

Molecular mimicry in Reiter's syndrome: cytotoxicity and ELISA studies of HLA-microbial relationships

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SUMMARY

The pathogenic links between HLA antigens, certain bacterial infections and arthritis have not yet been characterized. The hypothesis of cross-reactivity between HLA B27, the marker of disease susceptibility for these disorders, and the provocative microorganism has been suggested by studies of *Klebsiella* and ankylosing spondylitis. The present study examines the possibility of molecular mimicry between HLA B27 and two organisms implicated more directly in reactive arthritis, *Yersinia enterocolitica* and *Chlamydia trachomatis*. Antibodies against these organisms were obtained both from patients and from antisera raised in rabbits. Neither source of antibacterial antibody was specifically cytotoxic for HLA B27-positive lymphocytes, even when the target cells were derived from patients with recent infections due to these organisms. In addition, monoclonal antibodies against HLA B27 (M1 and M2) showed no reactivity with antigens from these organisms in an ELISA system. These data do not support the notion of molecular mimicry as being the basis of immunogenetic susceptibility to reactive arthritis and Reiter's syndrome following infections with *Y. enterocolitica* and *C. trachomatis*.

INTRODUCTION

Reiter's syndrome (RS) and reactive arthritis (ReA) are paradigms of rheumatic diseases in which preconditions of a particular immunogenetic susceptibility on the part of the host combined with a particular exogenous antigen must be met. For the former, HLA B27 is present in 75-90% of Rs and ReA (Leirisalo *et al.*, 1982), and the presence of this antigen clearly confers heightened risk of RS and ReA after certain bacterial infections. Such a risk has been estimated at 20-35% (Keat, 1983). Of the organisms implicated in a provocative role in these diseases, the predominant gastrointestinal pathogens are *Yersinia enterocolitica*, *Salmonella* sp., *Shigella* sp. and *Campylobacter*, while the genitourinary pathogens are *Chlamydia trachomatis* and *Ureaplasma urealyticum*.

The mechanism whereby the HLA B27 antigen, present on all nucleated host cells, confers disease susceptibility following such infections remains unknown, but several mechanisms have been postulated. The HLA-B27 gene, or a closely related gene on the sixth chromosome, may constitute an immune response gene, such that the gene products participate in an aberrant immune response to certain infections, which sets in motion the pathogenic sequence culminating in an immune-mediated synovitis. Secondly, the HLA B27 antigen expressed on all surface membranes may function as a receptor for the pathogens, thereby resulting in an abnormal host response to the organism. Finally, there may be a degree of cross-reactivity between the

HLA B27 antigen and the microbial antigens, the molecular mimicry theory. Such a phenomenon might result in a blunted immune response, since the exogenous antigen is not regarded as foreign, or alternatively might produce a true autoimmune reaction when the appropriate host immune response to the organism inappropriately includes self-antigens in its attack by virtue of immunological cross-reactivity.

This latter hypothesis has been lent support by the studies of Geczy *et al.* (1980) on *Klebsiella pneumoniae* and ankylosing spondylitis. These workers have reported that anti-*Klebsiella* antibodies raised against a particular serotype of that organism are specifically cytotoxic for lymphocytes from HLA B27-positive spondylitic patients (Geczy *et al.*, 1980). This is not seen when lymphocytes from B27-positive healthy individuals are examined. Additionally, these investigators report that lymphocytes expressing the B27 antigen may absorb a bacterial factor, possibly a plasmid, from culture supernatants of certain organisms *in vitro* (Cameron *et al.*, 1983). These observations have been a provocative contribution to this question, although not all investigators have reproduced this phenomenon (Beaulieu *et al.*, 1983).

If this model of molecular mimicry forms the basis for the HLA B27-microbial antigen interaction, then it would be anticipated that the same process might obtain in RS and ReA. In these conditions, there is frequently strong evidence implicating a particular organism in an antecedent extra-articular infection of either enteric or genitourinary origin. In the present study, we have examined the question of molecular mimicry between HLA-B27 and the organisms *Y. enterocolitica* and *C. trachomatis*.

