

RHEUMATIC DISEASE CLINICS OF NORTH AMERICA

Spondyloarthropathies

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Ankylosing spondylitis and related spondyloarthropathies form a family of rheumatic diseases that are characterized by inflammatory peripheral and axial arthritis, with predilection for sacroiliitis, and a remarkably strong association with a genetic marker, HLA-B27. The association with B27 has provided a great impetus to the epidemiologic studies of spondyloarthropathies and also helped broaden the clinical spectrum of these diseases. There are at least six subtypes of B27, and the x-ray crystallographic structure of the common B27 subtype (B*2705) is now known. Endogenous peptides bound in the B27 antigen-binding cleft have been isolated and all seem to be nonamers (i.e., nine amino acids long).

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The close association of HLA-B27 with arthritic conditions has led to the suggestion that these diseases are mediated by cytotoxic T lymphocytes that recognize self-peptides presented by HLA-B27 molecules. The further association with enteric bacterial infections suggests that bacterial antigens may prime the CTL that later crossreacts on self. Bacterial infections do not usually generate CTL responses. We speculate here that unusual properties of HLA-B27 molecules

may predispose to such responses. Thus, HLA-B27-related disease may be an unfortunate consequence of the generation of a suitable, self-mimicking HLA-B27-binding peptide by certain bacteria, plus an unusual propensity for the HLA-B27 molecule to bind and present such peptides.

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In recent studies, ileocolonoscopy has revealed ileal or colonic inflammatory changes in 30% to 60% of patients with spondyloarthropathies. In patients with a short duration of arthritis, the changes are similar to those observed in patients with bacterial enteritis. In those with prolonged or chronic spondyloarthropathy, the changes show chronic inflammation, with features of Crohn's disease in a quarter of the patients. It is tempting to speculate that infection leads to chronic gut inflammation with increased absorption of bacterial products that may contribute to the development of arthritis and spondylitis. The microbes probably do not persist in the host in a dividing form, because contrary to patients with chlamydia-triggered reactive arthritis, the prolonged use of antimicrobial chemotherapy in patients with acute enteroarthritis does not influence the natural course of the arthritis. The use of sulfasalazine in the treatment of spondyloarthropathies seems to be promising.

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Mahboob U. Rahman, Alan P. Hudson, and
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Rubén Burgos-Vargas and Ross E. Petty

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James T. Rosenbaum

An acute onset, unilateral anterior uveitis occurs during the course of either Reiter's syndrome or ankylosing spondylitis. Conversely, many patients who suffer from an acute anterior uveitis are HLA-B27-positive and have associated joint disease. The consistent presentation of the uveitis can aid in the process of differential diagnosis. This article includes a discussion of the recognition of the characteristic presentation, the complications, the role of B27 testing, the relevance of animal models, the pathogenesis, and treatment.

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Alfonse T. Masi

Ankylosing spondylitis (AS) has a striking disease marker, i.e., HLA-B27, indicating the major genetic predisposition; however, expression of disease is also strongly influenced by age- and sex-related factors. Sex steroids studies suggest greater androgenicity in AS than normal control persons. Therapeutic interventions that normalize such sex steroid status have shown clinical improvements in males and females. Muscle histopathology in AS shows frequent changes early in disease consistent with neuropathic and myopathic mechanisms of a noninflammatory nature. Accepting the available, aggregate data, one may infer that sex steroid imbalance in persons susceptible to AS may target axial and proximal muscle tissues, resulting in relative functional hypertonicity. Such phenomenon, developing in preteen and younger adult ages, may contribute to peripheral and axial manifestations of enthesopathy in this disease by complex and currently unknown mechanisms.

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Georges M.G.M. Verjans, Gerald Messer, Elisabeth H. Weiss, Sjeff M. van der Linden, and Aize Kijlstra

Family studies have indicated that other genetic factors, besides the HLA-B27 allele, might be involved in the pathogenesis of ankylosing spondylitis. In view of the localization of the tumor necrosis factor (TNF) genes within the HLA complex in close vicinity to the HLA-B locus, and the pleiotropic biologic activities of TNF- α and TNF- β in inflammation and the regulation of the immune responses, a possible involvement of polymorphic TNF genes has been postulated to contribute to the associations of certain HLA antigens with various diseases. The authors review recent data on the polymorphism of the TNF region in relation to ankylosing spondylitis and other diseases.

