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THE ROLE OF BACTERIAL COMPONENTS IN AUTOIMMUNITY AND CHRONIC INFLAMMATORY DISEASES

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CONTRIBUTIONS OF THE MICROBIAL FLORA TO AUTOIMMUNITY

I will first briefly review some of the work of other laboratories relating microbial antigens to autoimmunity.

Immunogens cross-reactive with mammalian tissue (Table 1)

The first example is the induction of antibodies against several antigens of mammalian heart, apparently through cross-reactivity with antigens of group A streptococci (*Streptococcus pyogenes*) These, and other autoimmune responses stimulated by bacterial immunogens, have been studied by several laboratories (*Dale* and *Beachey*, 1982; *van de Rijn* et al., 1977; *Cunningham* et al., 1984). It should be noted that in none of these examples it has been proven that the autoantibodies have a direct role in the pathogenesis of the associated diseases.

More recently, *Karounos* and colleagues (1988) have examined the immunogenesis of autoantibodies against DNA in patients with systemic lupus erythematosus (SLE). They report that

Table 1: Cross-reactive bacterial immunogens

Tissue	Immune response	Bacteria	Reference
Heart myosin actin	Antibody	*Streptococcus pyogenes* (M protein) *Streptococcus pyogenes* (membrane peptide)	Dale and Beachey, 1982 van de Rijn et al., 1977; Cunningham et al., 1984
DNA	Antibody	*Micrococcus lysodeikticus* *Staphylococcus epidermidis* *Escherichia coli* *Pseudomonas aeruginosa*	Karounos et al., 1988
Cartilage proteoglycan	T-cell	*Mycobacterium bovis* and over 50 other bacteria which share a common epitope in a heat shock protein	Holoshitz et al., 1983 Holoshitz et al., 1986 van Eden et al., 1988

anti-DNA in normal individuals and the anti-DNA, which is elevated in SLE patients, seems to be primarily a result of antigenic stimulation by DNA from a variety of bacteria which are part of the normal microflora.

The third example I have listed in Table I involve extensive studies by Cohen and colleagues in which they describe T-cells isolated from rats with adjuvant arthritis. Two T-cell clones specific for a protein antigen (MT) from the *Mycobacterium tuberculosis* organisms in complete Freund's adjuvant have been examined. One clone can induce joint inflammation when injected into irradiated Lewis rats. A second clone of the same specificity did not induce arthritis, but did protect rats against adjuvant-induced arthritis (*Holoshitz* et al., 1983). These T-cell clones also recognise a human cartilage proteoglycan antigen. T-cells from rheumatoid arthritis patients also respond to the MT antigen (*Holoshitz* et al., 1986). The 65 kD, MT antigen has been cloned in *E. coli* and the critical sequence is amino acids l80 to 188, with 4 of the 9 amino acids identical to the link protein of rat proteoglycan. The 65 kD MT antigen cannot induce arthritis, but does induce resistance to adjuvant arthritis. Finally, the MT antigen has some sequence homology with a heat shock protein shared with many other bacteria (*van Eden* et al., 1988). It is important to note that classical adjuvant arthritis requires a petidoglycan component and the MT protein is unrelated to the structure of bacterial cell wall petidoglycan. Therefore, it would appear that the T-cells specific for MT antigen do not have an essential role in adjuvant arthritis. Rather, when such T-cells cross-reactive with cartilage are induced in classical adjuvant arthritis their function may be to maintain and/or increase the severity of inflammation which has been initiated by cell wall peptidoglycan.

Non-antigen-driven autoimmunity

Another mechanism by which autoantibodies can be stimulated by bacteria may involve B-cell mitogens derived from bacteria, rather than antigenic mimicry. More evidence for this concept is presented by *Swartzwelder* et al. (1988). It has been known for some time that *Streptococccus mutans* vaccines can stimulate production of autoantibodies specific for heart antigens. However, autoantibody in rabbit anti-*S. mutans* serum, affinity purified from heart myosin antigen, does not react with *S. mutans* antigens. One interpretation is that the bacterial polyclonal B-cell mitogens can theoretically stimulate the expression of a large part of the B-cell repertoire, including antibodies against self antigens (*Swartzwelder* et al., 1988).

