Why HLA-B27? My Thirty-Year Quest

The Friedenwald Lecture

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The custom on the occasion of a named lectureship is to recount one's achievements and thank those who have contributed to it. A good scientist, however, must be an iconoclast. Consequently, I considered recounting my failures rather than the triumphs. I rapidly concluded, however, that the time was insufficient for the former and I have settled on the latter.

Jonas Friedenwald and Alan C. Woods were close friends. Friedenwald was chief of pathology at the Wilmer Eye Institute at Johns Hopkins Medical School. A. C. Woods was the director of the Wilmer for more than two decades, and he and Supreme Court Justice Felix Frankfurter delivered eulogies when Dr. Friedenwald passed away in 1955. Both Woods and Friedenwald are extremely important in shaping the remarks in this essay. Friedenwald's contribution is honored in the naming of the lectureship, and Woods' provocative observations on the pathogenesis of uveitis foreshadowed my own career.

A. C. Woods injected rabbits intravenously with the filtrate from cultured bacterial broth and frequently observed a uveitis in these animals. In 1916 Woods wrote, "Poisons can be isolated from non-pathogenic ferment producing bacteria which have a definite action on the uveal tract in the eye."¹ In addition, he commented, "Histologically the eyes showed a round cell infiltration about the ciliary processes..."¹ The observations from Woods and others were extremely influential in the effort to understand uveitis. For several decades, it was accepted that uveitis often results from occult bacterial infection. In a publication from the Mayo Clinic in 1932, Rosenow and Nickel² wrote, "In many cases streptococci having elective localizing power have been isolated in uveitis." "Different foci have been found. The most frequent were teeth, tonsils, prostate, or cervix. Removal of these foci...." In other words, hysterectomy or prostatectomy were potential forms of treatment for uveitis in the 1930s. The work of Rosenow and Nickel was presented at the second annual meeting of the Association for Research in Ophthalmology, an organization subsequently known as ARVO.

As implied in the title of this lecture, I want to discuss the potential mechanism by which the human leukocyte antigen HLA-B27 predisposes to uveitis. As background, I will outline briefly the HLA system, provide an overview of the clinical problem of uveitis, discuss selected observations which my laboratory has made in animal models, and then return to the

statement by A. C. Woods to ask whether his concept of occult infection could have been correct. If I succeed, I hope to persuade my reader that HLA-B27 and bacterial flora are closely related topics.

My scientific mentor was Hugh O. McDevitt, a professor at Stanford and a member of the National Academy of Sciences. By injecting short repetitive peptide sequences into mice and rabbits, McDevitt and Sela³ astutely observed that some animals made an immune response while other animals were incapable of an immune response. He further determined that the major histocompatibility complex was responsible for the ability to mount that immune response.⁴ For this observation, McDevitt is appropriately credited as the discoverer of immune response genes. In a seminal essay in the journal *Science* nearly 40 years ago, Baruj Benacerraf and McDevitt⁵ wrote, "This type of genetic control of specific immune response may play an important role in susceptibility to a variety of diseases in both animals and man." Within a few months, McDevitt's hypothesis was validated by the demonstration that HLA-B27, then known as HLA 27, profoundly influenced susceptibility to ankylosing spondylitis,^{6,7} reactive arthritis,³ and acute anterior uveitis.⁸ A great deal is now known about the human leukocyte antigen or HLA system, which constitutes the major histocompatibility complex in man. Several major loci code for HLA alleles which are highly polymorphic. The B locus alone has greater than 700 distinct alleles. In fact HLA-B27 is not a single allele; 65 distinct subsets of B27 are now recognized thanks to molecular typing techniques.⁹ All but 2 of the 65 subtypes increase susceptibility to the spondyloarthropathies—namely, ankylosing spondylitis and reactive arthritis.