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Henning Zeidler, Wilfried Mau, and Muhammad Asim Khan

The term undifferentiated spondyloarthropathy (uSpA) refers to patients with clinical and roentgenographic features suggestive of spondyloarthropathies but not fulfilling the diagnostic or classification criteria for any of the currently established disease categories. The frequency and clinical spectrum of uSpA have been ignored in previous epidemiologic and clinical studies. A generally accepted nosologic concept and definition of uSpA may be needed to overcome this issue. So far the recently developed ESSG criteria have the broadest basis of consent, at least for several European centers. With the use of the ESSG classification criteria the real prevalence may be better defined in the future and the early classification of such patients in clinical practice should be advanced. Nevertheless, the diagnosis of uSpA is only a working label with the implicit demand to solve the clinical conundra by follow-up or even better by identifying the causative or triggering infectious agents.

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Kanji Shichikawa, Yoshitaka Takenaka, Masao Yukioka, and Takashi Ikawa

Polyenthesitis is defined as a disease that has, as the main clinical feature, the inflammatory involvement of many entheses. Arthritis is occasionally associated with the disease but is always transient. The disease is uniformly negative for HLA-B27 and no sacroiliitis is shown on the radiographs. Histologic findings of enthesial lesions and results of bone scintigraphy are described. It is suggested that polyenthesitis may have an infectious origin.

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SAPHO (synovitis, acne, pustulosis, hyperostosis, osteitis) syndrome put together osteoarticular lesions described separately under numerous denominations, such as multifocal osteomyelitis, pustulotic arthroosteitis, acne rheumatism. The association of sterile inflammatory bone lesions and neutrophilic skin eruptions is the cornerstone of this new syndrome, which also has links with spondyloarthropathies and plain psoriasis.

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**There Is an Association Between Human Immunodeficiency
Virus Infection and Spondyloarthropathies** 257
Luis R. Espinoza, Luis J. Jara, Carmen G. Espinoza,
Luis H. Silveira, Pindaro Martinez-Osuna, and Mitchell Seleznick

The presence of inflammatory musculoskeletal manifestations during the course of human immunodeficiency virus (HIV) infection is well established. A wide spectrum of rheumatic disorders have been reported since the first reports of Reiter's syndrome with HIV infection. Other reported associations include forms of arthropathies, psoriatic arthritis, Sjögren's syndrome, polymyositis-dermatomyositis, vasculitis, and septic arthritis.

Human Immunodeficiency Virus Is Not Associated with Reiter's Syndrome: Data From Three Large Cohort Studies

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Marcus R. Clark, Alan M. Solinger, and Marc C. Hochberg

In this article, three large studies estimating the prevalence of Reiter's syndrome (RS) in persons infected with or at risk for infection with human immunodeficiency virus (HIV) are examined. The prevalence of RS, which was less than 1% in all three cohorts, was not associated with serologic evidence of infection with HIV or a diagnosis of AIDS. In one study, the occurrence of RS was correlated with a history of bacterial enteritis. Thus, it appears that the increased prevalence of RS in homosexual/bisexual men with HIV infection is related to a greater frequency of risk behaviors that increase exposure to arthritogenic organisms.

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POLYMORPHISM OF THE TUMOR NECROSIS FACTOR REGION IN RELATION TO DISEASE: AN OVERVIEW

Georges M.G.M. Verjans, MSc, Gerald Messer, MD,
Elisabeth H. Weiss, PhD, Sjef M. van der Linden, MD,
and Aize Kijlstra, PhD

The strongest association of a HLA allele with rheumatic diseases has been found between HLA-B27 and ankylosing spondylitis (AS).⁵³ Among Caucasian people, the prevalence of HLA-B27 in the general population is about 8%, whereas this antigen is found in more than 90% in patients with AS. The association shows a dominant or additive mode of inheritance. Among B27-positive individuals less than 2% will develop AS, although the prevalence of AS in adult B27-positive first-degree relatives of B27-positive AS patients is at least tenfold higher.^{57,58} This increase among relatives in affected families prompted the hypothesis that other genetic factors might participate additively with the B27 allele in the susceptibility to AS.