CHRONIC GRANULOMATOUS INFLAMMATION INDUCED BY PEPTIDOGLYCAN-POLYSACCHARIDE POLYMERS FROM BACTERIAL CELL WALLS

This section will summarise some of the recent work our laboratory has been doing on experimental inflammatory diseases induced by bacterial cell walls. The participation of autoimmunity in the pathogenesis of these diseases remains unproven. The petidoglycan-polysaccharide (PG-PS) structures which initiate and maintain the inflammation can be derived from a variety of infectious bacteria as well as bacteria which are part of the normal human microflora

(*Stimpson* et al., 1986a). The PG-PS polymers, which we use most frequently, are isolated from group A streptococci. The petidoglycan moiety has a number of relevant pro-inflammatory activities (*Stimpson* et al., 1986b). The polysaccharide also has biological activity (*Dalldorf* et al., 1988) but its primary function is to protect the petidoglycan from *in vivo* degradation which allows it to persist in tissue for a prolonged period. The relative degree of N- and O-acetylation also contributes to resistance of PG-PS to biodegradation.

Chronic, erosive, recurrent arthritis

Intraperitoneal injection of an aqueous suspension of PG-PS into rats induces an acute inflammation of joints, which reaches a peak in about 3 to 5 days and then recedes. About 2 to 3 weeks after injection, depending upon polymer size and fine structure, the inflammation of joints recurs and repeated cycles of waxing and waning occur over a period of several months. This prolonged, recurrent process can result in a severe erosive arthritis and loss of function (*Cromartie* et al., 1977).

Granulomatous enterocolitis

Sartor and colleagues (1985) have been investigating the intestinal and extra-intestinal pathology in rats injected locally or systemically with PG-PS. A chronic, recurrent, granulomatous enterocolitis, histologically resembling Crohn's disease, develops after submucosal injection of the small intestine or caecum. By about l2 weeks there appears to occur a reactivation of the inflammation at the injection sites, as evidenced by oedema and renewed infiltration of neutrophils, as well as accumulations of lymphocytes and macrophages. At 6 months there is still an active chronic inflammation with the presence of neutrophils. This prolonged disease can be induced by PG-PS from group D streptococci, part of the normal intestinal flora, but not by injection of a protein antigen such as human serum albumin.

Gastrointestinal lymphoid tissue haemorrhage (GALT)

Within 3 minutes after the i.v. injection of PG-PS into rats macroscopic haemorrhage appears in the lamina propria, organised lymphoid aggregates of the caecum and small intestine, and in the mesenteric lymph nodes (*Sartor* et al., 1986). Haemorrhage is not seen in the lung, kidney, liver, spleen, adrenal, or submandibular or popliteal lymph nodes. The response is maximal at 5 minutes and resolves completely by 3 days. Only erythrocytes appear in the tissue; neutrophils, oedema, vasculitis and necrosis are not seen. Since vascular changes are the initial event common to all inflammatory responses, we believe this haemorrhagic reaction provides a model for investigation of the earliest mediators of inflammation induced by PG-PS polymers.

Granulomatous hepatitis

Lichtman has been investigating the pathological consequences of small bowel bacterial overgrowth in the rat (*Lichtman* et al., 1990). This develops following surgical creation of a self-filling blind loop in which there is a 4-log increase in the number of anaerobic bacteria. Within 6 to l2 weeks, depending upon the strain of rat, an extensive hepatitis develops and granulomatous lesions occur in the mesenteric lymph nodes and spleen, PAS-positive macrophages are present in these lesions, which is presumptive evidence for PG-PS. The hypothesis is that the large amount of bacterial cell wall debris accumulating from the bacterial overgrowth is transported across the gut wall and systemically distributed. This

could represent one way by which PG-PS derived from the intestinal microflora can be delivered to joint and become part of the pathogenesis of inflammatory arthritis.

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