Editorial

How Much HLA-B27 Expression Is Needed for Spondyloarthritis?

Until relatively recently, quantitative effects of HLA-B27 expression have not attracted much attention in relation to explaining the allele's association with spondyloarthritis (SpA). The classical hypothesis, that HLA-B27 presents a "spondylogenic" peptide to CD8+ T cells in order to initiate an inflammatory arthritis[1], does not depend critically on the amounts of B27 expressed on the cell surface. CD8+ T cells are notoriously sensitive detectors of antigen — it has even been claimed that they may be able to detect a single HLA:peptide complex on a target-cell surface[2], although these observations apply to antigen-specific clones already selected *in vivo* and *in vitro* for high affinity recognition of antigen. However, in viral infection the amounts of class I MHC that are expressed must have a critical bearing on generation of specific immune responses, otherwise viruses would not invest in so many strategies to downregulate host cell class I MHC expression[3,4]. Intracellular bacteria have similar abilities, as demonstrated for the reactive arthritis-associated pathogen *Chlamydia trachomatis*[5].

There are additional reasons to consider in more detail the consequences of altered HLA-B27 expression in pathogenesis of SpA. First, in the B27 transgenic rat model of SpA, the requirement for multiple copies of B27 heavy-chain and/or ß$_2$m transgenes relates to a need for supranormal levels of B27 expression to produce both joint and gut pathology[6,7]. Similarly, in patients with ankylosing spondylitis (AS), one group has reported statistically higher levels of B27 expression by lymphocytes of patients with AS as compared to healthy HLA-B27+ controls[8]. These observations can be coupled with several of the more recent hypotheses put forward to explain what B27 is doing in SpA, in the absence of convincing data to clinch the concept of a spondylogenic peptide. These all have elements in which quantitative differences in B27 expression would play a part.

For instance, if the formation of B27 heavy-chain homodimers[9,10] is critical to SpA pathogenesis, this would be favored by increased quantities of surface B27 molecules containing suboptimal peptides. These peptides could then dissociate from the B27 trimeric molecule producing B27:ß$_2$m dimers, which rapidly fall apart to produce free heavy chains; these are then available to form dimers. Dimers may play a role in altering T cell or antigen-presenting cell responses through their interaction with class I MHC interacting receptors other than the T cell receptor; such receptors include those of the KIR (killer cell Ig-like receptor) and LILR (leukocyte Ig-like receptor) families[9,11,12]. These same receptors also modulate activation of classical CD8+ T cells, so even classical class I MHC-restricted responses could be influenced by quantitative changes in B27 expression.

A modification of the spondylogenic peptide hypothesis has also been proposed based on the interesting demonstration that B27 (specifically SpA-associated B*2705, but not nonassociated B*2709) is able to bind peptides in more than one configuration[13]. Given that both configurations are not likely to be present to the same extent, the quantity of the peptides whose conformation is required to elicit pathogenic responses might depend critically on the total amount of B27 expressed.

Lastly, Colbert's group have drawn attention to the consequences of inefficient folding of the B27 heavy chain in the endoplasmic reticulum[14]. Misfolded heavy chains accumulate and trigger the unfolded protein response (UPR), which in turn modulates cytokine production by antigen-presenting cells. In this case the amounts of B27 expressed are clearly critical to induction of the UPR. Thus the UPR was readily detected in cells from B27 transgenic rats[15]; in contrast, "normal" expression of B27 in a transfected monocyte-like cell line, U937, did not substantially alter either gene expression at rest, or the response to lipopolysaccharide (LPS) as assessed by gene profiling. Induction of the UPR was detectable when cells were stimulated with LPS, although not in the resting state[16].

