Workshop IV. Aetiopathogenetic factors in Reiter's syndrome

Adjuvant disease: pathology and immune reactivity

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Adjuvant disease, or adjuvant arthritis, is a disorder that is uniquely inducible in rats. After an injection of Freund's complete adjuvant, or its incorporation in the oily base of some subcomponents of various bacterial cell walls, a peripheral arthritis (Fig. 1) appears in about 10 to 14 days. This is sometimes associated with urethritis in male animals and also with other lesions which include an iritis, perivascular lesions of the external ears and sometimes elsewhere on the skin, and, occasionally, internal lesions such as small granulomas in the liver, etc. In addition a number of animals develop a significant arthritis and peri-arthritis in the caudal, or tail, joints and about the vertebrae, especially near the intervertebral discs, the lumbar vertebrae, and also the sacroiliac joints.

To the surprise of one of us,^{245b} when trying to induce an experimental myositis in rats by injecting

into the skin between their scapulae a skeletal muscle homogenate combined with Freund's adjuvant, most of the rats developed a peripheral polyarthritis after 10 to 14 days. This often affected all four extremities, including many of the small digital joints, the wrists, and the ankles but rarely the more proximal extremity joints. The tail seemed to be involved as did some of the lumbar vertebrae.

Shortly after this observation it was realised that this constellation of symptoms somewhat resembled one or more of the rheumatic diseases that afflict humans, notably Reiter's syndrome (RS), psoriasis with arthritis, rheumatoid arthritis, rheumatoid spondylitis, gonococcal arthropathy, arthritis and ulcerative colitis, erythema nodosum, and iridocyclitis.

Of major importance at that time was the observation that *plain* Freund's adjuvant *without* the added tissue homogenate could initiate the identical syndrome. Dr. Brian Newbould, who was at that time working in our laboratory, then discovered that even as little as 50 µg adjuvant induced the disease in a slightly shorter period of time when injected directly into one or two inguinal lymph nodes.^{234a, 234b} This amount of adjuvant was about 100 times less than the initial injection that had been given either into the skin of the back, into one footpad, or into the base of the tail—methods still used today.

A large number of studies were done on this model³⁴¹ to try to find out why this disease develops in rats. Moreover, the model has become a routine screening test for various anti-inflammatory compounds in laboratories throughout the world.

Clinical features

About 10 to 12 days after injection of adjuvant an arthritis and peri-arthritis suddenly appear in the

Fig. 1 Adjuvant arthritis.

joints of the extremities. These inflammatory lesions, especially in the distal small joints of the paws (Fig. 1), are characterised by redness, swelling of varying severity, and pain. The arthritis is often para-articular and sometimes migratory. In rare cases it almost wholly immobilises the animal, although the proximal limb joints are seldom noticeably affected. The intensity of the lesions may wax and wane for several weeks. Sometimes complete remission is followed by spontaneous recurrences, usually on or about the 60th and 100th postinoculation days.

Death rarely occurs except in maximally affected animals. The arthritis and peri arthritis are often accompanied by: (1) Reddish nodules in the external ears along the course of certain vessels. These basically are the result of perivascular inflammatory and oedematous reactions with secondary vascular congestion. (2) A skin rash (Fig. 2), sometimes diffuse, sometimes not very obvious, and best seen by carefully shaving the skin of the back or flanks of the animals. (3) An anterior uveitis (Fig. 3), which occurred in about 20% of at least two strains of rats that were carefully studied ophthalmologically and pathologically.^{320a} (4) A urethritis and balanitis (Fig. 4) and prostatitis (Fig. 5) in some male animals.^{245b}

Histological features

The histological features of the joint and periarticular lesions as well as the lesions in the eye, skin, genitals, and, occasionally, elsewhere have been the subject of

Fig. 2 Focal lesion in dermis of skin from the flank of animal with adjuvant disease and diffuse dermatitis. The appearance of this lesion, called an 'onion peel lesion', resembles that of human psoriasis.

Fig. 3 Acute uveitis or iritis in anterior chamber with many inflammatory cells and other features characteristic of iritis. Note retina in upper segment of photograph.