Although it has been nearly four decades since the discovery that HLA-B27 predisposes to these diseases, the mechanism for this predisposition is still uncertain. Three theories predominate.¹⁰ One hypothesis holds that B27 directly affects the immune response, a known function of major histocompatibility complex molecules. However, no triggering antigen has ever been confirmed as the inciting agent for this immune response. A second theory is based on the observation that HLA-B27 dimerizes on the surface of cells which express it and this activates a type of lymphocyte known as the natural killer cell. A third theory is based on the observation that the B27 molecule is unique in its intracellular instability, which could induce a series of metabolic changes within the cell known as the unfolded protein response. HLA-B27 increases the likelihood of developing ankylosing spondylitis approximately 100-fold.⁹ This is a far greater influence than what one discovers for susceptibility loci identified by genome wide association studies involving thousands of patients and testing the contribution of a million or more polymorphisms. Although the influence of HLA-B27 on predisposition to ankylosing spondylitis is profound, the mechanism of this association has eluded scientists.

I began my fellowship as a rheumatology trainee in McDevitt's laboratory at Stanford in 1978. He challenged me to explore the mechanism by which HLA predisposes to disease, and I responded by investigating the response of Lewis rats to

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Supported by the Stan and Madelle Rosenfeld Family Trust, the William C. Kuzell Foundation, the William and Mary Bauman Foundation, Research to Prevent Blindness and National Institutes of Health Grant EY019604.

Disclosure: **J.T. Rosenbaum, P**

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injections of killed Gram-negative bacteria, since it was well known that certain Gram-negative infections, such as *Shigella*, *Salmonella*, or *Yersinia*, could trigger a reactive arthritis, especially in HLA-B27 positive individuals. Although I did not observe any consistent joint swelling, the rats developed a near universal eye inflammation that histologically proved to be predominantly an anterior uveitis. Endotoxin was easily identified as the moiety responsible for the uveitis. These observations were published in *Nature*,¹¹ and the model has been the subject of more than 400 subsequent reports on the pathogenesis of endotoxin-induced eye inflammation in rats, rabbits, or mice. The model inspired me to speculate as to why HLA-B27 predisposes to disease in an essay published in 1981 in the *Annals of Internal Medicine*.¹² My colleague Michael Davey and I have updated this speculation in an essay in *Arthritis & Rheumatism*.¹³ The endotoxin-induced uveitis model has been useful for some of the initial publications on intraocular cytokines,^{14,15} adhesion molecules,^{16,17} complement,¹⁸ and intracellular pathways¹⁹ activated by a ligand for a toll receptor during inflammation within the eye. Like all animal models, however, this one has limitations. Unlike the human disease,²⁰ the uveitis is not dependent on the major histocompatibility complex. The human disease is typically unilateral, whereas the rat disease is bilateral. If one tries to induce sustained disease by repeating the endotoxin injection, the animal becomes resistant or tolerant to the endotoxin.²¹ Finally, most human immune-mediated disease is generally attributed to the adaptive immune system, whereas a ligand that activates a toll receptor is an example of an innate immune system response.

To explain, the immune system is generally conceptualized as having two arms: an innate arm and an adaptive arm. The innate immune system consists mainly of cells such as macrophages, neutrophils, and dendrites, which have receptors that can recognize microbial products. These cells respond rapidly but somewhat nonspecifically to microbial invasion. The adaptive immune system, on the other hand, is highly targeted but slower to react. Major components of the adaptive immune system include T- and B-cells which undergo somatic gene rearrangements to recognize specifically an antigen and mount a response to it. Until recently, the classic immune-mediated diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), were attributed to dysfunction of the adaptive immune system. This understanding was based on the presence of autoantibodies, which must indicate a B-cell response. Furthermore animal models that mimic aspects of RA and SLE are generally transferable by T-cells and not by any cell within the innate immune system. However, we have recently recognized so called autoinflammatory diseases that are inflammatory, affect multiple organs, and are clearly due to the innate immune system. Blau Syndrome is a classic example. This disease most frequently affects the uvea, the skin, and joints, in which it causes a granulomatous inflammation.²² The disease is autosomal dominant and due to a mutation in NOD-2,²³ a critical part of the innate immune system. Many experts now conceptualize immune-mediated diseases as resulting from both innate and adaptive immunity.²⁴ Consequently the endotoxin-induced model involving the innate immune response takes on greater clinical significance.

