The pathogenesis of HLA-B 27-related reactive arthritis

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Two major clues are provided to investigators regarding the pathogenesis of reactive arthritis. The first is that the arthritis is induced by infection with certain intracellular bacteria. These include Salmonella typhimurium, Shigella flexneri, Yersinia enterocolitica and Chlamydia trachomatis. The second is that most patients are HLA-B 27 positive. Because the incidence of HLA-B 27 is very high in a related arthritis, ankylosing spondylitis, and also because animals transgenic with the HLA-B 27 gene develop arthritis, it is certain that the HLA-B 27 itself, and not genes in linkage disequilibrium, is the predisposing factor.

Multiple hypotheses are being proposed as to how HLA-B 27 and bacteria jointly induce the arthritis. Although none of them have yet claimed to be the ultimate solution, two major ones have been the subject of most studies. The first is that HLA-B 27 provides the target for a T lymphocyte immune response. The second is that HLA-B 27 promotes persistence of the pathogens in the host tissues including the synovia.

HLA-B 27 is a major histocompatibility class I protein. Its physiological function is to present peptides to the T cell receptors of CD 8+ T lymphocytes. A critical question has been whether in the arthritis patients, there is an immune response against HLA-B 27 complexed with bacteria derived peptides. A positive immune response has indeed been discovered. CD 8+ T cell clones have been derived from synovial T cells which are specific for HLA-B 27 target cells infected in vitro with arthritis-causing bacteria. In patients with reactive arthritis, serum antibodies have also been discovered which are specific for HLA-B 27 cells transfected with the mycobacterial hsp 65 gene, or carrying peptides derived from the sequence of the corresponding heat shock protein. This protein is selected for study because it is an immunodominant antigen, and highly homologous among bacteria. These studies indicate that there is an active immune response against HLA-B 27 expressed on cells infected by arthritis-causing bacteria. Interestingly, there are also some CD 8+ T cells which are reactive with HLA-B 27 target cells even when they are not infected in vitro with bacteria. These autoreactive T lymphocytes are potential mediators of autoimmune response.

To identify the peptides which are the targets of these HLA-B 27-restricted immune responses, it is necessary to know how peptides which are presented by HLA-B 27 are different from those which are presented by other alleles. This particular question has been the focus of several groups of investigators. Many of the studies are based on the principle that peptides which are presented by HLA-B 27 can be distinguished by their ability to stabilize the complex between HLA-B 27 heavy chain and β 2-microglobulin. In our own laboratory, we have been able to recognize these HLA-B 27 molecules by monoclonal antibodies. We have distinguished two
groups of antibodies. One group is reactive with HLA-B27 molecules irrespective of the peptides they are complexed with. The prototype of this group is the ME1 antibody. Using this ME1 antibody, our studies have arrived at the conclusion that the critical residue in peptides which bind to HLA-B27 is an arginine at P23. This motif is in agreement with studies derived from crystallography and from sequencing of peptides extracted from HLA-B27. The other group of anti-HLA-B27 antibodies is more specific for the peptides. The Ye2 antibody is reactive with HLA-B27 complexed with the peptide of the sequence RRYQKSTEL4. The B27.M2 peptide is reactive with HLA-B27 complexed with the peptide with the sequence RRKAMFEDI5. In studies using a large number of analogues of the corresponding peptides, we have arrived at the conclusion that the specificities of the Ye-2 and the B27.M2 antibodies reside on the conformation of the HLA-B27 heavy chain which is in turn dependent on the residing peptides6. This is novel because the usual perception is that immune recognition of an HLA-B27-peptide complex should be strictly specific for the residues of the peptide as well as the residues of the heavy chain. Immune recognition of the conformation as described here on the other hand, is much more promiscuous and reminds one of an immune response cross-reactive between self antigens and pathogen derived antigens.

Using the above information, we have attempted to identify the peptides responsible for the HLA-B27-restricted CD8+T cell clones derived from the synovial fluids of arthritis patients. One auto-reactive cell T lymphocyte clone indeed carries the specificity of one which recognizes conformation dependent changes of the heavy chain. It is able to recognize HLA-B27 complexed with several peptides of very different sequences. This might explain why it is able to react with HLA-B27 carried on different cell lines even when they are not infected in vitro with bacteria. Several other clones recognize peptides derived from bacterial heat shock 65 KDa proteins8. How these T lymphocytes contribute to arthritis is currently a focus of study. Because the number of these lymphocytes is very small, it appears unlikely that they mediate arthritis by their cytotoxicity. Their cytotoxic potential might be fortuitously important by providing a convenient in vitro assay. More likely is the possibility that these cells participate in the immune response by releasing arthritis-causing cytokines such as TNFα. TNFα is detected in the synovial fluids as well synovia of these patients. In Rheumatoid arthritis, it has been demonstrated to be so important for that it is the target of specific therapies.

These and related studies do not explain why only a minority of HLA-B27 positive subjects develops arthritis. It is known that for peptides to complex with HLA class I molecules, the peptides need to enter the endoplasmic reticulum via TAP channels. We have studied whether polymorphism of human TAP imposes an additional restriction on the specificity of peptides. An assay is carried out in which the ability of a panel of peptides to enter the endoplasmic reticulum guarded by different TAP alleles are compared. No restriction appears to be imposed by TAP polymorphism9. Hence, TAP polymorphism is not a factor which contributes to reactive arthritis. Another factor to be considered in setting up animal models carrying HLA-B27 transgene is whether it is important for the animals also to carry human β2-microglobulin. We have carried out in vitro experiments using a panel of mutants of the human β2-microglobulin. Mutation is focused at those residues which are in contact with the HLA-B27 heavy chain. We find that β2-microglobulin does contribute to the conformation of the HLA-B27 heavy chain, as recognized by CD8+T lymphocytes or by monoclonal antibodies9. This might explain why HLA-B27 transgenic mice are unable to develop arthritis unless there is knockout in the endogenous mouse β2-microglobulin, the human β2-microglobulin playing a permissive role.

Overemphasis on the role of the immune response on HLA-B27-restricted immune response in arthritis is not without serious challenge. A major con-
fender is the possibility that arthritis is caused by persistence of bacteria in arthritis hosts. Discovery of Yersinia fragments in the joints of some patients several years after the initial infection has heightened this possibility. What has been lacking has been an explanation of how HLA-B27 induces this persistence. Recent in vitro studies have shown that after invasion into cultured cell lines, *Salmonella typhi* remain viable for several days in higher numbers inside those cells which carry transfected HLA-B27. This is so in contrast to cells carrying transfected HLA-A2. It indicates that HLA-B27 can alter the interaction between bacteria and the cells in multiple aspects independent of the immune response. A major question for future investigation is how HLA-B27 mediates these cellular activities. Since bacteria invasion involves signal transduction of the target cells, it is an area of promise for investigators. We have discovered for example, that when *Salmonella* invade into cells, a signal is induced to increase the alternate splicing of the HLA-B27 pre-mRNA.

**REFERENCES**


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