See: Identification of cytokines that enhance promoter activity of HLA-B27, *page 862*

Therefore, given that there is increasing evidence that quantitative aspects of B27 expression might be very important, it is relevant to examine factors that might modulate this. The most obvious first step is to examine factors that might alter transcription, with proinflammatory cytokines likely candidates for this role. Zhao, *et al* report just such an analysis in this issue of *The Journal*[17]. Their first step was an *in silico* examination of the 432-base pair 5'-promoter region of HLA-B*2705 and other B alleles. Interestingly, few B alleles had identical promoter sequences to B*2705, but they include B*2706, which is thought *not* to confer susceptibility to AS, so it is not likely that differences in this region of the gene are solely responsible for the difference in disease association seen with different B27 alleles. To determine influences on transcription, ~300 base pairs of the 5' untranslated region of B*2705 (i.e., the promoter region) were linked to a luciferase reporter system and transfected into 2 host cell lines, HeLa and CCL6. The majority of the results reported relate to one transfectant HeLa clone that was particularly responsive to cytokines tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), but similar results (data not shown) were obtained with the polyclonal transiently transfected population, making it unlikely that those shown represent a peculiarity of one particular clone. Results using the luciferase system were corroborated using real-time quantitative polymerase chain reaction on transfected CCL6. Transfectants were then treated with a large battery of cytokines alone or in combination — possibly an over-large battery since it is not clear that the transfectants expressed receptors for all the cytokines tested. In any case, enhancement of transcription (3- to 6-fold) was found to be the property of only 3 categories of cytokines — TNF-α, IFN-γ, and the type I interferons IFN-ß or IFN-γ with IFN-ß being particularly effective. These results were not unexpected since the B*2705 promoter was known to contain sites for the binding of nuclear factor-κB (NF-κB; induced by TNF-α) and also an IFN-stimulated response element (ISRE). Consistent with effects through these separate transcription factor binding sites, the effects of TNF-α and each of the interferons were additive and the effects of interferons abolished by mutation of the ISRE. One interesting finding was the different kinetics shown for the effects of TNF-α compared to interferons, particularly IFN-ß, which produced maximal and sustained responses between 72 and 96 hours, whereas TNF-α's effect was maximal at 16 hours and sustained at the same level up to 96 hours. This delayed kinetic for the response to IFN-ß was difficult to explain since separate constructs containing either an NF-κB binding site or an ISRE showed prompt responses to TNF-α and IFN-ß, respectively, peaking at 6 hours and being lost at 24 hours. Thus, the B27 promoter, while containing these elements, clearly has additional features that alter the response to cytokines.

Somewhat surprising was the failure of interleukin 1 (IL-1) or IL-1ß, also excellent inducers of NF-κB, to alter transcription from the B27 promoter; when a construct containing only an NF-κB site was tested (i.e., without an ISRE), IL-1 was (as expected) an efficient transcription inducer. The authors noted that much higher levels of the p50 and p65 components of NF-κB were induced by TNF-α than by IL-1, and suggest that the B27 promoter required these higher levels to show a response and therefore appeared unresponsive to IL-1. However, in this case one might expect to have seen some synergy between IL-1 and TNF-α if a full titration of both cytokines were to be tested — only optimal doses of TNF-α and IL-1 were tested for synergy.

What are the implications of these findings? Taken at face value they imply that situations in which both TNF-α and Type 1 interferons are present would lead to upregulation of HLA-B27 expression, assuming increased transcription leads to increased protein synthesis. This might be important if a threshold of B27 expression has to be exceeded in order to see pathogenic effects such as induction of the UPR. The study does not show that B27 is peculiarly sensitive to this combination of cytokines, but if other B alleles such as B7 do not misfold or form heavy-chain homodimers, the increased expression in response to cytokines would not have deleterious effects. Indeed the B7 promoter is known to respond to both TNF-α and IFN-γ but the effects of type I interferons have not been tested — something that should be done in case this turns out to be another peculiarity of HLA-B27. The other message of the study is that it may be worthwhile looking at sites or stimuli where type I interferons are likely to be present, or cells that produce them, such as plasmacytoid dendritic cells[18], in view of their potent effect on B27 expression.

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