Fig. 4 *Intense balanitis and urethritis in male rat 16 days after adjuvant injection. Material exuding from urethra was sterile to culture by usual bacteriological means. Note also intense inflammation, deformity, and swelling of hind paws.*

considerable study.^{46a, 171a, 245e} The dominant early joint lesion is oedema and sparse mononuclear (chiefly lymphocyte) inflammatory infiltration. Within two or three days the inflammatory response becomes intense. There is synovial proliferation, fibrin deposition, joint effusion, and profound proliferation of the connective tissue elements, including fibroblasts and osteoblasts adjacent to a peripheral joint (Fig. 6) and to an intervertebral disc of the tail vertebrae (Fig. 7).

The osteoblastic reaction causes a periosteal

osteogenesis (Fig. 6). Hence, in rapid fashion in the more severe cases, within a matter of 20 to 30 days after the clinical onset of the disease the small paw joints may be invaded, destroyed, and fused by

Fig. 6 *Intense periosteal osteogenesis with new bone formation as well as cartilage formation adjacent to bone and intense inflammatory reaction with fibroblastic reaction. Photograph taken adjacent to a metatarsal joint.*

Fig. 5 *Histopathology of prostate and seminal vesicles. Note intense inflammatory infiltrate made up of mononuclear cells, primarily lymphocytes with a few plasma cells, and intense fibroblastic proliferative reaction.*

fibrous and bony bridges. In less severe cases synovial proliferation, some subchondral erosions, and periosteal new bone formation may cause only minor or moderate limitation of joint function. The lumbar spine and tail (or caudal) vertebrae and

adjacent tissues may be affected in severe cases, leading to significant ankylosis (Fig. 8) with profound bony bridging (Fig. 9). Sometimes the lumbar spine is also involved, but not usually the thoracic or cervical spines. These features can readily be seen by x-ray examination.^{314a}

Immunogenesis

After this syndrome was first described many investigators tried to (1) define the mechanisms that produced the disease, especially during the latency period; (2) ascertain the role of lymphocytes and lymph nodes in the genesis of the disease, and also the role of circulating antibodies; (3) vary the incidence and severity of the disease by experimental manipulations, including its complete inhibition; and (4) provide a model of inflammatory arthritis in the search for therapeutically effective anti-inflammatory drugs.

When the disease was originally observed it was thought likely that a latent infection of some sort was being activated in the rat. As the results of other studies accumulated, however, an infection of the usual variety seemed less likely. This was because (1) a bacterium or mycoplasma could never consistently or even occasionally be cultured from the affected tissues^{285a}; (2) penicillin and broad-spectrum antibiotics were ineffectual against the disease; (3) a 'germ-free' group of animals easily developed the entire disease syndrome²⁴⁵; (4) an inability to transfer the disease by contact between affected and healthy rats or by inoculation of serum or affected articular tissues into normal controls; (5) the disease was transferred to highly inbred controls by inoculating them with viable sensitised lymphocytes taken from the lymph nodes and spleen or the thoracic duct of previously inoculated animals; and (6) this transfer failed if the lymphocytes were previously disrupted by repeated freezing and thawing.^{245f, 320c, 323a}

Fig. 7 Intense inflammatory infiltration adjacent to intervertebral disc separating two tail vertebrae. On the adjacent vertebrae was also significant new bone formation or periosteogenesis.

Fig. 8 Ankylosed tail in affected animal secondary to pathological reactions described in the previous figures.

Fig. 9 Dissection of entire spine of rat showing profound bony fusion of tail and lumbar vertebrae but sparing most thoracic and all cervical vertebrae. This type of picture was common.

On the other hand, some workers showed that a virus might be activated and, in turn, provoke an immune response, giving rise to arthritis and other lesions.^{60c, 173b} The interferon-inducing substance Tilorone or purified interferons in small doses that do not possess anti-inflammatory reactions (the interferon was induced in a tissue culture of fibroblasts by infection with Sindbis virus) depress or completely inhibit the appearance of adjuvant disease, especially when the Tilorone or interferon is given one or two days before and one or two days after the injection of adjuvant.

