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Meeting Report

Murine Models of Chronic Inflammation

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Acute rodent models such as the carrageenan edema and the ultraviolet light edema tests of inflammation, and the phenylbenzoquinone writhing test of analgesia have been instrumental in the discovery and development of nonsteroidal antiinflammatory drugs (NSAIDs) for treatment of rheumatoid arthritis (RA). The usefulness of these models has been validated in the clinic. The search for drugs to treat arthritis currently is focused on models for identifying drugs which may treat the chronic aspects of the disease. On September 26, 1985 the Inflammation Research Association met at the New York Academy of Sciences to discuss several murine models of chronic inflammation to access their contribution to an understanding of the human disease and their potential utility for discovery of new therapy for RA.

The methylated bovine serum albumin (mBSA) model of antigen-induced monoarticular arthritis was first described in the mouse by Brackertz in 1977. Although attention has been primarily focused on the Dumonde-Glynn monoarticular arthritis rabbit model for identifying penicillamine-like drugs, the difficulty of doing extensive studies in the rabbit has led to a resurgence of interest in the mouse model, both for studying mechanisms of the arthritis and potential therapeutic agents. Dr Ian M. Hunneyball (Boots Company, Nottingham, England) presented his findings on the effects of anti-arthritic drugs in the mouse model compared to the rabbit model. Drug effects were determined by examining the pathology of the knee joint, specifically the extent of synovitis and bone erosion.

NSAIDs (flurbiprofen, ibuprofen and indomethacin) did not inhibit the murine arthritis. As in the rabbit arthritis, corticosteroids (prednisolone and dexamethasone) inhibited synovitis and erosion in the mouse knee joint. Moreover, sulphasalazine (but not sulphapyridine), dapsone, and immunosuppressants (azathioprine, methotrexate and cyclosporin A) inhibited both the synovitis and bony erosions. In the rabbit immunosuppresants, but not sulphasalazine, were active.

While NSAID, corticosteroids and immunosuppressants have similar activities in both the rabbit and the mouse, Dr Hunneyball showed that disease-modifying antiarthritic drugs (DMARDs) had a unique profile in each of the two models: d-penicillamine and gold were therapeutic in the rabbit but without effect in the mouse model. Chloroquine was inactive in both models. Because of size and ease of handling, the mouse model would appear to be more adaptable to the discovery of novel therapeutic agents than the rabbit. Moreover, the profile of antiarthritic drugs in the mouse may indicate that this model may be best used for identifying therapeutic agents with unique mechanisms.

Dr Wim B. van den Berg (University Hospital, St Radboud, Nijmegan, the Netherlands) reported his mechanistic studies in the mBSA model. He showed that BSA made cationic by methylation (mBSA) or amidation (aBSA) deeply penetrates the dense hyaline cartilage and is retained in the mouse knee joint in higher amounts and for longer periods than other antigens. Charge-mediated antigen retention in the joint was demonstrated to be an important factor in the chronicity of the inflammation as shown by ^{99m}Tc uptake in the joint and by histological evaluation.

Oxygen radicals and hydrogen peroxide (H_2O_2) were considered as potential mediators of the mBSA arthritis. When the respective inactivating enzymes, i.e. superoxide dismutase (SOD) and catalase were made cationic, only catalase suppressed the arthritis. Catalase was also effective in

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inhibiting a zymosan-induced, nonimmune arthritis, while SOD was not. Swelling and plasma leakage were inhibited by the cationic catalase while only a slight effect on cellular infiltrate was shown.

To further examine the role of H_2O_2 in chronic inflammation, Dr van den Berg localized glucose oxidase, a hydrogen peroxide-generating enzyme, in the synovial cartilage by cationization. An arthritis resulted which was characterized by endothelial cell destruction and plasma leakage. He then evaluated the therapeutic effects of various treatments by measuring ^{99m}Tc uptake and the histology of cartilage destruction. As found previously in the mBSA arthritis, cationic catalase significantly inhibited the glucose oxidase-induced arthritis. Ebselen, a selenium compound from Nattermann & Cie, also inhibited the arthritis. NSAIDs (indomethacin and piroxicam) had no effect at day 2; however, at day 4 slight inhibitory activity was seen.

Charge appears to be an important determinant in local joint retention of potential antigens as well as other proteins such as scavenging enzymes (catalase) and destructive enzymes (elastase). The destructive potential of enzymes such as elastase may be determined in part by its cationicity. Elastase is taken up by cartilage in high amounts and, therefore, may escape from normal inhibitors like α-1 antitrypsin and α-antiprotease.

Collagen-induced arthritis (CIA) has been thought to be closely related to RA since it shares similarities with the human disease. Therefore, the model has been studied in an attempt to understand the possible role of an immune response to Type II collagen in the pathogenesis of human arthritic diseases, and to identify possible pharmacologic and biologic interventions.

Dr Paul H. Wooley (Ayerst Laboratories, Princeton, NJ) described murine CIA which, although not identical to human RA, he feels consists of a constellation of pathological mechanisms representing a portion of the athritic disease spectrum and one that is open to modulation by biological approaches. He has shown that susceptibility to CIA is through the I-A region of the immune response genes. Genetic studies of disease susceptibility with collagen from various sources (chick, bovine, porcine, deer, human) suggests that two epitopes on the collagen molecule are able to induce CIA.

CIA-susceptible strains generally show high antibody responses to type II collagen; however, some resistant strains show high antibody titers, suggesting that this represents an immune response against a foreign antigen rather than an autoimmune response. Polyclonal antibody to type II collagen, but not monoclonal antibody, can passively transfer a transient acute disease. This suggests that the immunoglobulin involved represents only one component of the chronic disease to type II collagen. The antibody-mediated response does appear to be more important than the cell-mediated response in murine CIA.

