The Journal of Rheumatology

VOLUME 15: NO. 7 JULY 1988

Editorials

Fibromyalgia: Whither Treatment?
F. Wolfe 1047

Molecular Relationships Between the Class II HLA Antigens and Susceptibility to RA.
C.T. McCusker, D.P. Singal 1050

The Natural History and Prognosis of AS.
A. Calin 1054

Clinical Trials with Biological Response Modifiers in Rheumatic Diseases.
M.A. Scheinberg 1056

Articles

Functional Heterogeneity of Human Rheumatoid Synovial Tissue Macrophages.
A.E. Koch, P.J. Polverini, S.J. Leibovich 1058

Effect of Metal Containing Compounds on Superoxide Release from Phorbol Myristate Acetate Stimulated Murine Peritoneal Macrophages: Inhibition by Auranofin and Spirogermanium.
C.K. Mirabelli, C-P. Sung, D.H. Picker, C. Barnard, P. Hydes, A.M. Badger 1064

The Occurrence of RF Isotypes in Early Definite RA.
K.B. Eberhardt, B. Svensson, L. Truedsson, F.A. Wollheim 1070

Resorptive Arthropathy in RA.
G.M. Mody, O.L. Meyers 1075

Concurrent Use of Folinic Acid and Methotrexate in RA.
P.S. Hanrahan, A.S. Russell 1078

Patients with RA at High Risk for Noncompliance with Salicylate Treatment Regimens.
N.C. Beck, J.C. Parker, R.G. Frank, E.A. Geden, D.R. Kay, M. Gamache, N. Shivvers, E. Smith, S. Anderson 1081

Multipotent Hemopoietic Progenitor Cells in Patients with SLE.
T. Otsuka, S. Okamura, M. Harada, N. Ohhara, S. Hayashi, S. Yamaga, K. Nagasawa, Y. Niho 1085

The Binding of Native DNA to the Collagen-Like Segment of Clq.
A.M. Rosenberg, P.A. Prokopchuk, J.S. Lee 1091

Papular Lesions and Cutaneous LE: A Comparative Clinical and Histological Study Using Monoclonal Antibodies.
N.J. McHugh, P.J. Maddison, T.I.F. MacCleod, S.G. Dean, I.E. James, N.J. Goulding, R. S-H. Tan 1097

Complete Congenital Heart Block: Risk of Occurrence and Therapeutic Approach to Prevention.
J. Buyon, R. Roubey, S. Swersky, L. Pompeo, A. Parke, L. Baxi, R. Winchester 1104

Clinical Features and Prognosis of Patients with Possible AS.
W. Mau, H. Zeidler, R. Mau, A. Majewski, J. Freyschmidt, W. Stangel, H. Deicher 1109

Restriction Fragment Length Polymorphism of T Cell Receptor α and β Chain Genes in Patients with AS.
J.P. Durand, F.A.K. El-Zaatari, A.M. Krieg, J.D. Taurog 1115

Lack of Hybridization Between *Yersinia Enterocolitica* and HLA-B27 DNA

ANNA-MARI VIITANEN, RIITTA LAHESMAA-RANTALA, ELISABETH WEISS, and AULI TOIVANEN

Abstract. **No homology was observed between *Yersinia enterocolitica* O:3 and HLA-B27 at DNA level when *Yersinia enterocolitica* chromosomal probes were hybridized with human HLA-B27 positive leukocyte DNA or in the hybridization of *Yersinia* DNA with HLA-B27 specific probe. Our results do not exclude the existence of molecular mimicry between *Yersinia* proteins and HLA-B27 antigen, since the crossreactive epitope might be a conformational determinant not detected with hybridization.** (*J Rheumatol 1988;* **15:1123–1125)**