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SUBJECTS AND METHODS

Patients

Reiter's syndrome was defined as a seronegative peripheral arthritis in combination with at least one of the following characteristics: mucocutaneous lesions, inflammatory eye disease, or urethritis. Reactive arthritis was a seronegative peripheral arthritis in the absence of extra-articular features, which followed an antecedent infection of either gastrointestinal or genitourinary tract. In both cases, sterile synovial fluid on culture was a necessary diagnostic criterion. Post-*Yersinia* ReA was diagnosed in a group of patients who developed polyarthritis within 2 weeks of enteritis due to *Y. enterocolitica*. All had titres of antibodies to *Y. enterocolitica* 0:3 greater than 1:1600, and all were HLA B27-positive. Post-urethral ReA was diagnosed in patients with polyarthritis beginning within 2 weeks of the onset of urethritis, and having either positive cultures for *C. trachomatis* or high titre antibodies against this organism. The patients were all HLA B27-positive. Normal HLA BL27-positive individuals were identified from normal blood donors, and with the assistance of Dr Dharam Singl, McMaster University.

Microorganisms

Y. enterocolitica, serotypes 0:8; 0:5, 27; 0:3 phage 8; and 0:3 phage 9b were generously provided by Dr A. Toma, Ontario Ministry of Health Microbiology Laboratories, with the assistance of Ms Marie Kennedy. The organisms were grown on trypticase soy agar plates at 22° in the dark for 48 hr then harvested and washed in phosphate-buffered saline (PBS), killed with 5% formalin, then washed three times.

C. trachomatis serotype L2 was grown on HeLa monolayer cell cultures following the method of Brunham (Brunham *et al.*, 1981). The cells were harvested after 48–60 hr, washed with PBS, and disrupted by sonication. The cell debris was pelleted by centrifugation at 250 g for 30 min using an CRU 5000 centrifuge (IEC). The supernatant was then centrifuged at 45,000 g for 30 min and the pellet resuspended in sucrose phosphate buffer. This was applied to a 35% diatrizoate sodium solution (Renografin 76, Squibb Canada Inc., Montreal) and centrifuged in a Beckman L2 ultra-centrifuge for 60 min at 78,000 g. The resulting pellet was washed in sucrose phosphate buffer. The purification of the elementary bodies of *C. trachomatis* was monitored by examining the sequential precipitates with a fluoresceinated monoclonal anti-*Chlamydia* monoclonal antibody (SYVA Corporation, Palo Alto, CA).

Uninfected HeLa cells were cultured, harvested and purified by the sequence outlined above as a control for the immunization studies.

Antimicrobial antibodies

Anti-*Chlamydial* antibodies were measured by a microimmunofluorescence assay using the test system of Electro-Nucleonics Inc. (Columbia, MD). Anti-*Yersinia* antibodies were detected by microagglutination reaction.

ELISA for antimicrobial antibodies

The procedure followed that outlined by Levy, Munoz & McCormack (1983) with certain modifications. Purified elementary bodies of *C. trachomatis* at a concentration of 10 µg/ml in bicarbonate buffer were added in 100 µl volume to wells in a

micro ELISA plate (Immunol I, Fisher Scientific, Don Mills, Ontario). After overnight incubation at 37°, 100 µl of 10% formaldehyde in PBS were added to each well and incubated at 4° for 15 min. The wells were washed three times with PBS-Tween containing 1% bovine serum albumin. Two-hundred µl of test serum or of a selected monoclonal antibody were added to the wells and incubated for 1 hr at 37°, and washed with PBS-Tween. Two-hundred µl of horseradish peroxidase-conjugated F(ab')₂ goat anti-human IgG (Cooper Biomedical, Malvern, PA) or of conjugated anti-mouse immunoglobulin (Cooper Biomedical) were added to the wells and incubated at 37° for 2 hr. The wells were washed three times with PBS-Tween and 20 µl of substrate were added to each well (25 mM citric acid, 51 mM sodium dibasic phosphate, pH 5.0, containing 40 mg *o*-phenylenediamine and 40 µl 30% H₂O₂). The reaction was stopped with 50 µl 4 M H₂SO₄ and absorbance read at 492 nm using a micro ELISA Autoreader MR 580 (Dynatech, Santa Monica, CA). A similar procedure was followed for the anti-*Yersinia* ELISA, with the coating concentration of antigen being 10 µg/ml.

