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HLA-D Region Genes and Susceptibility to Rheumatoid Arthritis

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HLA-D REGION genes play a crucial role in immune response, transplantation, and disease susceptibility. It is known that polymorphisms in structural genes as well as level of cell–surface expression of these molecules contribute to disease susceptibility.[1] In the present study, we have therefore examined the role of polymorphisms in DR genes, and Y box-binding nuclear proteins, which play a dominant role on level of expression DR molecules, in susceptibility to rhematoid arthritis (RA).

MATERIALS AND METHODS

Mononuclear cells from peripheral blood from 20 adult Caucasian patients with classical seropositive RA and 20 unrelated healthy Caucasian subjects were separated by the Ficoll–Hypaque density gradient centrifugation. B lymphocytes were obtained by the nylon wool adherent method and transformed with Epstein–Barr virus (EBV). In addition, 11 EBV-transformed homozygous typing cell (HTC) lines were used as controls. Patients and normal healthy controls were typed for HLA-DR antigens by oligonucleotide typing of PCR-amplified genomic DNA.[2]

Y box-binding proteins were examined by the gel-mobility shift experiments using nuclear proteins from patients, normal controls and HTCs, and two polymorphic Y box oligonucleotides: YB1.1=5′-GCTG*ATTGG*TTCTCCAACAC-3′, containing an inverted CCAAT motif, and YB1.2 5′-GCTG*ATTCG*TTCTCCAA CAC-3′, with an imperfect inverted CCAAT sequence.[3]

RESULTS AND DISCUSSION

The prevalence of RA-susceptibility DRB1-epitope carrying amino acid sequence motif QKRAA or QRRAA at position 70 to 74 in DRB1 chain was significantly (P = .028; RR = 4.3) higher in patients (65%) as compared to normal healthy controls (30%).

The results from the gel-mobility shift experiments showed that the nuclear protein(s) which binds to YB1.2 oligonucleotide was present in all patients, all healthy subjects, and all HTCs. In contrast, when YB1.1 oligonucleotide was used as a probe, specific DNA-protein complex was observed in all normal healthy controls and HTCs, but not in all RA patients. The absence of YB1.1-specific nuclear proteins in patients (50%) was significantly different ($P = 1.4 \times 10^{-5}$; RR = 31.0) from that in normal controls (0%). Further analysis of the data showed that all patients (100%) either carried the RA-susceptibility DRB1 amino acid sequence motif and/or lacked *trans*-regulatory nuclear protein that binds to the YB1.1 oligonucleotide, which contains an inverted CCAAT sequence. The relative risk associated with the presence of RA-associated DRB1 epitope and/or lack of YB1.1-binding protein for development of RA is 46.6 ($P < 1.6 \times 10^{-6}$), highest reported so far for RA.

We and others have recently shown that the affinities of Y box-binding proteins (NF-Y binds to YB1.1 and NF-YB binds to YB1.2 sequence) have inverse relationships to expression levels of DR molecules. Lack of NF-Y will therefore result in an enhanced expression of DR genes (eg, DRB1*15) which carry an inverted CCAAT sequence in the Y box of their promoters. These data suggest that an aberrant immune response due to the presence of DRB1 amino acid motif QKRAA or QRRAA and/or an enhanced expression or inappropriate expression of certain DR genes will affect presentation of both foreign and self antigens and result in the development of RA.

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