The distribution of non-B27 alleles of the HLA-B locus among B27-positive patients with AS has been studied to detect any additional HLA-B allele(s) that may act in conjunction with B27 to increase susceptibility to AS. HLA-Bw60 (or B40 when the Bw60,61-split of B40 was not typed for) was statistically shown to be increased significantly among B27-positive AS patients in each of five independent data sets. Susceptibility to AS in B27-positive individuals was further increased by a factor of about three when Bw60 was also present.⁴⁴

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RHEUMATIC DISEASE CLINICS OF NORTH AMERICA

Two major theories have been proposed to explain the association between B27 and AS.³ The one gene theory suggests that the HLA-B27 antigen itself is directly involved in the pathogenesis of the disease, either through its function as restriction element for T lymphocytes or through another yet unknown function of the B27 molecule.^{16,51,62} With regard to this theory, the additional genetic factors could be either HLA-linked or segregate independently of the HLA complex. The two gene theory postulates that the B27 molecule itself is not involved but is a marker for a second HLA-linked gene in strong linkage disequilibrium with HLA-B27 that causes or permits the development of the disease.³¹

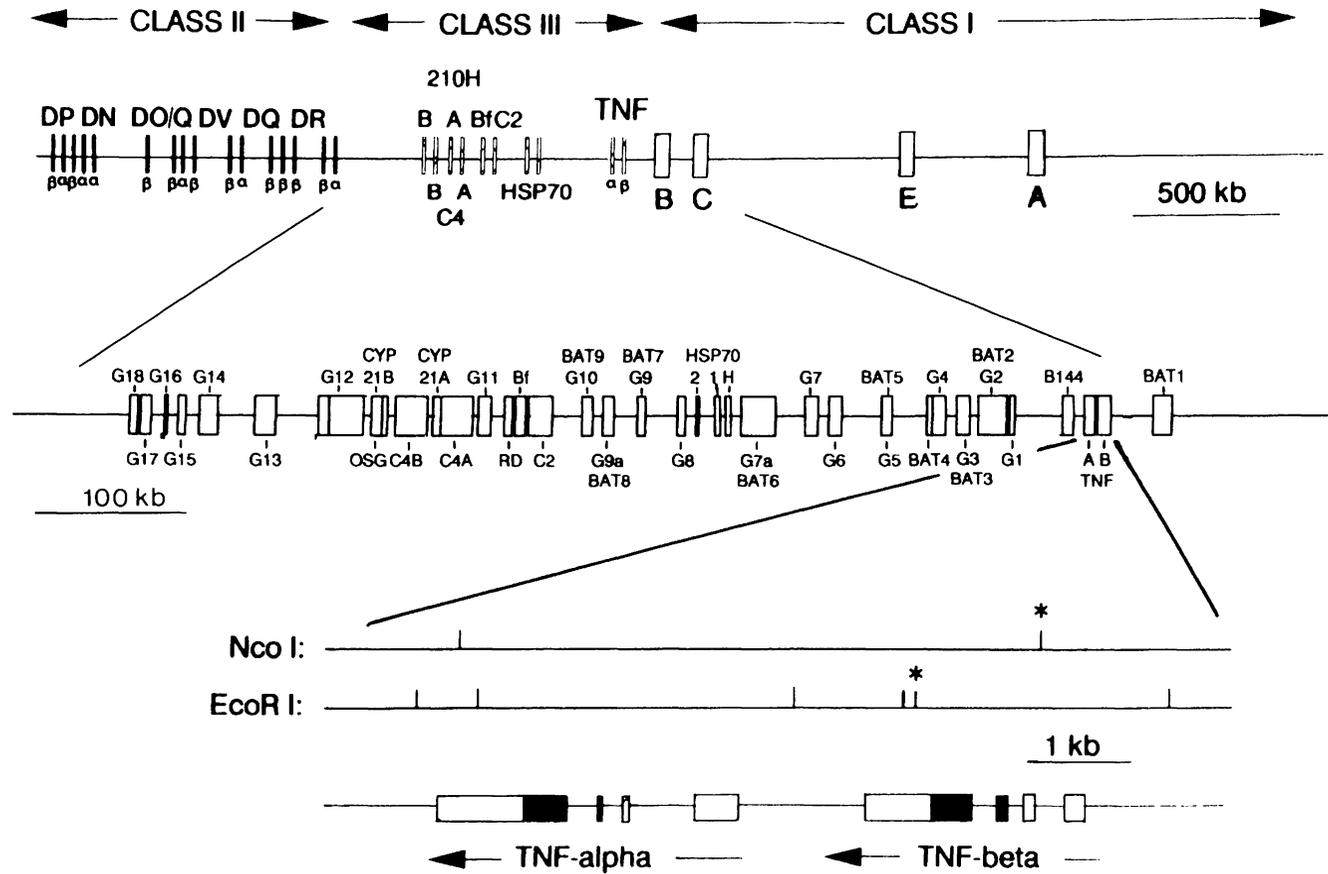
GENETIC ORGANIZATION OF THE HLA SYSTEM

The human major histocompatibility complex (MHC), located on the short arm of chromosome 6, comprises a chromosomal segment of 3500 kb (Fig. 1).²⁶ A molecular linkage map of the HLA complex has been established by linkage analysis and recently by pulsed-field gel electrophoresis.^{8,12,13,42} It contains a large number of loci that, based on structure and function of the encoded proteins, have been organized into three gene clusters. Located at the telomeric end of the HLA complex is the class I region encoding the classical transplantation antigens HLA-A, -C, and -B. At the centromeric end, the class II genes are located, consisting of HLA-DP, -DO, -DV, -DQ, and -DR. The class I and class II molecules are cell-surface glycoproteins regulating antigen recognition by T lymphocytes. Between the class I and class II region is the class III region, originally characterized as a heterogeneous group of genes encoding complement factors (C2, Bf, C4A, and C4B).⁶ Several genes for previously known proteins have been localized in the class III region: steroid 21-hydroxylase (21OHA and 21OHB),⁷ the tumor necrosis factor (TNF) genes TNF- α (cachectin), and TNF- β (lymphotoxin),^{8,13,20,36,42} and the heat shock proteins (HSP 70), two highly homologous copies and one more divergent gene.