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REVIEW

Autoimmunity, infection and adjuvants

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The effect of infection in initiating autoimmune disease has been debated for many years. There are, even now, few instances of a human autoimmune disease clearly caused by prior infection, probably due to the frequent separation in time and space from the clinical outcomes. As our understanding of the immunologic consequences of the infectious process has deepened, we can re-think some of the issues by focusing attention on the varied adjuvant effects of microbial products. We are now able to distinguish some of the critical steps in progression from virus infection to benign autoimmunity to autoimmune disease in an experimental model of myocarditis. Immune regulators, such as cytokines and costimulatory molecules, serve as signposts in the process. The lessons learned may be broadly applicable to autoimmune disorders. *Lupus* (2010) **19,** 354–358.

Key words: adjuvant; autoimmunity; cytokines; infection; receptors

Introduction

The role of infection in autoimmune disease has been a topic of speculation and investigation for over a century. Many research articles, review papers and books have been devoted to the topic and yet major questions remain unanswered. How often is infection linked to autoimmune disease? When does infection promote and when does it prevent autoimmune disease? What are the fundamental mechanisms dictating the relationship? This brief article will deal with one aspect of the problem; namely, infection as adjuvant.

Space and time conundrum

In assessing the association of infection with autoimmune disease, we first encounter the problems of space and time. By space, I refer to the frequent finding that more than one microorganism can induce virtually the same autoimmune condition. Indeed, substantial investigations have identified over 50 distinct microorganisms that show association with multiple sclerosis. The accumulated literature supports a similar situation of multiple infectious agents linked to type 1 diabetes and to lupus. In studies of experimental autoimmune disease, a similar situation prevails. Using our model of experimental autoimmune myocarditis, for example, we have discovered that three different microorganisms, coxsackievirus B3, encephalomyocarditis virus and murine cytomegalovirus, produce essentially the same pathologic outcome. Equally important as a space problem is the observation that the same infectious agent can be associated with entirely different autoimmune disorders. Epstein–Barr virus has been seriously implicated in lupus, rheumatoid arthritis and multiple sclerosis. The lack of a one-to-one association between the putative causative agent and the disease outcome is a significant impediment in research aimed to establish a cause and effect relationship between infection and autoimmunity in humans.

The difficulties imposed by timing are equally daunting. Epidemiologic evidence suggests that an infectious exposure in early childhood may be expressed years later in the form of an autoimmune disease. Multiple sclerosis exemplifies the concept that an environmental influence before puberty sets the stage for enhanced susceptibility to clinical disease decades later. Type 1 diabetes and lupus appear to be other instances where the infectious

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trigger occurs long before the disease is clinically expressed.

It is in those rare instances where the time and space conundrum has been overcome that we can discern a causal relationship between an infectious process and an autoimmune disease outcome. Rheumatic fever and its association with *Streptococcus pyogenes* is our leading example. Establishing this connection has lead to the virtual elimination of rheumatic fever and rheumatic heart disease as major public health problems in the industrialized countries. The benefit that can arise from establishing a linkage between infection and autoimmunity can pay enormous dividends in the control of autoimmune diseases.

Infections as adjuvants

A number of years ago we pointed out the importance of distinguishing the two general mechanisms by which viruses might induce autoimmunity (Figure 1).[1] The greatest amount of attention has been directed to the role of an infectious agent in providing or presenting the disease-initiating antigen. The proposed mechanisms include the release of intracellular or otherwise masked antigens or epitopes, alteration of host-cell antigens, incorporation of host-cell antigen into the infectious agent and, most commonly, molecular mimicry, the concept of a partial sharing of antigenic determinants between a microorganism and the host. It has now become evident that a large variety of autoantibodies rise following infection. A similar increment in autoantibody production can be observed after vaccination. The progression from an autoimmune response (usually detected by circulating antibodies) to autoimmune disease requires a number of discrete steps and helps to explain why the occurrence of autoimmune disease following infection is a relatively rare event.

In addition to the mechanisms involved in antigen presentation, infections modify the immune response of the host. Such changes can be recognized in the earliest stages of infection during the innate immune response but have consequences in the quality as well as quantity of subsequent adaptive immunity. These early modulations of the immune response can be referred to as the adjuvant effect of infection.

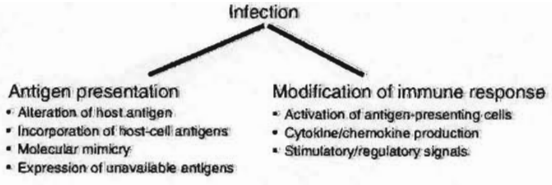

Figure 1 Antigen-specific and antigen-non-specific effects of infection.

The adjuvant effect in infection

In 1928 Louis Dienes observed that if he injected a foreign antigen into the granulomatous tubercle of a *Mycobacterium tuberculosis*-infected guinea pig, he obtained a greatly enhanced immune response.[2] Not only were antibodies increased, but the animals developed vigorous delayed hypersensitivity reactions. A decade later Jules Freund harvested these observations to develop the adjuvant to which his name is attached.[3] He prepared a water and oil emulsion and included killed mycobacteria in the mixture. After adding an antigen to the emulsion he obtained an enhanced immune response involving both humoral and cell-mediated immunity. The value of Freund's adjuvant was rapidly demonstrated in the induction of experimental allergic encephalomyelitis and by Freund himself in inducing experimental orchitis.