Rabbit antisera

After purification of the organisms as outlined above, suspensions of *C. trachomatis* (0.56 µg/ml) and *Y. enterocolitica* 0:3 pH 8, (0.58 µg/ml) were emulsified with equal volumes of incomplete Freund's adjuvant. Uninfected HeLa cells were used for immunization of other rabbits as a control. After preimmune serum was obtained, two New Zealand White rabbits were immunized with 0.1 ml suspensions of the bacterial antigens by subcutaneous inoculation. Repeated inoculations were performed 2 and 3 weeks later. Successful immunization was confirmed by examining the rabbit antisera against the immunogens in a Western blotting system (Towbin, Staehlin & Gordon, 1979).

Patient sera and cells

For serum studies, blood was allowed to clot at room temperature, then the serum removed and stored at -70° in 0.4-ml aliquots. Peripheral blood lymphocytes were obtained by centrifugation of heparinized blood through a Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Piscataway, NJ).

Cytotoxicity studies

Studies for the cytotoxicity of patient sera or rabbit antimicrobial antisera were carried out using standard methodology with Terasaki microtitre plates. Cytotoxicity was scored from 0 (absent) to 8 (greater than 50% cells killed) using an inverted microscope. All experiments were conducted at both 4° and 37°.

Monoclonal antibodies

Monoclonal antibodies against β₂-microglobulin and Class II MHC antigens, together with control mouse ascites fluid, were obtained from Becton-Dickinson Inc. (Mountain View, CA). Monoclonal anti-B27 antibodies M1 and M2 developed by Grumet *et al.* (Grumet, Fendly & Engleman, 1981; Grumet *et al.*, 1982), with specificity for two epitopes of the B27 glycoprotein, were obtained from Cooper Biomedical (Malvern, PA). Monoclonal anti-*C. trachomatis* antibody, directed against the major outer membrane protein of the organism was the generous gift of SYVA Corporation, Palo Alto, CA.

RESULTS

Y. enterocolitica-B27

Cytotoxicity studies. Two HLA B27-positive patients with post-*Yersinia* ReA were identified who had high titre antibodies against their infecting serotype, 0:3. In order to evaluate whether these human antibodies might demonstrate cross-reactivity with the HLA B27 antigen, these sera were examined for lymphocytotoxicity against B27-positive or B27-negative human peripheral blood lymphocytes (Table 1). In the test system, rabbit anti-lymphocyte globulin as a positive control showed cytotoxicity against all human lymphocytes tested. The sera from the post-*Yersinia* ReA patients showed no cytotoxicity either against HLA B27-positive or negative lymphocytes.

The rabbit anti-*Yersinia* antisera were then examined for reactivity with peripheral blood lymphocytes from a B27-positive post-*Yersinia* RS patient, three B27-positive post-*Yersinia* ReA patients and a B27-positive normal individual (Table 2). The post-*Yersinia* lymphocytes were probed with the anti-*Yersinia* antibodies to evaluate the presence of microbial antigen absorbed onto the surface of the lymphocytes which either alone, or in combination with the B27 antigen on the cell surface, would have bound the anti-*Yersinia* antibodies. Neither the rabbit antisera against *Y. enterocolitica* 0:3 (the arthritogenic strain isolated from the patients examined) nor against *Y. enterocolitica* 0:8 demonstrated cytotoxicity for the lympho-

Table 1. Cytotoxicity of sera from patients with post-*Yersinia* reactive arthritis (Post-*Ye* ReA) and a normal person against B27-positive and -negative PBL

Patient sera	Target cells			
	B27-positive		B27-negative	
	Normal	Normal	Normal	Normal
B27-positive Post- <i>Ye</i> ReA	0	0	0	0
Post- <i>Ye</i> ReA	0	0	0	0
B27-neg normal	0	0	0	0
ALG	8	8	8	8

Anti-lymphocyte globulin (ALG) is included as a positive control.

