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

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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
- 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 *Yersinia 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 TaqI 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 the 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 TaqI. 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 TaqI 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 TaqI was chosen based on the findings by Trapani, *et al*\(^14,15\), demonstrating the presence of a 3.5 kb TaqI 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...
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). 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. 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. Coppin, et al did not find any difference between HLA-B27 antigen of a patient with AS and that of normal individuals. Ness and Grumet 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. We analyzed bacterial colonies isolated from stool samples of patients with Yersinia triggered arthritis and control persons to detect a homologous fragment possibly hiding in the intestine, but no homology was found between normal flora bacteria 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.

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REFERENCES