We were first convinced of the importance of adjuvants in the induction of autoimmune disease during our early studies on the initiation of experimental autoimmune thyroiditis by immunization with thyroglobulin. The injection of thyroglobulin plus complete Freund adjuvant induced production of both thyroglobulin-specific autoantibodies as well as inflammatory lesions in the thyroid. On the other hand, injection of thyroglobulin with incomplete Freund adjuvant lacking the mycobacterial component gave rise to antibody production but no thyroiditis, thus featuring the importance of the microbial component in the induction of actual autoimmune disease.[4] In later studies with Yi-Chi Kong we were able to follow up these observations by showing that incomplete Freund adjuvant plus muramyl dipeptide, the component of the mycobacterial cell wall responsible for its immunity-promoting effects, lead to the induction of the characteristic lesions of thyroiditis. Injection of thyroglobulin and concurrent, separate administration of bacterial lipopolysaccharide also induced the full picture of autoimmune disease with the production of both autoantibodies and thyroid lesions. In contrast, a great variety of other adjuvants tested at the time that lacked any microbial component, including alum and silica, enhanced autoantibody

production, but did not induce autoimmune thyroiditis. These experiments convinced us that the administration of microbial products together with self antigen greatly increases the probability that autoimmune disease ensues.

Subsequently we investigated the effect of viral infection in inducing autoimmune disease.[5] We found that we could produce a florid acute myocarditis in virtually all of the strains of mice we tested by infecting them with a cardiotropic strain of coxsackievirus B3. In most of the mouse strains the disease receded spontaneously, leaving virtually no signs of inflammation three weeks after infection. In a few strains, however, cardiac inflammation continued, producing chronic myocarditis. This chronic phase of disease was associated with an autoimmune response to cardiac myosin and could be reproduced in the absence of viral infection by direct immunization with cardiac myosin in the presence of complete Freund's adjuvant. Induction of disease by immunization with cardiac myosin was successful only in the few strains of mice genetically susceptible to post-viral chronic myocarditis.

In the first instance, then, the complex genetics of the host determines whether an infection will resolve or proceed to an adverse autoimmune outcome.[6] Identifying the particular traits that favor susceptibility or resistance to an autoimmune sequel helps us to understand how infectious disease can culminate in autoimmune disease. Like most autoimmune disorders, experimental myocarditis susceptibility relates to how major histocompatibility complex (MHC) genes deal with antigen presentation and H-2^a, H-2^b and H-2^s haplotypes are all associated with vigorous responses to cardiac myosin. The expression of the response, however, is modified by non-MHC background genes, so that A.SW mice are strong responders whereas B10.S, which share the H-2^s allele with C57BL/10.S mice, are poor responders. Gene mapping has led us to identify some of the more prominent non-MHC traits that account for resistance to autoimmune disease in mice. Candidate genes include cytotoxic-T lymphocyte antigen (CTLA)4, inducible T-cell co-stimulator (ICOS) and CD27. All of these gene products are known to regulate the immune response and have been implicated in other autoimmune diseases.

When we looked further at resistant C57BL/10 mice, we were quite surprised to learn that severe autoimmune myocarditis could be induced in this strain by infecting with coxsackievirus B3 and simultaneously administering bacterial lipopolysaccharide. (Lipopolysaccharide alone had no such effect.) We further found that administration of two early, critical pro-inflammatory cytokines, interleukin (IL)-1β and tumor necrosis factor (TNF)α, would also render the normally resistant C57BL/10 mice susceptible to autoimmune myocarditis.[7] Furthermore, injection of C57BL/10 mice with myosin in combination with complete Freund's adjuvant failed to induce any heart disease whereas myosin immunization with complete Freund's adjuvant and additional lipopolysaccharide induced disease. These findings clearly indicate that a potent adjuvant effect can overcome the relative genetic resistance to autoimmune disease. At the same time we showed that blocking either IL-1β or TNFα inhibits the development of disease even in highly susceptible A/J mice.

These experiments convinced us that the production of particular cytokines early in the course of viral infection, or administration of these cytokines at the time of immunization with antigen and adjuvant, determines whether the mouse will subsequently develop chronic autoimmune myocarditis or even a life-threatening dilated cardiomyopathy. Together with DeLisa Fairweather we found that there was a relative difference in the innate response of susceptible or resistant strains of mice to coxsackie B3 infection.[8] Susceptible animals developed significantly greater increases TNFα and IL-1β in the heart. These differences were observed within 6 h after infection. This enhanced response in the mice susceptible to chronic autoimmune disease is reminiscent of the greater delayed hypersensitivity demonstrated so many years ago by Louis Dienes and bespeaks an adjuvant effect of infection.