Table 2. Cytotoxicity of rabbit anti-*Yersinia* antibodies against PBL from Reiter's syndrome (RS), post-*Yersinia* reactive arthritis (Post-*Ye* ReA) and a normal control

Rabbit antisera	RS	Target cells			
		Post- <i>Ye</i> ReA		Post- <i>Ye</i> ReA	
		Post- <i>Ye</i> ReA	Post- <i>Ye</i> ReA	Post- <i>Ye</i> ReA	Normal
Anti- <i>Ye</i> 0:3 (1)	0	0	0	0	0
Anti- <i>Ye</i> 0:3 (2)	0	0	0	0	0
Anti- <i>Ye</i> 0:8 (1)	0	0	0	0	0
Anti- <i>Ye</i> 0:8 (2)	0	0	0	0	0
ALG	8	8	8	8	8

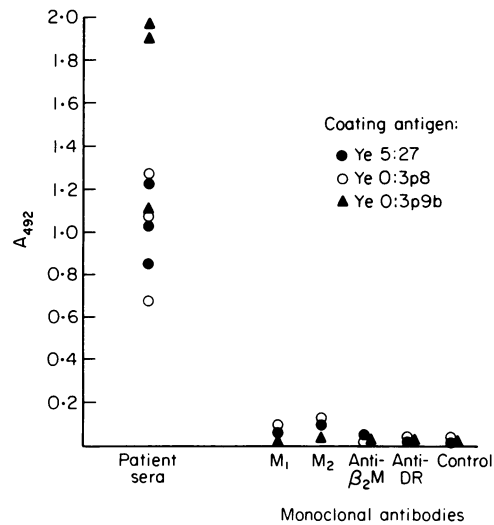


Figure 1. Binding of selected monoclonal antibodies to solid-phase *Y. enterocolitica* antigens by ELISA. M1 and M2 refer to monoclonal antibodies against HLA B27, anti-β₂M refers to monoclonal antibody against β₂-microglobulin, anti-Dr refers to monoclonal antibody against Class II HLA antigens.

cytes from RS, post-*Yersinia* ReA patients or from the B27-positive normal individual.

ELISA studies. Positive binding of sera from post-*Yersinia* ReA patients in the solid-phase ELISA system was demonstrated and revealed highest binding to the serotype 0:3 phage 9b (Fig. 1). In order to probe for cross-reactivity of HLA-B27 and the *Y. enterocolitica* antigens, two monoclonal anti-B27 antibodies were reacted in the solid-phase ELISA with antigens from the three strains. No significant binding was observed with either the M1 or M2 monoclonal antibodies. Monoclonal antibodies to the Class I MHC framework determinant (β₂-microglobulin) and to the Class II MHC antigens (DR) also failed to show cross-reactivity with the *Yersinia* antigens.

Chlamydia-B27

Cytotoxicity studies. The design of these experiments evaluated the possible role of bacterial antigens and HLA B27 in both

Table 3. Cytotoxicity of sera from B27-positive and -negative patients and normals against PBL from Reiter's syndrome (RS), non-specific urethritis (NSU) and normals

Patient serum	Serum Anti-Ct	Target cells			
		B27-positive		B27-negative	
		RS	Normal	NSU	Normal
B27-positive	RS POS	0	0	0	0
	Normal POS	0	0	0	0
	Normal NEG	0	0	0	0
B27-negative	NSU POS	0	0	0	0
	Normal NEG	0	0	0	0
ALG		8	8	8	8

Anti-lymphocyte globulin is included as a positive control.