Key Indexing Terms:
YERSINIA ENTEROCOLITICA ARTHRITIS HLA-B27

One popular theory to explain the pathogenetic mechanisms leading to reactive arthritis and other HLA-B27 associated diseases is the potential molecular mimicry between microbes and the HLA-B27 antigen[1,2]. Such a mimicry has been described for *Shigella flexneri* and *Yersina enterocolitica*[3], both known as triggers of reactive arthritis, and for *Klebsiella pneumoniae*[4], often connected to the etiology of ankylosing spondylitis (AS)[5,6]. Pulkkinen, *et al* using DNA hybridization and *Yersinia* plasmid as a probe, demonstrated that the potential crossreactivity of *Yersinia enterocolitica* is not mediated by the virulence plasmid[7]. Recently a sequence of 6 amino acids shared by HLA-B27 antigen and *Klebsiella* nitrogenase reductase has also been reported[8,9]. On this basis, our study was designed to investigate the existence of homology between *Yersina enterocolitica* total DNA and the HLA-B27 gene.

MATERIALS AND METHODS

Experimental design. Total DNA of a strain of *Yersinia enterocolitica* isolated from a patient with reactive arthritis was digested with the restriction enzyme Taql and fractionated by electrophoresis into 19 fractions. These were used as probes on Southern blot hybridization with total DNA from HLA-B27 positive and negative human leukocytes. In the reverse experiments, genomic and cDNA probes cloned from the HLA-B27 gene were used for *Yersinia enterocolitica* DNA as target.

In an additional experiment, we studied bacterial colonies isolated from stool samples of 11 patients with *Yersinia* triggered arthritis, 5 patients with *Yersinia* enteritis without arthritis and 8 controls. Strains were analyzed with colony hybridization technique using HLA-B27 cDNA as a probe.

Patients. Female and male Caucasian patients with arthritis were used as blood donors. In addition to the clinical features the diagnosis of *Yersinia* triggered reactive arthritis was verified by the isolation of *Yersinia enterocolitica* and/or serology. HLA-B27 negative healthy persons without a history of yersiniosis and *Yersinia* antibodies were used as controls. HLA-B27 antigen was determined by a cytotoxicity test (Histognost-B27, Behring Institut, Behringwerke AG, Marburg, W. Germany).

Bacterial strains. The *Yersinia enterocolitica* O:3 strain (No. 4147) used to prepare the probes was isolated from a patient with *Yersinia* triggered reactive arthritis and a high level of persistent IgA antibodies against he causative strain. The strains of *Yersinia enterocolitica* O:3 used for DNA preparations were isolated from patients with *Yersinia* enteritis (No. 5432, 4846, 2973, 3065) and from patients having reactive arthritis as a post-infection complication (No. 2230, 6874, 4147, 1881, 5991, 2883, 5870, 3668, 7326, 7532). *Salmonella typhimurium* strain (No. 5637) was isolated from a patient with reactive arthritis, and *Shigella flexneri* from a clinical patient.

In an additional experiment, bacteria of normal intestinal flora were analyzed. Stool samples from the patients and controls were collected every other day during one week and 10 colonies were picked from each sample[10]. The bacterial strains analyzed included *E. coli, Klebsiella, Proteus, Hafnia* and fecal streptococci.

Purification of Yersinia enterocolitica DNA. The strains of *Yersinia enterocolitica* O:3 were grown in brain-heart infusion (Difco, Detroit, MI) at room temperature overnight. Total DNA was isolated as described by Silhavy, *et al*[11] and digested with the restriction enzyme Taql. DNA digests were run on a preparative 0.7 (w/v) low gelling temperature agarose gel electrophoresis for 5 h at 65 mA. The total DNA was separated into 19 fractions according to the molecular weight, by cutting the gel and eluting the bands from the low gelling temperature agarose (Sigma, St. Louis, MO, USA)[12]. These fractions were employed directly as hybridization probes, 1 μg of DNA in each hybridization (Figure 1). *Yersinia* DNA digests (10 μg) used as target were separated in agarose gel electrophoresis and transferred onto Gene Screen filters. *Salmonella* and *Shigella* strains used as positive controls were prepared in the same way.

