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Hypothesis: Time for a gut check: HLA B27 predisposes to ankylosing spondylitis by altering the microbiome

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Nearly four decades have passed since two groups first reported the remarkable relationship between HLA B27 and ankylosing spondylitis [1, 2]. This is arguably the strongest association between a genetically determined factor and a genetically complex, immune-mediated disease. The more technologically sophisticated approach through genome wide scanning tends to discover genes that are two orders of magnitude less influential in predisposing to a disease compared to the effect of B27. And equally remarkable as the discovery of the B27-ankylosing spondylitis relationship is the frustrating observation that we are still unable to determine precisely the mechanism of this biological effect.

A recent report on an international workshop on the role of HLA B27 in spondyloarthritis summarizes three leading theories as to why HLA B27 predisposes to inflammation: a) HLA B27 presents a distinct peptide repertoire and thus has a direct effect on the immune response as would be expected by an MHC molecule ; b)HLA B27 misfolds and activates a series of intracellular events known as the unfolded protein response; c) HLA B27 forms a dimer on the cell surface and this serves as a target for NK cells. [3]. None of these theories, however, adequately explains the pathogenesis of spondyloarthropathy. This summary [3] of the proceedings of a meeting sponsored by the National Institute of Arthritis and Musculoskeletal Diseases for members of IGAS (International Genetics of Ankylosing Spondylitis), SPARTAN (Spondylitis Research and Therapy Network), as well as interested members of PANLAR (PanAmerican League Against Rheumatism), does not include mention of the role of endogenous flora in the pathogenesis of this disease.

Several features of ankylosing spondylitis overlap with inflammatory bowel disease. Patients with inflammatory bowel disease may develop sacroiliitis, peripheral arthritis, uveitis, or aphthous stomatitis, all potential manifestations of ankylosing spondylitis or its first cousin, another HLA B27-related disease, reactive arthritis. Conversely, patients with ankylosing spondylitis or reactive arthritis frequently have inflammatory bowel disease, although it may be clinically occult [4]. There is now general agreement that bowel flora play a causative role in inflammatory bowel disease [5-8]. And several of the genetic factors identified as predisposing to inflammatory bowel disease including NOD2 and CARD9 [8, 9] participate in the recognition or response to microbial products.

A variety of other immune-mediated diseases, including all the diseases in the spondyloarthropathy family, are believed to have a bacterial pathogenesis. Some have argued, for example, that psoriasis is a disease induced by skin flora [10]. Psoriasis, of course, is associated with psoriatic arthritis. Sarcoidosis [11], Behcet's disease [12], and possibly rheumatoid arthritis [13] might be caused by a response to bacteria.

Potential conflict: James Rosenbaum and Oregon Health & Science University have filed a pending patent application on the concept to treat or inhibit the development of spondyloarthritis through the manipulation of bowel flora.

A number of investigators have noted a relationship between spondyloarthropathy and bacterial flora. The most obvious connection is the acknowledged ability of Chlamydia and certain strains of Salmonella, Shigella, Yersinia, or Campylobacter to trigger reactive arthritis. Some have noted a colonization with Klebsiella in bowel flora of patients with ankylosing spondylitis [14] and this colonization might affect flares of anterior uveitis [15]. Others have reported a cross reactivity between antisera to a specific isolate of Klebsiella and HLA B27 itself [16]. Observations regarding Klebsiella have been controversial and difficult to replicate [17, 18]. However, HLA B27 does have an unusual degree of sequence identity with proteins derived from Gram negative bacteria [19, 20]. Monoclonal antibodies to HLA B27 may cross react with specific Gram negative bacteria [21]. And endotoxin from Gram negative bacterial cell walls when injected into the footpad of a rat, induces an acute anterior uveitis with some similarity to what is characteristic of the HLA B27-related spectrum of disease [22].

While these observations are sometimes decades old, their failure to gain widespread acceptance might relate in some cases to the inability to replicate the findings as well as the inability to link the pathogenesis of ankylosing spondylitis to any of the above conclusions. That linkage is becoming more apparent. We hypothesize that HLA B27 influences the composition of the body's endogenous flora and that this 'B27-shaped flora' causes ankylosing spondylitis.

More than a decade ago, Nobel laureate Joshua Lederberg called attention to the human microbiome and noted that for every cell in a human body, an individual plays host to ten genomes of bacterial, viral, or fungal origin [23]. Characterizing the diversity of the microbes to which we play host is daunting. Stool, for example, contains at least 1000 species of bacteria and we routinely culture only a small percent of these. Furthermore, the microbiome between individuals can vary in genes encoding functionally interconnected pathways that are directly influenced by the host [24]. Although the NIH has a dedicated, specific interest in elucidating the entire microbiome, cataloging this diversity remains an incomplete task at present. What is clear is that these microbes profoundly influence the immune response. For example, animals which are reared in a germ free environment fail to develop a normal immune system or normal lymphoid architecture [25]. Development of multiple cell lineages (B cells, Th17, Treg, Th1/Th2 balance and CD8 T cells) is abnormal in germ free animals [25]. Accordingly an HLA B27 positive rat that normally develops colitis, skin lesions, and arthropathy remains generally healthy in a germ free environment [26] and several murine models of colitis are also cured by a germ free environment [27]. Most models of autoimmunity in laboratory animals require an adjuvant, which usually acts by activating the innate immune system, the body's way to identify microbial pathogens. More recently we have learned that Bacteroides can ameliorate a mouse model of colitis [28], that Clostridium influences the presence of regulatory T cells [29], and that a mouse model of the allergic response is dependent on bowel flora as well [29]. Mice which lack TLR5 develop obesity and changes that mimic the metabolic syndrome [30]. The transfer of gut flora from the TLR5 deficient mouse to a healthy, germ free mouse results in metabolic syndrome features in the recipient [30]. Studies such as these provide clues to mechanisms by which altered flora could influence susceptibility to disease.