Uveitis is a major clinical problem. Its prevalence is only about 1 person per 1000.²⁵ Any portion of the uveal tract can be inflamed, but anterior uveitis is approximately five times as common as intermediate or posterior uveitis combined.²⁵ Although uveitis is not frequent, it is roughly comparable to diabetes or macular degeneration in terms of years of visual morbidity.²⁶ Unlike diabetes or macular degeneration, both of which tend to affect individuals late in life, uveitis often occurs during one's 20s or 30s and may even affect infants as it does classically in a disease known as juvenile idiopathic arthritis.

Uveitis has many distinct subsets meaning that the differential diagnosis for uveitis is broad and ranges from infections to immune-mediated diseases.²⁷ For reasons that are incompletely understood, uveitis often occurs in conjunction with arthritis. Table 1 lists 16 discrete diagnostic entities that can be characterized by both uveitis and arthritis.

Inflammation anywhere within the body is characterized by an invasion of leukocytes and an increase in vascular permeability. The eye is unique in that a slit lamp examination of a patient with anterior uveitis allows a clinician to observe both of these hallmarks of inflammation quantitatively and noninvasively.

I want to digress for a moment to describe how my laboratory has made use of animal models to clarify the pathogenesis of uveitis. Matthias Becker,²⁸ one of my former fellows, introduced the use of intravital fluorescence microscopy in our laboratory. This has allowed us to visualize the movement of leukocytes within the anterior uveal tract as an immune response evolves.^{29–32} With the use of transgenic animals that express green fluorescent protein or related fluorescent molecules, we can readily track specific cell populations in real time or with time lapse photography.³³

Holly Rosenzweig developed an interest in the pathogenesis of arthritis induced by aggrecan, the predominant proteoglycan within the enthesis. Spondyloarthropathies are often considered enthesopathies because the inflammation generally involves the enthesis or tendinous insertion. Previous investigators had characterized an arthritis in this model³⁴ and had shown that in the absence of the cytokine γ -interferon, the arthritis was diminished.³⁵ Immunologists refer to T-cell responses that are γ -interferon dependent as Th1. Using intravital microscopy, Dr. Rosenzweig³⁶ observed that a uveitis was also present in many of these mice. Surprisingly, she and a postdoctoral fellow, Jelena Kezic noted that the absence of γ -interferon markedly exacerbated the uveitis although it reduced the intensity of the joint inflammation.³⁷ Thus, Dr. Rosenzweig had discovered the first mouse model that reflects a common clinical situation—the coexistence of uveitis and arthritis—and she had shown convincingly that despite the co-occurrence, the inflammation in the joint was mediated far differently than its regulation within the eye.

Other approaches to understand uveitis from the laboratory have included genome-wide scanning under the direction of Tammy Martin,³⁸ gene expression arrays with the collaboration of Dongseok Choi and Christina Harrington (Sharma et al.),³⁹ novel treatment paradigms with biologics under the supervision of Eric Suhler,^{40,41} and testing a hypothesis with regard to vascular diversity within the eye, as undertaken by Justine Smith.⁴²

With this background in mind, I want to return to the observations of A. C. Woods claiming that occult bacterial infection is a frequent cause of uveitis. Ankylosing spondylitis is universally considered to be the prototype of a disease called a spondyloarthropathy, meaning arthritis of the spine. Other diseases overlapping clinically with spondyloarthropathy include psoriatic arthritis,⁴³ reactive arthritis,⁴⁴ inflammatory

TABLE 1. Examples of Diseases Characterized by Uveitis and Arthritis

Ankylosing spondylitis	Psoriatic arthritis
Behçet's disease	Reactive arthritis
Blau syndrome	Relapsing polychondritis
Crohn's disease	Sarcoidosis
Juvenile idiopathic arthritis	Systemic lupus erythematosus
Kawasaki's disease	Ulcerative colitis
Lyme disease	Vasculitis, such as Wegener's
Neonatal-onset multisystem	granulomatosis
inflammatory disease	Whipple's disease

bowel disease,⁴⁵ and Behçet's disease.⁴⁶ These diseases share many spine, peripheral joint, eye, skin, and bowel manifestations. In each of these four latter conditions, a bacterial trigger is either definite or highly likely.