Table 4. Cytotoxicity of rabbit antisera to *Chlamydia trachomatis* and HeLa cells against PBL from B27-positive and -negative normals

Reciprocal serum dilution	Target cells			
	B27-positive	B27-positive	B27-negative	B27-negative
N	8	8	8	8
2	8	8	8	8
4	8	8	8	8
8	8	8	8	8
16	8	8	8	6
32	6	6	2	0
64	0	0	0	0
128	0	0	0	0
	Anti-Ct	Anti-HeLa	Anti-Ct	Anti-HeLa
	Rabbit antisera			

host serum and target cells (Table 3). Serum examined for cytotoxicity derived from a B27-positive post-urethritis RS patient with high titre anti-*Chlamydia* antibodies, and two B27-positive normals with and without anti-*Chlamydia* antibodies. Serum samples were also obtained from a B27-negative patient with non-specific urethritis (NSU) and high titre anti-*Chlamydia* antibodies, and from a B27-negative normal person. Target lymphocytes were selected to examine the following possibilities: B27-positive with recent *Chlamydia* urethritis, B27-positive without *Chlamydia* infection, B27-negative with *Chlamydia* urethritis, and B27-negative without *Chlamydia* infection. No cytotoxicity was observed in these experiments, arguing against the presence of any antibodies with specificity for native B27 or an altered B27 having complexed with bacterial antigens on the cell surface.

Rabbit anti-*Chlamydia* antibodies were cytotoxic for human peripheral blood lymphocytes, but this was seen regardless of

the HLA-B27 status of the target cells (Table 4). Furthermore, the control immunization with uninfected HeLa cells demonstrated cytotoxicity indistinguishable from that seen with the anti-*Chlamydia* antibodies. Thus, it was not possible to demonstrate specific cross-reactivity of HLA-B27 and *Chlamydia* antigens using this method.

ELISA studies. Patients with RS and NSU with anti-*Chlamydia* antibodies by MIF were positive for anti-*Chlamydial* antibodies by solid-phase ELISA (Fig. 2). Positive binding was also seen in the case of the monoclonal anti-*Chlamydia* antibody (Fig. 2) developed with the anti-mouse immunoglobulin conjugate. However, no binding was observed with anti-B27 monoclonal antibodies M1 and M2. Similarly, anti- β_2 -microglobulin and anti-DR monoclonal antibodies failed to show binding to the solid-phase *Yersinia* antigens.

DISCUSSION

This study has addressed the possibility that there may exist a degree of immunological cross-reactivity between putative arthritogenic microorganisms and the HLA determinant that characterizes the population at risk for RS and ReA. The question has broad implications in biology and disease pathogenesis. Gene products of Class I MHC appear to play an important role in immune response to virus-infected cells, but their role in bacterial infections remains less well-defined. The interest in this interface of host and microbe has been heightened by the provocative observation that certain strains of *Klebsiella* may demonstrate cross-reactivity with surface determinants of lymphocytes from B27-positive patients with ankylosing spondylitis (Geczy *et al.*, 1980). Avakian *et al.* (1980) reported studies indicating that human monospecific HLA B27 typing sera had increased binding activity for *Klebsiella* extracts when compared to non-B27 tissue typing sera. Although there is *a priori* no strong reason to implicate this organism in the pathogenesis of ankylosing spondylitis, certain groups have reported a positive association between faecal carriage of *Klebsiella* and ankylosing spondylitis (Ebringer *et al.*, 1977; Kuberski *et al.*, 1983). Other reports, however, have questioned not only the faecal carriage association (Shinebaum *et al.*, 1984; Warren & Brewerton,