^{33,47} In addition, a large number of sequences have been described, characterized by unmethylated CpG-rich sequences that are constitutively transcribed; either called, owing to their location in between C2 and HLA-B, B-associated transcripts (BAT 1-9)^{48,49} or G-loci (G 1-10).⁴⁶ Moreover, eight additional genes (G 11-18) have been identified by the same approach centromeric of C2.²⁵ In analogy to the MHC of the mouse, a human sequence homologous to the murine B144 gene⁵⁵ has been characterized 5' of the human TFN- α gene (Holzinger I et al, unpublished data).

Because of the localization and the biologic activities of the genes encoding HSP70 and TNF, it has been suggested that these loci might contribute to the susceptibility of HLA-associated diseases.^{8,27,47}

The HSP70 proteins, induced in response to cellular stress, belong to the family of so called stress proteins (HSP60, HSP70, and HSP90) that are evolutionary conserved between prokaryotes and eukaryotes. Stress proteins play a cell protective role and are involved in post-translational protein assembly and protein translocation.²⁴

Figure 1. Genetic map of the HLA complex with the genes encoded by the class I, II, and III regions. The middle line shows an extension of the class II region of 900 kb between HLA-DR and HLA-B. The bottom graph displays the organization of the TNF- α and - β genes, with the introns (*open bars*) and exons (*closed bars*). The Nco I and EcoR I restriction sites are shown, and the polymorphic restriction sites are indicated (*asterisks*). The arrows signify the transcriptional orientation. (Data from references 12, 25, 34, 37, 43, 47 and 50.)



The TNF genes encode closely related cytokines with a broad range of biologic activities and are primarily produced by macrophages and activated lymphocytes.⁴ Biologic effects of TNF include the regulation of the immune response by the induction of gene expression, such as HLA antigens,^{10,41} activation of polymorphonuclear leukocytes, and cytotoxic effects against tumor cells.^{28,39}

RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS IN ANKYLOSING SPONDYLITIS

Genetic polymorphism is essential for the biologic function of proteins such as class I and class II MHC antigens. Recombinant DNA technology facilitates and increases the identification of genetic polymorphisms. In addition, the use of restriction fragment length polymorphisms (RFLPs), by hybridization of restriction enzyme digested genomic DNA with radio-labeled cloned DNA probes, provides a powerful tool for the diagnosis of hitherto undetectable disease states and enables the chromosomal localization of the loci responsible.