More than a decade ago, the Nobel laureate, Joshua Lederberg, described the concept of the microbiome. Our bodies are host to approximately 100 trillion bacteria, most of which live in our bowel. In fact, our bacterial cells outnumber our mammalian cells by approximately 10 to 1.^{47,48} If we think of this in terms of RNA transcripts, approximately 99% of the transcripts within our body derive from microbes rather than from our personal genome. Moreover, our understanding of this microbial world within us is primitive. We are capable of culturing only a fraction of these microbes. Only through modern molecular techniques have we begun to appreciate the diversity of these bacteria. Furthermore, these bacteria are essential to our health. They are a major source of vitamin K. If we raise mice in a sterilized, germ-free environment, the immune system fails to develop without the needed stimulation from gut microbes.⁴⁹ Recently, a series of remarkable papers has begun to demonstrate how important our microbiome is in shaping our immune system. For example Vijay-Kumar and colleagues⁵⁰ reported in *Science* that the deletion of the TLR-5 receptor results in metabolic syndrome in mice. TLR-5 recognizes the bacterial protein flagellin. In the absence of TLR-5 there is a change in gut flora, and if the flora is transferred from the TLR-5 knockout mice to healthy mice, the previously healthy mouse now develops aspects of metabolic syndrome as well. Atarashi and colleagues⁵¹ have shown that *Clostridium* species can influence the number and function of regulatory T-cells within the mouse. For example, a model of allergic disease can be modified by certain *Clostridium* species and a model of inflammatory bowel disease can be treated as well. Round and Mazmanian,^{52,53} have reported that bacteroides produce a polysaccharide that also influences regulatory T-cells and ingestion of this polysaccharide alone can cure mice of a model of inflammatory bowel disease Hammer and colleagues⁵⁴ described a model of spondyloarthritis developing in rats which expressed multiple copies of the HLA B27 gene along with the light chain, β_2 -microglobulin, which is required for its cell surface expression. These rats develop bowel inflammation and the spinal and peripheral arthritis characteristic of ankylosing spondylitis. When the rats are raised in a germ-free environment, they are mostly spared both the bowel and joint disease.⁵⁵ Dieleman and colleagues⁵⁶ have shown that antibiotics are an alternative way to prevent disease development in this model. Once treated with antibiotics, the use of the probiotic, *Lactobacillus rhamnosus* GG, can maintain the absence of disease, whereas other lactobacillus isolates do not have this property. Recently, Yan and colleagues⁵⁷ have shown that a protein with molecular weight 40,000 can be isolated from *Lactobacillus rhamnosus* GG. This protein can mimic the beneficial effects of the probiotic in a mouse model of inflammatory bowel disease.

Some observations strongly suggest that HLA-B27 alters bowel flora. Although the studies have been controversial and difficult to replicate,⁵⁸ some evidence suggests that *Klebsiella* colonizes the bowel before an attack of HLA-B27-associated anterior uveitis.⁵⁹ Several investigators have described monoclonal antibodies that cross-react with both HLA-B27 and specific Gram-negative bacteria^{60,61} When monocyte lines are transfected with HLA-B27, the response to endotoxin is altered.⁶² Patients with ankylosing spondylitis also have an increased antibody to certain bacterial cell walls or cell wall components.^{63–65} Finally, HLA-B27 is unique in that it shares six amino acids that are identical with a sequence of a protein from *Klebsiella*, specifically a nitrogenase.⁶⁶ The known functions of a major histocompatibility complex antigen are such

that it is highly likely that it will indeed alter bowel flora. What has not been appreciated until recently is how profoundly bowel flora might change the immune response. Accordingly, I submit to you a testable hypothesis: HLA-B27 alters the microbiome in such a way that predisposes an individual to ankylosing spondylitis, uveitis, and reactive arthritis. In other words, HLA-B27 alters endogenous flora, which in turn shapes the immune response, which in turn is the cause of both spondylitis and uveitis. If indeed this hypothesis is correct, it should be possible to manipulate the flora by antibiotics, probiotics, or dietary changes that alter bowel flora in such a way that we can prevent both spondylitis and its close cousin, acute anterior uveitis.

The work that I have discussed has resulted from collaboration with many colleagues, fellows, and technicians over three decades, some of whom I have mentioned specifically. Stephen Planck in particular has worked closely with me for more than two decades, and his influence is considerable in much of what I have shared.

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