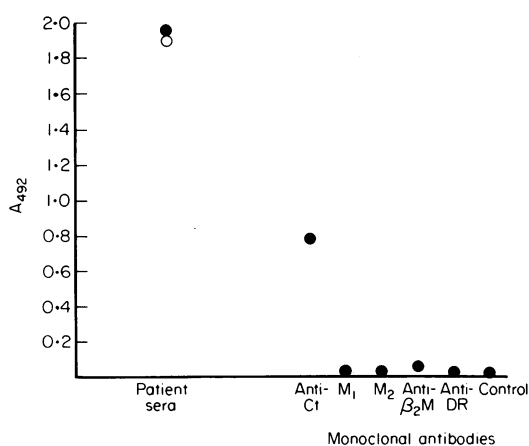


Figure 2. Binding of selected monoclonal antibodies to solid-phase *C. trachomatis* antigens by ELISA. Abbreviations are as used in Fig. 1. Anti-Ct refers to a monoclonal antibody against *Chlamydia trachomatis*. In the patient sera, the closed circle represents a patient with RS and the open circle a patient with NSU.

1980), but also the restricted cytotoxicity of anti-*Klebsiella* antisera (Prendergast *et al.*, 1984).

In view of these controversial reports linking *Klebsiella* with ankylosing spondylitis and the potential importance of this hypothesized phenomenon, we have examined this construct in clinical situations where the identity of the antecedent microbial infection was more firmly established. The evidence linking *Chlamydia* to RS has been based largely on seroepidemiological grounds (Keat *et al.*, 1980), but the recent report of Martin *et al.* (1984) has provided additional data supporting a pathogenic relationship. In this study, elevated antibody titres against *Chlamydia* were seen in post-urethral RS in comparison to post-diarrhoeal RS or rheumatic control patients. In addition, heightened lymphocyte transformation to *Chlamydia* antigens was present in lymphocytes from RS compared with controls. In the case of *Yersinia*, there is considerable data linking antecedent enteritis due to this organism with the subsequent development of RS and ReA. The mechanism underlying this relationship has remained incompletely defined. Granfors *et al.* (1980) have reported that persistence of IgA anti-*Yersinia* antibodies is a serological feature distinguishing such patients from those with uncomplicated *Yersinia* infection. In addition, a depressed lymphocyte transformation to *Yersinia* *in vitro* has been reported to be a characteristic immunological abnormality in the ReA and RS group (Vuento *et al.*, 1983). A recent report by Brenner *et al.* (1984) has demonstrated enhanced responsiveness of peripheral T lymphocytes to *Yersinia* antigens in RS. This was somewhat unexpected, in that the patients lacked anti-*Yersinia* antibodies and had no history of an antecedent diarrhoeal illness. Thus, although precise pathogenic pathways have not been defined, there is strong circumstantial evidence implicating *Chlamydia* and *Yersinia* in these B27-related reactive phenomena. If the concept of molecular mimicry is valid, it should be testable in the clearly 'reactive' processes.

In patients with post-*Yersinia* ReA, having positive stool cultures, we were unable to demonstrate by a cytotoxicity assay any serum with anti-B27 reactivity despite the presence of high titre anti-*Yersinia* antibodies in these sera. Similarly, rabbit antisera against the same arthritogenic strain of *Yersinia*, 0:3, failed to show reactivity with B27-positive lymphocytes. It has been postulated (Cameron *et al.*, 1983) that there may be a microbial factor adsorbed onto the surface of B27-positive lymphocytes, thereby rendering them susceptible to lysis by antimicrobial antisera. The absence of cytotoxicity by the anti-*Yersinia* antisera against B27-positive lymphocytes from the post-*Yersinia* ReA patients does not support such a mechanism in ReA. In screening patient sera with anti-*Chlamydia* antibodies for anti-B27 reactivity, both B27-positive (with RS) and -negative (with non-specific urethritis) patients were included. Target cells included B27-positive patients with and without *Chlamydia* infection, and B27-negative patients with and without *Chlamydia* infection. No reactivity was observed, even in the B27-positive infected patient. The rabbit anti-*Chlamydia* antisera did demonstrate lymphocytotoxicity, but this was not restricted to B27-positive lymphocytes. In fact, the control antisera raised against the HeLa cells in which the *Chlamydia* were grown showed cytotoxicity in comparable titres, stressing the importance of this control in any assessment of anti-*Chlamydia* antisera.