Purification of human DNA. Genomic DNA was isolated from human leukocytes according to Vanderplas, *et al*[13]. DNA (10 μg) was digested with the restriction enzyme Taql in the assay conditions recommended by the manufacturer (Amersham International, Amersham, UK), separated in an agarose gel electrophoresis and transferred onto a Gene Screen filter (NEN Research Products. Boston, MA). The restriction enzyme Taql was chosen based on the findings by Trapani, *et al*[14], demonstrating the presence of a 3.5 kb Taql restriction fragment in human genomic DNA to correlate with the expression of HLA-B27 antigen.

HLA-B27 derived probes, the genomic EcoRI fragment (6.7 kb) containing the HLA-B27 gene and 2 overlapping cDNA fragments (1.372 and 0.605 kb) derived from the HLA-B27 mRNA[15,16], were used.

DNA hybridization. The hybridizations were performed as described by

From the Departments of Medical Microbiology and Medicine, Turku University, Turku, Finland and the Institute of Immunology, University of Munich, Munich, Federal Republic of Germany.

Supported by a grant from the Sigrid Jusélius Foundation.

A-M Viitanen, MD, R. Lahesmaa-Rantala, MD, E. Weiss, PhD, A. Toivanen, MD, Professor of Medicine.

Address requests for reprints to Dr. A-M Viitanen, Department of Medical Microbiology, Turku University, SF-20520 Turku 52, Finland.

Submitted September 21, 1987 revision accepted April 20, 1988.

Fig. 1. Southern hybridization of *Yersinia enterocolitica* chromosomal DNA (positive control) with 9 of the probes used in the present study. To prepare the probes 130 μg *Yersinia* DNA was digested with TaqI and run in low gelling temperature agarose gel electrophoresis for 5 h at 65 mA. After electrophoresis the gel was cut in 19 pieces, DNA was isolated from the gel fragments, labelled by nick translation and used as probes. Numbers represent different probes.

Southern[17]. Three washing temperatures (40, 50 and 65°C) were used when hybridizing *Yersinia* DNA with HLA-B27 probes. Colony hybridization filters were analyzed according to Thayer[18]. Nick translation kit (^{32}P deoxycytidine triphosphate [50 μCi] in each labelling) and deoxyribonucleotide triphosphates (dATP, dGTP, dTTP) were used in oligonucleotide labelling[19]. The HLA-B27 DNA probes were purchased from Amersham International (Amersham, UK). The oligonucleotide primer was produced by Pharmacia Biotechnology International AB (Uppsala, Sweden) and the Klenow enzyme by Boehringer (Mannheim, Federal Republic of Germany).

RESULTS

Genomic DNA of *Yersinia enterocolitica* O:3 (strain 4147, derived from a patient with reactive arthritis) was fractionated into 19 fractions, which were used, each separately, as hybridization probes on digests of human leukocyte DNA isolated from 3 healthy, HLA-B27 negative persons and 4 HLA-B27 positive patients with *Yersinia* triggered arthritis. The results were negative. Total DNA of *Yersinia enterocolitica* O:3, *Salmonella typhimurium* and *Shigella flexneri* were used as positive controls.

In the reverse experiments genomic and cDNA probes prepared from the HLA-B27 gene were applied. The target DNA were prepared from 14 strains of *Yersinia enterocolitica* O:3 (10 strains from patients with reactive arthritis). To improve the sensitivity of hybridization, low washing temperatures were used. Even at low stringency no cross-hybridization signal was detected, and thus no indication for and homology between the bacterial and human DNA was observed. Plasmid containing HLA-B27 DNA was used as positive control of hybridization test and showed a clearly positive hybridization pattern.