During the past years, several groups have employed RFLP analysis, using class I MHC cDNA or genomic probes, to identify additional genes besides HLA-B27 involved in the pathogenesis of AS. No RFLP linked with AS has been identified so far.⁶¹ A 9.6 kb Pvu II fragment³⁷ and a 3.5 kb Taq I fragment⁵⁴ were equally present in B27-positive AS patients as in B27-positive controls. A 9.2 kb Pvu II fragment was reported by one group to be increased significantly in B27-positive AS patients compared to B27-positive controls³⁰; however, this could not be confirmed by two other studies.^{15,61}

In addition, it has been postulated that polymorphic T-cell receptor (TcR) α and/or β genes might confer susceptibility to AS. A preliminary RFLP analysis of the TcR α and β genes in patients with AS showed no disease association with the TcR alleles studied.¹⁴

GENETIC POLYMORPHISM OF THE TUMOR NECROSIS FACTOR REGION

In mouse model systems, autoimmunity has been attributed to a low TNF- α response in NOD-mice with diabetes²¹ and mice prone for lupus nephritis (NZWxNZB, F1).²³ Application of TNF could prevent the autoimmune phenomena. A RFLP has been described for the TNF- α gene in the mouse. No studies, however, have been analyzed so far as to whether one of the TNF- α alleles might correlate with a low TNF- α response.⁴³

The localization of the human TNF genes, analogue to the murine TNF genes,^{34,35} in the HLA complex initiated investigations to detect polymorphisms in the human TNF genes. Two of 40 endonucleases tested, Eco RI and Nco I, revealed a polymorphic pattern when hybridized with TNF cDNA probes.^{17,38} The polymorphic Eco RI site maps to the 3' untranslated region of TNF- β gene (exon 4) (Fig. 1) resulting in either a 2.4 kb or a 2.5 kb Eco RI fragment.³⁸ The polymorphic Nco I recognition sequence has been detected by Nedospasov et al³⁶ by comparison of the cloned genomic TNF- β sequences when the MHC localization of the TNF genes was not known. The polymorphic Nco I site was later wrongly assigned to the TNF- α gene.^{2,11,17,18} The location within the first intron of the TNF- β gene was finally confirmed by genomic Southern blot analysis using both a TNF- α and a TNF- β probe, by restriction site mapping of cloned genes, and by direct genomic sequencing.^{1,32,59,60}

In Nco I-digested genomic DNA, the TNF- α probe hybridizes with either a fragment of 5.5 kb (indicate for the TNF β *1 allele) or a 10.5 kb fragment (encoding the TNF β *2 allele), whereas the TNF- β probe detects both a 5.5 kb and a 5 kb Nco I fragment or the 10.5 kb fragment (Fig. 1). The Eco RI and the Nco I RFLP of the TNF- β gene segregate independently, enabling four TNF- β alleles to be analyzed by RFLP typing.³²

It has been shown recently that the polymorphic Nco I restriction site is linked to an amino acid substitution in the native TNF- β protein.³² The amino-terminal asparagine at position 26 is conserved in the TNF β *1 allele and threonine in the TNF β *2 allele. The significance of this single amino acid substitution with regard to the biologic activities of TNF- β is not known.