Colitis in the HLA B27 transgenic rat model can be effectively prevented with antibiotics, and this benefit can be maintained if the rat is colonized with Lactobacillus rhamnosus but not by Lactobacillus plantarum [31]. In contrast, Lactobacillus bifidus will induce arthritis in germ free mice which lack the IL-1 receptor antagonist [32].

HLA B27 itself affects the immune response to HIV [33] and hepatits C [34], and virus derived products stimulate the innate immune system. Transfection of HLA B27 into a monocyte-

derived cell line reduces the proliferative response to endotoxin[35], an effect that could relate indirectly to HLA B27 altering bacterial flora. The replication of Salmonella is increased in a monocyte cell line if it expresses HLA B27 [36]. HLA B27 also impacts humoral immunity to several Gram negative bacteria [37, 38]. Patients with spondyloarthritis have increased antibody titers to bacterial cell wall [39]. Adjuvant arthritis in rats is induced by cell walls from mycobacteria. Adjuvant arthritis bears many similarities to spondyloarthropathy including spinal disease, periosteal new bone formation, uveitis, and nongonococcal urethritis [40]. Susceptibility to adjuvant arthritis is increased in rats which express HLA B27 [41]. Ankylosing spondylitis is predominantly a male disease and gender also has a powerful influence on bacterial colonization [42]. These types of observations provide circumstantial support for the hypothesis that HLA B27 predisposes to ankylosing spondylitis by virtue of the gene's effect on the body's endogenous flora.

The value of any hypothesis regarding pathogenesis rests with the ability to stimulate studies that lead to a novel understanding which could in turn lead to improved therapy. Ideally the hypothesis should be testable, but the very complexity of the bacterial genome hosted by each of us makes this test a daunting task. The availability of transgenic rats which express HLA B27 and beta two microglobulin and which develop a disease that resembles spondyloarthritis offers a surrogate for human studies. We have embarked on studies to characterize the gut flora of these rats and their cage mate controls prior to the onset of clinical disease (Rosenbaum, J, Taurog, J, Van Gelder, R, in progress). HLA B27 is now recognized to include at least 65 subtypes, only two of which do not predispose to disease [43]. It is feasible to create transgenic animals with these subtypes in order to determine if the subtype differentially affects endogenous flora. Devising studies in humans is more problematic, but these could lead to the ultimate reward: to be able to prevent ankylosing spondylitis. Although sacroiliitis usually lacks a discrete onset, the acute anterior uveitis that often accompanies AS has a definable onset and attacks can be readily quantified. The isolate of Lactobacillus which prevents the onset of colitis in antibiotic treated B27 positive rats, Lactobacillus rhamnosus GG, is available without prescription in the United States. Accordingly one test of the hypothesis could involve assessing the ability of this probiotic to prevent attacks of acute anterior uveitis in patients diagnosed with AS.

In the last few years we have begun to learn how profoundly the microbiome shapes the immune response. As a gene that codes for a protein that presents antigen to induce an immune response and that also regulates positive and negative selection of T cells in the thymus, HLA B27 almost certainly does have an effect on normal human microbial flora. It is possible that additional properties of HLA B27 such as dimerization, its effect on the unfolded protein response, or the peptide identity between B27 and bacterially derived proteins all affect bacterial colonization. However, the vast diversity of gut flora and the rather primitive understanding of this diversity make it difficult to quantify how HLA B27 alters this flora. While it is possible that other HLA-disease associations are also due to an effect of the MHC molecule on host bacteria, the HLA B27 association is the strongest of these associations and therefore, the one that should be easiest to subject to a test of this hypothesis. The authors hope that this essay will stimulate additional studies on the effect of HLA B27 on endogenous flora and the effect of that flora on the pathogenesis of ankylosing spondylitis. Modern molecular tools should succeed in categorizing the species of bacteria in the human microbiome far better than the approach by culture that was used several decades ago.

Clarifying the pathogenesis of ankylosing spondylitis will undoubtedly have therapeutic implications. If innate immunity is primarily responsible for its pathogenesis, it makes sense that the inhibition of tumor necrosis factor alpha would be an effective therapeutic. But the inhibition of TNF alpha could also itself have an effect on bacterial flora and this effect

could potentially be counter therapeutic. We have entered an era in which some have begun to explore the benefit of fecal transplantation to alter endogenous flora [44]. The reduction of arthritogenic flora or the induction of non-arthritogenic flora are potential avenues of therapy which might be efficacious with fewer risks and even a safety profile that would justify their use for prophylaxis. Perhaps, nearly forty years after HLA B27's impact on susceptibility to spondyloarthropathy was discovered, we at last have the tools to elucidate the mechanism for this remarkable association.

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