed, few nucleotide differences could be detected in the sequence of the gene isolated by us and that isolated by Weiss *et al* [16] from a non-diseased subject. None of the nucleotide differences changed the amino acid sequence. Thus when translated into an amino acid sequence the HLA-B27 gene we cloned encoded a protein identical to that encoded by the HLA-B*2705 allele (fig 4).

Discussion

More than 95% of white patients with AS are positive for the MHC class I antigen HLA-B27, but only about 20% of all HLA-B27 positive subjects are likely to develop some stigmata of the disease, though the incidence seems to be higher in families with a history of AS.[18] This might be due to the influence of other genetic as well as environmental factors on the pathology of the disease. As RFLP analysis has proved useful in associating particular DNA polymorphisms with diseases[19] an extensive search for RFLPs preferentially associated with AS alone or together with HLA-B27 was carried out. McDaniel and coworkers described a 9·2 kb *Pvu*II DNA fragment which was detected with an HLA-B27 cDNA probe,[10] and in a study of patients with AS from Alabama (USA) it was found to be present in 73% of HLA-B27 positive patients with spondylitis compared with only 27% of the healthy subjects with HLA-B27. The 9·2 kb *Pvu*II gene seems to increase the relative risk from 90 (for HLA-B27 alone) to a relative risk of 297 when both markers are present.[10] The 9·2 kb *Pvu*II fragment was later identified as a polymorphism of the HLA-A3 or the HLA-A9 gene,[12] suggesting the existence of either a second MHC linked AS susceptibility gene, a disease gene different from HLA-B27 but in linkage disequilibrium with the HLA-B27 gene, or a particular allelic form of the HLA-B27 gene associated with AS.

RFLP studies of patients with AS in Baltimore,[12] Minnesota and Texas,[11] and in Germany,[13] however, have all failed to confirm the preferential association of the 9·2 kb *Pvu*II fragment and AS in HLA-B27 positive subjects as reported by McDaniel *et al.*[10] Indeed none of these RFLPs identified by MHC class I polymorphisms preferentially associated with AS in HLA-B27 positive subjects. We analysed patients with ankylosing spondylitis from two geographically isolated, but in themselves homogeneous, groups from England and Poland. We found no increase in the prevalence of the haplotype HLA-A3/A9, HLA-B27 among patients with spondylitis compared with normal subjects. We did find, however, a preferential association between AS and a 14 kb *Pvu*II fragment among HLA-B27 positive patients compared with HLA-B27 healthy subjects in the English group (but not in the Polish group), though this failed to reach statistical significance when corrected for the number of fragments examined. None of the

other studies[10–13] reported a similar association. Only seven healthy subjects in our English control group had the HLA-B27 class I allele so this preferential association might not persist if a larger control group were to be studied. There is insufficient tissue typing data of our patient group to identify the MHC class I gene containing the 14 kb polymorphism. In addition, 4·6 kb and 3·7 kb polymorphic fragments were found which, in the English control group, were more prevalent in HLA-B27 negative subjects than in those who were HLA-B27 positive, and the 3·7 kb fragment was more prevalent in healthy subjects than in patients with AS irrespective of whether the subjects were positive for HLA-B27 or not. This seems to be a negative correlation among English patients with AS, but this association was not seen in the Polish group.

The conflicting results might be due to genetic differences between the groups studied. The HLA-A3/A9 allele might be linked with the disease allele in the Alabama group studied by McDaniel *et al*,[10] whereas such a linkage might not exist in the other groups with AS. Likewise, the 14 kb *Pvu*II fragment, which appears to be preferentially linked together with HLA-B27 to AS in the English group as reported here, but absent in the other group studied, might reflect the genetic diversity between different populations. This implies that no additional MHC class I gene is involved in the pathology of AS. If the HLA-B27 gene is not the disease gene itself or if additional MHC encoded susceptibility genes exist then they must be very close to the HLA-B27 allele.

The discrepancy between the number of people carrying HLA-B27 and those developing AS could be explained if there existed particular susceptibility alleles of HLA-B27 which cannot be identified by restriction fragment analysis. We sequenced the entire structural HLA-B*2705 gene from a patient with AS. Our results show that the translated amino acid sequence is identical with the sequence of an HLA-B*2705 antigen isolated from a healthy person.[16 20] This is in complete agreement with Coppin and McDevitt,[9] who found no differences between the translated sequence of exon II, III, and IV encoding the extracellular portion of the MHC class I antigen in HLA-B27 proteins from patients with AS and healthy subjects. Furthermore, our DNA sequence shows that the promoter and regulatory region of the HLA-B27 gene isolated by us is virtually identical to the non-diseased gene characterised by Weiss *et al*.[16] This eliminates the possibility that the pathology of AS might be due to altered expression of the HLA-B27 antigen. We found very few nucleotide differences between the HLA-B*2705 gene isolated by us and the nucleotide sequence of a similar allele from a normal subject cloned and sequenced by Weiss *et al*[16] and Seeman *et al*.[20] We noted some nucleotide differences in exon II and IV but none which would result in amino acid differences. We also found sequence differences within intron I and II when compared with previously published sequences for non-diseased HLA-B*2705 alleles.[16 20] Interestingly, some of these differences create or destroy potential binding sequences for the transcription factors SP1 or AP2. It has not been shown that such binding sites are used in regulation of the transcription, however.

The absence of any differences between the coding sequence of the HLA-B27 genes from patients and healthy subjects does not eliminate a direct role for HLA-B27 in the pathogenesis of the disease through its function as restriction element for antigen presentation to cytotoxic T cells.[21] Many organ specific autoimmune diseases, like insulin dependent diabetes mellitus and Hashimoto's disease, are characterised by a cellular as well as a humoral immune response. Ankylosing spondylitis might well be initiated by an infection of klebsiella or related bacteria.[22] The fact that the concordance rate for AS in identical twins is well below 50% clearly suggests the involvement of an environmental factor in a genetically susceptible host[23] who may have more than one susceptibility gene. Family studies suggest a possible role for a second susceptibility gene, not necessarily located in the MHC region. Such a second susceptibility gene could code for a self protein sharing a T cell epitope with a bacterial protein, which might have a restricted tissue distribution, and this might account for the tissue specificity of the disease. A shared T cell epitope would then only be presented by HLA-B27 or a related MHC class I antigen with a nearly identical antigen binding groove.[24]

Clearly, further studies are required to resolve the problem of the association of HLA-B27 with AS.

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