SENSITISING ANTIGEN

The precise sensitising 'antigen' has not been identified. It may be some component of the acid-fast bacillus (a prime component of Freund's adjuvant) such as one of the wax fractions, which in almost pure form, free of all detectable protein including tuberculo-protein, can readily induce the disease. Several smaller protein molecules that have been prepared from various other bacterial cell walls can also induce adjuvant disease when injected in an oily emulsion. These other bacterial cell wall preparations include, among others, those obtained from *Nocardia*, *Streptomyces*, *Streptococcus*, and *Lactobacillus*. Moreover, other studies have shown that relatively small molecules such as peptidoglycans (comprised of a disaccharide and a hexapeptide) in mineral oil may induce adjuvant disease.^{186a-f, 297a} The possible role of peptidoglycans from various human sources or from bacterial cell walls in the induction of certain human diseases such as rheumatoid arthritis, RS, and other human inflammatory joint diseases as well as adjuvant

disease have been speculated upon extensively by Hadler.^{143b}

In a somewhat parallel series of investigations Schwab^{278a} showed that a polyarthritis can be produced in rats by intraperitoneal inoculation of water soluble peptidoglycans obtained from the cell wall of group A streptococci. He showed, on the one hand, that the disease was chronic and, on the other hand, that the peptidoglycans were generally non-degradable, that they were transported and sequestered in macrophages, the persistence of the (possibly antigenic) initiating stimulus being due to an inability or inefficiency of macrophages to degrade these bacterial cell wall constituents. He further speculated that the defect in the handling of bacterial cell walls, observed in certain strains of animals, could be related to an immune deficiency.

As had been found by many workers before,^{186a,c,f} Schwab^{278a} described the basic structure of these water-soluble preparations as oligosaccharides consisting of a backbone of N-acetylglucosamine and N-acetylmuramic acid which were associated with several peptide side chains that include a variety of amino-acids, among them alanine, glutamine, and lysine.

There has as yet been no proof that a virus is involved in the syndrome. Only circumstantial evidence has been deduced, including the above-mentioned inhibition by Tilorone or interferon or other interferon-inducing agents. Furthermore, if a virus is involved it may be indirectly via the immunological system.

The theory that a hypersensitivity immune reaction is at least partially responsible for adjuvant disease, and also for certain human disorders such as

RS, is based upon the following: (1) The results of the lymphocyte transfer experiment mentioned earlier. (2) That development of the disease is inhibited when all the lymph nodes that drain the site of inoculation are resected.^{74a} (3) That the latency between the time of inoculation and the onset of disease is invariably at least 10 days. (4) That a refractory state similar to immune tolerance is induced when the animals are exposed early in life to adjuvant, or even when more than 100 µg of dead tubercle bacilli are given in suspension during the incubation period after adjuvant inoculation. (5) The suppressive effect of whole-body irradiation when given immediately before adjuvant inoculation. (6) That the disease is suppressed by simultaneous administration of heterologous antilymphocyte globulin.^{74b} (7) That the arthritis and other lesions are accentuated by tuberculin (PPD) in doses of 4 to 100 µg given during the incubation period.^{74a} (8) The observation that the arthritis can be transferred only to native inbred animals by sensitised lymphocytes between the seventh and eleventh post-inoculation days, when these cells must be competent (earlier they appear to lack full competence and hence need a lymph node environment in which to develop further). (9) That the disease is produced with greater regularity and more severely when smaller amounts of adjuvant are injected directly into one or more of the inguinal lymph nodes.

AUTOIMMUNITY

An alternative explanation is that adjuvant disease may be an autoimmune phenomenon resulting in autoantibodies or specific sensitised lymphocytes, or both, acting against the animal's own tissues.^{234a, 245f, 320b} There is so far no clearcut evidence of autoimmunity in rats with adjuvant disease. Hence attempts to induce arthritis and other lesions in rats by the intradermal inoculation of oil suspensions of glycogen, silica, dried bovine cartilage, extracts of a variety of other tissues, cardiolybin, and other materials has been totally unsuccessful.^{127a} But Trentham *et al.*^{314c} have described the induction of a somewhat similar polyarthritis by type II collagen when placed in incomplete adjuvant. Other collagen types did not induce any disease in similarly injected rats. Possibly in this instance the type II collagen could be replacing the wax D, the tubercle bacillus, or the peptidoglycan and thereby be acting as a *complete* adjuvant rather than conveying something specific to the injected rats by type II collagen.