To find out potential DNA homology between bacteria of the normal intestinal flora and HLA-B27, 810 bacterial colonies isolated from stool samples of 11 patients with *Yersinia* triggered arthritis, 8 patients with *Yersinia* enteritis without arthritis and 8 control persons were analyzed with colony hybridization technique using HLA-B27 cDNA as a probe. The bacterial strains were *E. coli, Klebsiella, Proteus, Hafnia* and fecal streptococci. No evidence for homology was found in these experiments.

DISCUSSION

According to the molecular mimicry hypothesis, HLA-B27 shares the antigenic activity of a microbial agent. The results presented here do not reveal that *Yersinia enterocolitica* O:3 chromosomal DNA codes for a sequence corresponding to the mRNA of HLA-B27. As no *Yersinia* sequences are present in the available (EMBL, NBRF) data banks, it was not possible to determine the codon usage of the *Yersinia* genome. Since the majority of polymorphic determinants are made of amino acids encoded by 2 triplets differing in the 3rd position, the DNA homology should be high enough to be detected under the hybridization conditions employed.

It is possible that the crossreactive epitope is a conformational determinant, which is not based on a stretch of consecutive amino acids and sufficient homology at the DNA level. In addition, the antigenic crossreactivity observed between HLA-B27 positive lymphocytes and microbial agents might be due to a small shared epitope of a few amino acids, which could not be discovered with common hybridization probes. In the hybridization conditions used in our study, the stable hybrid would not be formed between our relatively large probe and a very small homologous target DNA fragment (e.g., 20 kb)[20]. Therefore, our findings do not either exclude or confirm the possible homology between *Yersinia enterocolitica* and the HLA-B27 antigen.

The HLA-B27 probes used were isolated from a healthy individual[15,16]. Whether different subtypes of HLA-B27 have influence on the ability to trigger the disease is not quite clear. Mölders, *et al* have shown 2 types of HLA-B27 antigens (K and W type) among patients with AS[21]. Coppin, *et al* did not find any difference between HLA-B27 antigen of a patient with AS and that of normal individuals[22]. Ness and Grumet[23] detected 4 restriction length polymorphisms for the B27 gene. None of these fragments was significantly associated with AS and the polymorphic restriction site was located outside of the gene. We avoided this problem in the reverse experiments, when *Yersinia* chromosomal fragments

were used as probes for hybridizations with human leukocyte DNA as a target.

Prendergast, *et al* described crossreactivity between normal intestinal flora of patients with AS and HLA-B27 antigen using immunological techniques[24]. We analyzed bacterial colonies isolated from stool samples of patients with *Yersinia* triggered reactive arthritis and control persons to detect a homologous fragment possibly hiding in the intestine, but no homology was found between normal flora bacteria of patients with *Yersinia* triggered arthritis and HLA-B27 DNA. Thus, it is left to sequencing studies to finally reveal whether a molecular mimicry between HLA-B27 and *Yersinia enterocolitica* exists.

ACKNOWLEDGMENT

We thank Leena Pulkkinen, MSc, for technical advice and support at the start of the study.