There are correlations between murine CIA and human RA: the presence of anti-collagen antibody, major histocompatibility complex (MHC) control of susceptibility, and MHC regulation of antibody formation. The classic association of DR4 with RA was confirmed at the Mayo Clinic. However, no association of DR4 with high antibody response to type II collagen was found. Moreover, although DR2 is under-represented in the RA population, it is associated with a high antibody titer to type II collagen and seronegative RA. Dr Wooley suggested that collagen arthritis in humans could be part of the rheumatoid spectrum but also it could appear as a distinct disease subject to different pharmacological and biological treatment. Using an Ig fraction containing anti-type II collagen from a patient, he was, in fact, able to transfer a transient disease from man into the mouse. The severity of CIA disease is able to be altered by biological modulation: pretreatment with α-la antibody, anti-collagen antibody or collagen causes low incidence and/or suppression of the disease in mice. This opens the question of suitability of such treatment in man.

Much has been published on the effects of NSAIDs, immunosuppressants and DMARDs in the rat model of CIA, but there have been only sporadic reports of drug effects in mice. Dr Kalindi Phadke (Eli Lilly, Indianapolis, IN) reported the first extensive study of antiarthritic agents in murine CIA. Assessment of disease and evaluation of drug therapy was done by soft tissue score, and radiologic and immunologic analyses. Of the many antiarthritic and antiinflammatory drugs tested, only paramethasone and cyclophosphamide inhibited the soft tissue swelling of the joint. Only cyclophosphamide inhibited radiologic changes. When cyclophosphamide was discontinued, the disease rebounded. D-penicillamine at a high dose caused an earlier onset of the disease but had no effect later.

Aspirin, chloroquine, levamisole, d-penicillamine and gold chlorophosphene failed to reduce serum levels of anticollagen antibody. Benoxaprofen and naproxen showed some suppression of antibody levels but only at high doses. Paramethasone and cyclophosphamide suppressed antibody at pharmacological levels. Delayed type hypersensitivity was suppressed by high doses of benoxaprofen, naproxen, levamisole and d-penicillamine, and by pharmacologic doses of paramethasone and cyclophosphamide. Aspirin, chloroquine and gold chlorophosphene were inactive. Since cyclophosphamide and paramethasone inhibit both antibody and DTH responses to type II collagen, this may be the mechanism of their therapeutic effect.

Dr Phadke concluded that NSAIDs and DMARDs are ineffective in murine CIA. This model is distinguished from adjuvant arthritis in the rat where NSAIDs improves the radiologic score. Therefore, it is hoped that murine CIA could be used to identify drugs with novel mechanisms of action.

The MRL/Ipr mouse is one of the several strains used as a model of autoimmune disease. However, the MRL/Ipr mouse is distinguished from the others in that it develops a low incidence of articular erosion and pannus, produces high levels of rheumatoid factor, develops elevated erythrocyte sedimentation rates, shows a hyperproliferation of nodular T cells, and develops a fatal immune-complex glomerulonephritis. Dr Vicki E. Kelley (Harvard Medical School, Boston, MA) described her studies with these autoimmune mice and the role of arachidonic acid metabolites in the resulting renal disease. When menhaden oil containing eicosapentanoic acid (EPA) was administered in the mouse diet, mortality was retarded, weight gain was normal, and proteinuria was reduced. There was no evidence of IgG in the glomerular peripheral capillary loops. Safflower oil, rich in arachidonic acid metabolites, was not therapeutic. When the arachidonic acid metabolites were measured separately in the kidney medulla and cortex, mice treated with menhaden oil made less PGE_2, TXB_2 and 6-keto $PGF_{1\alpha}$ than the mice given safflower oil. There was also a modest shift from PGE_2 to PGE_3.

MRL/Ipr mice have greatly increased levels of renal TXB_2 compared to normals, although there are no differences in PGE_2 or 6-keto $PGF_{1\alpha}$ levels. Increased levels of TXB_2 are also seen in the NZB/W mouse and the elevation correlates with urinary protein and histologic score. When large amounts of PGE_2 were administered to MRL/Ipr mice, the development of the renal pathology was inhibited and normal renal TXB_2 levels were seen. Elevations of TXB_2 levels are associated with renal pathology in other models of kidney disease: anti-glomerular basement membrane disease, adriamycin nephrosis, unilateral obstruction, and endotoxemic rat models. A thromboxane synthetase inhibitor (dazoxiben) administered to those animals with endotoxemia prevented loss of renal function although kidney TXB_2 levels were not suppressed for the full length of the disease.

The source of TXA_2, the unstable precursor metabolite of TXB_2, in the kidney may be the macrophage. Peritoneal macrophages from fish oil-treated MRL/Ipr mice produced decreased TXB_2 and PGE_2, and showed decreased Ia positivity.

Thromboxane appears to be the more important arachidonic acid correlate of renal disease. Dr Kelley suggested that future studies of renal disease will involve identifying TXB_2 inhibitors such as receptor antagonists, clarifying the cell type responsible for TXB_2 production, examining the role of leukotrienes, and improving our understanding of the role of arachidonic acid metabolites in renal hemodynamics.

Chronic inflammation, whether expressed in the kidney or the arthritic joint, is probably not the result of a simple disease mechanism. It is clear that no one animal model can represent all the varied aspects of chronic inflammation. But hopefully, by utilizing animal models, relevant mechanisms can be isolated and be incorporated into a composite picture of the human arthritic disease(s) and lead the way to safer, more effective treatment.

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