We have continued to dissect factors that determine the progression from a benign, limited autoimmune response to amplified pathogenic autoimmunity (Figure 2).[9] IL-18, produced early in the course of viral infection, and T helper cell 1

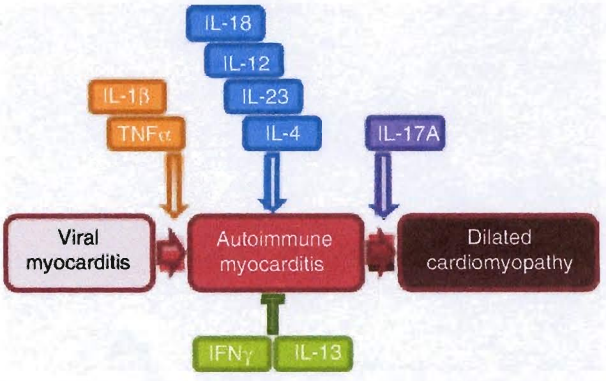

Figure 2 Progression from viral myocarditis to autoimmune myocarditis to dilated cardiomyopathy.

(Th-1) cytokines track closely with cardiac inflammation. Both IL-12, the prototypic inducer of Th-1 responses and, in the most highly susceptible strains of mice, IL-4, the classical mediator of Th-2 responses, promote cardiac inflammation. On the other hand, IFNγ, considered an effector cytokine of Th-1 responses is downregulatory. Similarly IL-13, which usually tracks with IL-4, reduces rather than increases myocarditis. IL-17, produced early following infection has relatively little effect on the severity of inflammatory autoimmune myocarditis, but is required for the robust fibrotic response that gives rise to subsequent dilated cardiomyopathy. These experiments carried out at the mechanistic level point to opportunities for interrupting the progression from infectious disease to a harmful autoimmune response.

The innate immune response as the decisionmaker

Our experience with the model of experimental autoimmune myocarditis focused our attention on the innate immune response to an infectious agent as a decider of the later development of autoimmune disease. The innate immune system includes numerous germ line encoded pattern recognition receptors (PRRs) that recognize highly conserved pathogen-associated molecular patterns (PAMPs) on microbial invaders.[10,11] These receptors include the toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and other families. Expression of these receptors on antigen-presenting cells such as macrophages and dendritic cells determine not only innate immunity but the subsequent adaptive immune response. We found, for example, that mice deficient in TLR4 have significantly reduced acute myocarditis, but still develop severe chronic autoimmune myocarditis. Mycobacteria stimulate both TLR2 and TLR4. Myeloid differentiation molecule (MyD)88 deficiency also prevents the autoimmune disease. Thus, the ability of PAMPs present on the microorganism or in the adjuvant can provide the signals to shape the function of antigen-producing cells and set the course of future autoimmune responses.

PRRs collectively recognize not only microbial peptides but carbohydrates, lipids and nucleic acids. Both pathogenic and non-pathogenic microorganisms that enter the body are recognized and responded to. Their combined action modulates inflammation and immunity. The normal flora of the gut is constantly signaling the immune system and exerts profound effects on the autoimmune response.[12] Under experimental conditions, most models of autoimmune disease, such as thyroiditis, lupus and inflammatory bowel disease, worsen in the presence of normal intestinal bacteria, but in the non-obese diabetic mouse model of type 1 diabetes, disease is dramatically reduced. Some specific types of parasites such as helminthic worms can shift the cytokine profile and decrease susceptibility to autoimmune disease.[13] Administration of muramyl dipeptide actually protects mice from experimental colitis acting through NOD2.[14] Thus, microbial adjuvants can up-regulate or down-regulate autoimmune responses.

Summing up

These recent findings about the early immune response provide fresh insight into the questions posed at the beginning of this review. Autoimmune responses are a common occurrence with infection. The stimulus may be exogenous or endogenous. But, as long as normal controls are in place, the immune response remains limited and these self-directed reactions are usually harmless. The non-antigen-specific signals of the response often determine whether pathogenic autoimmunity ensues. They represent the adjuvant effects of infection.

By dissecting each step and each checkpoint in the process we begin to understand when and how autoimmune disease follows infection. Invasion of the body by microorganisms may be due to infection, vaccination or even casual intrusion by normal microbial inhabitants of body surfaces. The intruders are first sensed by an array of the host's cellular recognition receptors that evolution has selected based on their ability to initiate protective immunity. The most critical steps in shaping the total immune response seem to occur in the first days or even hours after infection, although the consequence may not be evident for days, months or years. In the first instance, the events are determined by the genetics of the host and by the molecular signals given by the microorganisms. Most of the time, evolution dictates that the autoimmune response to infection is dampened by the regulatory mediators that restore physiologic homeostasis. When these regulators are inadequate, pathologic consequences in the form of autoimmune disease follow.

Perhaps the regulatory mechanisms that are now coming to light through the agency of modern molecular biology are the *Einrichtungen*.

('Contrivances') that Paul Ehrlich envisioned a century ago which usually prevent autoimmunity from leading to autoimmune disease.[15]

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