REFERENCES

1. Inman RD, Chiu B, Johnston MEA, Falk J: Molecular mimicry in Reiter's syndrome: cytotoxity and ELISA studies of HLA-microbial relationships. *Immunology 1986;*58:501-506.
2. Toivanen A, Toivanen P, eds: *Reactive Arthritis.* Boca Raton, CRC Press, In press.
3. van Bohemen CG, Grumet FC, Zanen HC: Identification of HLA-B27M1 and -M2 cross-reactive antigens in Klebsiella, Shigella and Yersinia. *Immunology 1984;*52:607-610.
4. Avakian H, Welsh J, Ebringer A, Entwistle CC: Ankylosing spondylitis, HLA-B27 and Klebsiella. II. Cross-reactivity studies with human tissue typing sera. *Br J Exp Pathol 1980;*61:92-96.
5. Ebringer RW, Cawdell DR, Cowling P, Ebringer A: Sequential studies in ankylosing spondylitis. Association of *Klebsiella pneumoniae* with active disease. *Ann Rheum Dis 1978;*37:146-151.
6. Trull AK, Ebringer R, Panayi GS, Colthorpe D, James DCO, Ebringer A: IgA antibodies to *Klebsiella pneumoniae* in ankylosing spondylitis. *Scand J Rheumatol 1983;*12:249-253.
7. Pulkkinen L, Vuorio E, Hyypiä T, Toivanen A: Lack of DNA homology between arthritis triggering bacteria and plasmid of *Yersinia enterocolitica* or *Chlamydia trachomatis* (letter). *J Rheumatol 1986;*13:831-833.
8. Oldstone MBA, Schwimmbeck P, Dyrberg T, Fujinami R: Mimicry by virus of host molecules: Implications for autoimmune disease. In: Cinader B, Miller RG, eds. *Progress in Immunology VI.* Toronto: Academic Press, 1986;787-795.
9. Schwimmbeck PL, Yu D, Oldstone M: Autoantibodies to HLA-B27 in the sera of HLA-B27 patients with ankylosing spondylitis and Reiter's syndrome. *J Exp Med 1987;*166:173-181.
10. Viitanen A-M, Pulkkinen L, Lahesmaa-Rantala R, Huovinen P, Toivanen A: *Yersinia enterocolitica* plasmid in fecal flora of patients with reactive arthritis. *J Infect Dis 1986;*154:376.
11. Silhavy T, Berman ML, Enquist LW: *Experiments of Gene Fusion.* Cold Spring Harbor: Cold Spring Harbor Laboratory, 1984.
12. Maniatis T, Fritsch EF, Sambrook J: *Molecular Cloning. A. Laboratory Manual.* Cold Spring Harbor: Cold Spring Harbor Laboratory, 1982.
13. Vanderplas S, Wild I, Gropler-Rabie A, *et al:* Blot hybridization analysis of genomic DNA. *J Med Genet 1984;*21:164-172.
14. Trapani JA, Mickelson CA, McKenzie IFC: A 3.5 kilobase TaqI restriction fragment of genomic DNA segregates with HLA-B27. *Immunogenetics 1985;*21:189-192.
15. Weiss EH, Kuon W, Dörner C, Lang M, Riethmüller G: Organization, sequence and expression of the HLA-B27 gene: A molecular approach to analyse HLA and disease association. *Immunobiology 1985;*170:367-380.
16. Szöts H, Riethmüller G, Weiss E, Meo T: Complete sequence of HLA-B27 cDNA identified through the characterization of structural markers unique to the HLA-A, -B, -C allelic series. *Proc Natl Acad Sci USA 1986;*83:1428-1432.
17. Southern E: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol 1975;*98:503-517.
18. Thayer RE: An improved method for detecting foreign DNA in plasmids of *Escherichia coli. Anal Biochem 1979;*98:60-63,
19. Feinberg AP, Vogelstein B: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem 1983;*132:6-13.
20. Meinkoth J, Wahl G: Hybridization of nucleic acids immobilized on solid supports. *Anal Biochem 1984;*138:267-284.
21. Mölders HH, Breuning MH, Ivanyi P, Ploegh HL: Biochemical analysis of variant HLA-B27 antigens. *Hum Immunol 1983;*6:111-117.
22. Coppin HL, McDevitt HO: Absence of polymorphism between HLA-B27 genomic exon sequences isolated from normal donors and ankylosing spondylitis patients. *J Immunol 1986;*137:2168-2172.
23. Ness DB, Grumet FC: New polymorphisms of HLA-B27 and other B locus antigens detected by RFLP using a locus-specific probe. *Hum Immunol 1987;*18:65-73.
24. Prendergast JK, McGuigan LE, Geczy AF, Kwong TSL, Edmonds JP: Persistence of HLA-B27 cross-reactive bacteria in bowel flora of patients with ankylosing spondylitis. *Infect Immun 1984;*46:686-689.