THE Nco I TNF- β RFLP IN RELATION TO CYTOKINE PRODUCTION

The molecular analysis of the TNF- β RFLPs led to the question whether these polymorphisms might be involved in differential cytokine secretion. A recent study analyzed the secretion of interleukin 1 beta (IL-1 β) and TNF- α of monocytes, stimulated with lipopolysaccharide and phytohemagglutinin.⁴⁰ Homozygosity for TNF β *2 correlated with high secretion levels of both IL-1 β and TNF- α , whereas heterozygosity was associated with low secretion levels of IL-1 β and TNF- α . In contrast, no association of the TNF- α production on lipopolysaccharide or mitogen stimulation of monocytes or peripheral blood mononuclear cells with the Nco I RFLP has been observed by Jacob et al.²² In an analysis of the TNF- α and TNF- β production by phytohemagglutinin-stimulated peripheral blood mononuclear cells in individuals homozygous for the Nco I TNF- β alleles, no significant differences in TNF- α secretion was detected, whereas donors homozygous for the TNF β *1 allele showed a significantly higher TNF- β production compared to individuals homozygous for the TNF β *2 allele.³² Furthermore, Sachs et al.⁴⁵ reported a significantly reduced TNF- β response in insulin-dependent diabetes mellitus (IDDM) patients. The genetic basis responsible for the differences in the production of TNF- α is not known so far. In conclusion, these data show that the Nco I TNF- β alleles are associated with distinct cytokine secretion profiles.^{32,40}

TNF- β RFLP ANALYSIS IN VARIOUS DISEASES

In humans, the Eco RI RFLP of the TNF- β gene is low informative: the less common fragment (2.5 kb) is present at a low frequency (6%) in the Caucasian population.^{38,59} Moreover, the 2.5 kb fragment is strongly associated with HLA-Bw60.⁵⁹

The frequencies of the Nco I fragments are 67% to 71% and 29% to 33%, respectively.^{17,32} The TNF β *2 allele is associated with HLA-DR4 and -B15, whereas the TNF β *1 is strongly associated with the HLA-A1, -B8, -DR3 haplotype.^{2,11,40} Because the A1/B8/DR3 haplotype is known to be strongly associated with various immunoinflammatory diseases, it has been suggested that a particular TNF- β allele might be involved in the pathogenesis of these diseases.^{11,50}

Fugger et al.^{17,18} have analyzed the frequency of the Nco I TNF- β RFLP in small groups of patients with various diseases, including primary biliary cirrhosis (PBC), pauciarticular juvenile rheumatoid arthritis (P-JRA), rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS), and systemic lupus erythematosus (SLE) (Table 1).

Table 1. TUMOR NECROSIS FACTOR REGION Nco I RFLP PHENOTYPES IN PATIENTS AND CONTROL SUBJECTS*

Study Group	Nco I Fragment			N
	5.5 kb+, 10.5 kb-	5.5 kb+, 10.5 kb+	5.5 kb-, 10.5 kb+	
Patients				
PBC ¹⁷	6 (27.3)	9 (40.9)	7 (31.8)	22
PBC ^{32a}	6 (10)	24 (40)	30 (50)	60
RA ¹⁸	3 (12.5)	6 (25)	15 (62.5)	24
RA†	4 (11)	19 (52.8)	13 (36.1)	36
SLE ¹⁸	6 (30)	7 (35)	7 (35)	20
P-JRA ¹⁸	9 (18.4)	24 (49)	16 (32.7)	49
pSS ¹⁸	2 (10.5)	14 (73.7)	3 (15.8)	19
Controls				
Random controls ¹⁸	8 (6.1)	60 (45.8)	63 (48.1)	131
Random controls ^{32a}	20 (11.2)	78 (43.6)	81 (45.2)	179
Patients⁴⁰				
Random IDDM	3 (11.1)	17 (63)	7 (25.9)	27
DR3+, DR4+, DQw8+ IDDM	3 (11.5)	17 (65.4)	6 (23.1)	26
Controls⁴⁰				
Random controls	3 (12)	9 (36)	13 (52)	25
DR3+, DR4+, DQw8+ controls	4 (30.8)	8 (61.5)	1 (7.7)	13

* Values are the number (%), RFLP = restriction fragment length polymorphism.

† Messer G et al, personal communication.

Data from references 17, 18, 32, 32a, and 40.

The TNF β *2 allele was decreased significantly in primary biliary cirrhosis and systemic lupus erythematosus patients. In the latter group, this is probably secondary to the strong association between HLA-B8 and the TNF β *1 allele.¹¹ Moreover, in a larger group of primary biliary cirrhosis patients analyzed by Messer et al,^{32a} no deviation in the frequency of the Nco I TNF- β alleles was observed.