Using an ELISA, we demonstrated appropriate binding of patient sera to the bacterial antigens of their respective ante-

cedent infections, either *Yersinia* or *Chlamydia*. There was, however, no evidence of binding by the anti-B27 monoclonal antibodies M1 and M2 to either *Chlamydia* or to arthritogenic (0:3) and non-arthritogenic (0:5,27) strains of *Yersinia*. Similarly, there was no antibacterial binding of monoclonal antibodies directed against β_2 -microglobulin, the structural monomeric component of the Class I MHC molecule, nor against Class II MHC determinants. Van Bohemen, Grumet & Zanen (1984) have reported a degree of cross-reactivity of M2 with *Shigella flexneri*, and of M1 with *Yersinia enterocolitica* type 0:9 and *Klebsiella pneumoniae* type K1 and K43. Neither monoclonal antibody reacted with the arthritogenic strain of *Y. enterocolitica*, 0:3. Kono *et al.* (1985) have recently described a monoclonal antibody, termed Ye-1, that cross-reacts with HLA-B27 lymphoblastoid cell lines and *Yersinia enterocolitica* 0:3. This will prove a useful reagent in future studies, although precise definition of cross-reacting antigens was impaired by the low-affinity characteristics of the monoclonal antibody. These studies indicate that monoclonal antibodies may define cross-reacting antigens between bacteria and HLA molecules.

Molecular mimicry has remained an attractive model to explain variable immunological sequelae to microbial infections. Early studies in rheumatic fever demonstrated that patients' sera contained heart cross-reactive antibodies that were directed against streptococcal membrane antigen (Zabriske & Freimer, 1966). In studies of Chagas' disease, a monoclonal antibody raised against dorsal root ganglia has been found to bind to *Trypanosoma cruzi* organisms, incriminating common neuronal and trypanosomal antigens in the neuronal degeneration of this disease (Wood *et al.*, 1982). Among viral infections that have implicated pathogen-host mimicry are the cross-reactions of the T protein of simian virus 40 and a common mammalian nuclear protein of similar molecular weight (Lane & Hoeffler, 1980), and the homology of human T-cell leukaemia virus envelope gene and Class I HLA gene (Clark, Gelmann & Reitz, 1983). The report of cross-reactivity between *Klebsiella pneumoniae* and lymphocytes from B27-positive ankylosing spondylitis patients thus remains a provocative observation in understanding the pathogenesis of this B27-related disease. Our data, however, do not support this model in other B27-related diseases in which the provocative bacterial infection is characterized with greater confidence than is *Klebsiella* for ankylosing spondylitis.

The particular immunogenetic predisposition of persons bearing the HLA B27 antigen to complications of certain bacterial infections may derive from factors other than molecular mimicry. It may be that certain immune response genes are located close to the B27 gene and are in linkage disequilibrium with that gene. Up to 20% of patients with RS are B27-negative, and in our patients there is no clinical or immunological feature differentiating these patients from their B27-positive counterparts. Alternatively, the B27 gene or related genes may encode a receptor for the particular microorganisms. Such receptor would not *a priori* be expected to result in cytotoxicity with antibacterial antisera as examined in the present study. In this regard, it is of interest that Robinson *et al.* (1983) have reported a differential adherence of bacteria to HLA B27-positive and -negative buccal mucosa cells, although this was not restricted to arthritogenic organisms. From the initial contact of a pathogen to a mucosal surface to the development of a reactive synovitis is a sequence with multiple steps. Continued effort at

defining the HLA-related event in this process should yield insight, not only into the pathogenesis of RS, but also into fundamental mechanisms underlying immunogenetic susceptibility to disease.

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