ENDOGENOUS IMMUNOGEN

We have recently obtained evidence suggesting that the immunogen(s) responsible for adjuvant disease

N,N-di-octadecyl-N',N'-bis(2-hydroxyethyl)propanediamine (CP-20,961)

Fig. 10 Structural formula for alkylldiamine.

is/are endogenous rather than something introduced in the Freund's complete adjuvant.^{60c} We found that a low molecular weight (MW=714), apparently non-immunogenic synthetic compound (called an alkylldiamine (Fig. 10)) induced classic chronic polyarthritis in Lewis rats that was morphologically indistinguishable in both time of appearance and clinical presentation as well as pathology from the classic adjuvant disease induced by Freund's complete adjuvant.

Various anti-inflammatory compounds such as phenylbutazone and cortisone, or its derivatives, can inhibit the inflammatory lesions of adjuvant disease if they are given two or three days before and during the expected time of appearance of the arthritis, etc. If they are given at the time of adjuvant injection and then discontinued during the 'incubation' period they have no effect. On the other hand, the interferon-inducers such as Tilorone and poly I:C are most effective when given *before* and *during* the first two or three days after the injection of adjuvant and/or alkylldiamine. They may be discontinued after that, but still prevent adjuvant disease.

To try to sort out the role of virus, humoral response, and cell-mediated immunity Chang^{60c} devised a technique whereby he could test both humoral and cell-mediated immune responses to a strain of cells (EL₄) when injected intraperitoneally in Lewis rats. By prelabelling these cells with ⁵¹Cr it was possible to ascertain, by release of ⁵¹Cr from these cells, that either the alkylldiamine or Freund's adjuvant was capable of enhancing both cell-mediated and humoral immune responses to the immunogen (either peptidoglycan or alkylldiamine) when coadministered. These findings strongly suggest that the immunogen responsible for the development of adjuvant disease is endogenous—for example, a constituent of host tissue, a viral protein, or some complex of the two.

Genetic influence

Beyond biochemical, immunological, and potentially infectious mechanisms there may well be a major genetic influence in adjuvant arthritis. Indeed, a number of studies have shown that there are clear strain differences in the response of inbred strains of rats to adjuvant injections. One study showed that adjuvant arthritis was induced in 100% of the Lewis inbred strains, in 45% of Long Evans, and in 10.8% of the AVN strains of rats.^{34a} The incidence in a hybrid of the Lewis and AVN strains was 55%. A back-cross of the latter F_1 with the Lewis strain produced a strain with a 70% incidence of adjuvant disease, whereas a back-cross with the AVN strain produced a 35% incidence.

A somewhat similar study^{186d} showed that the Lewis strain was the most highly susceptible to adjuvant arthritis, whereas incidence in the Fisher strain was about 30%. The Buffalo strain of rats, with an incidence of less than 10% was almost completely resistant. Not only was the arthritic response quite variable from one strain to another^{173a} but also, in the four inbred strains tested, there was a widely variable release of a ConA-reactive lympho-

cyte subpopulation that exhibited a low response to PHA and PWM.

Because of the striking similarity between adjuvant arthritis and RS or AS, our view is that adjuvant disease is linked to the Ag-B or H-1 histocompatibility system in rats as RS and AS are linked to the histocompatibility gene HLA-B27. A similar linkage was postulated in rats. The major histocompatibility complex has recently been investigated in considerable details in rats,^{126a, 143a} and it would appear to harbour, just as in mice, one or several immune-response (Ir) loci that define lymphocyte response (Fig. 11). However, there has not been a systematic search for an allele linked with adjuvant arthritis in the rat.

The mechanism(s) that underlie(s) the adjuvanticity of either complete Freund's adjuvant or of a peptidoglycan component of the bacterial cell wall or of the alkylamine is at present unclear. These agents may aid the development of the immune response by a non-specific stimulation of plasma cell or reticulum cell proliferation; by activating antigen-processing macrophages; by intensifying the traffic of lymphocytes and local lymph nodes draining the immunisation site; or by a combination of these mechanisms.

Fig. 11 Structure of the major histocompatibility complex of mouse and man and a tentative model of a similar genetic complex in the rat. Note similarity between the complexes in man and rat, especially between the Ir loci and two loci SDs and SDw, which control respectively the stronger and weaker histocompatibility antigens in both man and the rat (from Gill and Kunz^{126a}).

Conclusion

The induction of adjuvant disease either by complete Freund's adjuvant or an alkylidiamine compound or other stimulants in inbred rats seems to be a reasonable model of RS since, in this animal species, an arthritis, an iritis, a urethritis, and sometimes a dermatitis can be produced. These are features in common with human RS.