Susceptibility to IDDM is associated with HLA-DR3 or -DR4 or both, with heterozygosity for both alleles conferring the highest risk.^{50,53} It has been postulated that loci within the class III MHC region contribute to IDDM.^{5,52} Two groups have studied the TNF- β RFLP in IDDM patients.^{2,40} Badenhoop et al² analyzed 43 informative diabetic families (79 IDDM patients), but owing to the small number of HLA-matched controls, the gene frequencies in patients could only be compared with a randomly selected control group. In patients, the DR4 specificity was strongly associated with the TNF β *2 allele (77%), and 30% of DR3 haplotypes also carried the TNF β *2 allele. Similar results have been obtained by Pociot et al⁴⁰ studying DR3, 4 heterozygous IDDM patients (N = 26) in comparison to DR-matched healthy controls (N = 19). In HLA-DR3, 4 individuals, the DR3 haplotypes carried the TNF β *2 allele three times more frequently in IDDM patients than in controls. It was suggested that the TNF β *2 allele, when present on the DR3-haplotype, may contribute to susceptibility to IDDM in HLA-DR3, 4 heterozygous individuals.⁴⁰ In addition, sibpair analysis in multiplex families showed that sibpairs concordant for IDDM were identical for the TNF- β alleles,⁴⁰ even if they were only haploidentical for HLA-B-DR haplotypes.²

In addition, the frequency of the Nco I RFLP has also been analyzed in patients

with multiple sclerosis and optic neuritis. No association with a TNF- β allele was found.¹⁹

TNF- β RFLP ANALYSIS IN PATIENTS WITH ANKYLOSING SPONDYLITIS

Recently, we tested whether a polymorphism of the TNF- β gene confers an additional risk of AS in B27-positive individuals.⁵⁹ Because the Nco I TNF- β RFLP frequency was not significantly different between patients with AS and controls, it was concluded that neither Nco I TNF- β alleles are associated with AS.⁵⁹

In view of the suggested synergistic effect of B27 and Bw60 in AS,⁴⁴ we investigated the possible association between Bw60 and the Nco I and EcoR I RFLP, respectively, of the TNF- β gene. Although a significant association was found between Bw60 and the EcoR I 2.5 kb fragment, RFLP analysis with Eco RI of B27, Bw60 heterozygous AS patients and control subjects suggests that the increased risk for AS in this group of individuals is probably not due to the presence of the particular TNF- β allele characterized by the 2.5 kb EcoR I fragment.⁵⁹ Because this study was limited to RFLP analysis in unrelated individuals only, it does not exclude the possibility that TNF polymorphisms or differences only detectable by allelic sequencing are involved in AS susceptibility.

Although the search for additional genes in the HLA complex, contributing to the disease susceptibility of HLA-B27, was unsuccessful so far and current knowledge favors that B27 itself is directly involved, substantial evidence remains that other genetic factors besides HLA-B27 are involved in the pathogenesis of AS.^{3,44} At this moment, there is no direct evidence that these alleles are carried by the B27 haplotype,²⁹ but are more likely located on the non-B27 haplotype (possibly the Bw60 haplotype) or elsewhere in the genome.⁴⁴

SUMMARY

HLA antigens have been shown to be associated with several immunoinflammatory diseases. The mechanisms by which these antigens confer susceptibility to disease continue to be of major interest. Rapid progress has been made in the elucidation of the structure and function of class I and II MHC molecules, and several genes located within the HLA complex have been identified which are potentially involved in immunologic processes.

Because of the HLA localization of the TNF- α and - β genes and the biologic activities of the gene products, recent investigation has focused on a possible role of polymorphic TNF genes in the pathogenesis of HLA-associated diseases. Allelic variations have only been detected in the TNF- β gene. No evidence has been found so far that a particular TNF- β allele contributes significantly in the susceptibility to the diseases studied.

Although it has been postulated that the TNF β *2 allele contributes to susceptibility to IDDM in HLA-DR3, 4 heterozygous individuals, a larger group of HLA-typed patients and controls is needed to provide more conclusive evidence for this hypothesis. The increasing number of genes of unknown function encoded by the class III region leaves the possibility that the observed HLA associations in some diseases may be related to the presence of these genes.⁹

In AS, the lack of association with the TNF- β alleles furthermore supports the function of the HLA-B27 molecule in the disease and underlines the improbability that HLA-B27 is merely a marker for a closely linked susceptibility gene.

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