The weight of available evidence favours the view that immunological phenomena underly the generation of adjuvant disease. The induction of this experimental disease by intradermal inoculation of an oily preparation of an apparent non-immunogenic, low molecular weight, synthetic alkylidiamine suggests that the responsible immunogen is endogenous to the animal. Such a conclusion could not have been drawn specifically when complete Freund's adjuvant or peptidoglycans from bacterial cell wall sources were used.

The possible role of a virus is indicated by the finding that interferon, prepared *in vivo* or *in vitro*, effectively suppressed the development of adjuvant disease without effecting the immune response. These findings suggest that the disease may have resulted from an aberrant immune response to some endogenous material(s)—for example, a viral protein or some complex of viral protein and host tissue. That the variable host responses, probably immunological, are genetically dictated is suggested by the finding that some inbred rat strains are highly susceptible to the disease whereas other strains are partially or wholly resistant. This suggests the influence of the major histocompatibility system in the rat (Ag-B or H-1 system), much the same as HLA-B27 is associated with RS in humans.

General discussion

DR. E. ALBERT: Have you done a segregation study where you cross this resistant strain with a susceptible strain and then look in the F2 hybrids for histocompatibility antigen and resistance or susceptibility, which would be the classical linkage test?

PROF. PEARSON: We have not done any histocompatibility studies as yet. We did some cross-breeding of the Lewis strain and the Buffalo strain several years ago and compared the severity of the arthritis and the incidence. The arthritis appears in about 50% of the F-1 offspring, which again suggests the importance of genetic factors in addition, perhaps, to other factors.

DR. ALBERT: Susceptibility is dominant over resistance?

PROF. PEARSON: Yes, apparently.

DR. A. CALIN: Are there instances of spontaneous adjuvant disease in rats?

PROF. PEARSON: I have never seen or heard of any spontaneously occurring polyarthritis. However, Dr. Byron Waxman, while inducing experimental allergic encephalomyelitis (EAE) in his animals, occasionally observed polyarthritis instead of EAE. I think it is a matter of the relative balance between the amount of brain extract and the amount of tubercle bacilli that were placed in Freund's adjuvant. I have noticed the same phenomena using muscle extract and adjuvant. Other researchers, when inducing experimental allergic nephritis, have occasionally seen arthritis also when the amount of renal tissue antigen was small versus the amount of stimulating microbacterial agent in the adjuvant.

DR. J. C. WOODROW: Do you think it might be worth looking at cross-reactivity between some of these synthetic determinants and the host tissues? Shared specificities would at least take into account the possibility of impaired immune response in some strains.

PROF. PEARSON: Yes indeed. Impaired degradation might also be important. A number of bacterial cell walls and their metabolites are non-digestible in the human for lack of adequate enzyme systems. Until they are disposed of by macrophage ingestion they may remain on the spot for a long time and could act as the primary stimulating factor or as an adjuvant for something else that is responsible for the induction of the disease. Similar phenomena might be operative in some of the B27-associated diseases.

DR. D. A. BREWERTON: Have any of the injections been given into the urethra or the gut instead of into the skin or the lymph glands?

PROF. PEARSON: No, because other sites (subcutaneous, intramuscular, or intraperitoneal) are not very satisfactory. It seems to be necessary to inject in an area of potent lymphoid drainage. Indeed, one can block the disease completely by resecting the regional (popliteal, inguinal, and periaortic) lymph nodes within five days after the paw injection. But from day seven onwards resection of the nodes will not alter the normal course of the disease.

DR. G. W. CSONKA: How does adjuvant disease compare with mycoplasma-induced arthritis in rats? After all, mycoplasmas have no cell walls?

PROF. PEARSON: I have not had too much experience with mycoplasma-induced arthritis in the rat. I have seen some when Dr. John Ward was at the Massachusetts General Hospital. Those animals developed a transient polyarthritis. They do not

develop any of these other features that I have described. I believe that mycoplasma arthritis is a direct synovial infection and probably is relatively if not totally unrelated to what we are seeing in adjuvant-induced arthritis.

PROF. B. AMOR: Using your new compound, did you find any difference in the reactivity of various strains of rats?

PROF. PEARSON: We have only tested it in the Lewis strain so far.