Induction of Experimental Chronic Arthritis in Rabbits by Cell-free Fragments of *Erysipelothrix*

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A cell-free crude extract of *Erysipelothrix rhusiopathiae* injected by high pressure jet into the knee-joint of rabbits stimulated an acute, mild inflammatory reaction. Additional injections at 3-day intervals induced a chronic condition characterized by hyperplasia of the synovial cells and hypertrophy of the villi, due to infiltration by lymphocytes and plasma cells which formed aggregates resembling Allison-Ghormley bodies. There was also extensive proliferation of stroma vasculature and fibrous tissue. A similar jet injection of the diluent produced an early, transient, acute, and mild inflammation. A mechanism is postulated for fixation of one or more of the chemically characterized antigens in or near the synovium as a means of inducing the localized inflammatory response that predisposes the joint to infection.

The experimental production of rheumatoid arthritis, as reviewed by Gardner (2), has been attempted with a variety of biological materials. These were restricted largely to systems related only remotely, if at all, to natural arthritis. In contrast, Schwab et al. (7) described a pathologic relationship between group A streptococcal cell wall and rabbit articular tissue and postulated a role for this relationship in rheumatic fever.

Willkens et al. (11) induced an arthritis-like condition by the intra-articular injection of autologous aggregated gamma globulin in rabbits whose serum contained a rheumatoid-like substance. They postulated that the leukocytic phagocytosis of this immunologic complex induced the development of joint inflammation.

This preliminary report describes results which show that arthritis can be induced in the knees of rabbits by injection of the cell-free extract of *Erysipelothrix* described earlier (4, 10). These results were obtained by use of a jet injection procedure which appreciably reduces the consequences of trauma.

**MATERIALS AND METHODS**

An acetone-dried powder of *E. rhusiopathiae* strain S192 (kindly supplied by R. D. Shuman, National Animal Disease Laboratory, U.S. Department of Agriculture, Ames, Iowa) was extracted overnight at 4°C in aqueous phosphate buffer (pH 7.5). The preparation of this crude extract was described previously (10). A crude filtrate was passed through HA membranes (Millipore Corp., Bedford, Mass.) and stored at 2°C in sterile bottles with rubber caps. Sterility was checked in beef infusion broth and on slants containing 7% horse serum. A nonantigenic, high-protein preparation was made by removal of spleens from normal rabbits, extraction in phosphate buffer, and filtration; the protein content (5) was adjusted to that of the crude extract, and the material was then re-filtered through HA membranes. This high-protein preparation was stored in the same manner as the crude extract. Phosphate buffer for injection was sterilized and stored similarly.

New Zealand white, female rabbits, each weighing about 2.5 kg, were obtained from a single source. The left knee of each rabbit was shaved, and the appropriate reagent was injected into the flexed joint just lateral to the patellar tendon. Jet injection of 0.2 ml, containing 200 µg of protein, was made with a Hypospray manufactured by R. P. Scherer Corp., Detroit, Mich. This unit was mounted with a pointed injection tip designed by T. J. Rankin of this hospital for use in the management of the arthritides of human joints (6). It permits accurate and precise injection of irregular surfaces. Nine injections were made at 3-day intervals during the 28 days of each experiment. One or more rabbits of each treatment group was sacrificed at 1, 3, 7, 16, and 28 days. Immune rabbits were prepared by administration of a total of 20 intravenous injections of thimerosal-killed *Erysipelothrix* organisms (strain S192) adjusted to a density of 30% transmittance in a Bausch & Lomb Spectronic-20 colorimeter. The challenge dose was similarly prepared from viable organisms, and on day 28 one normal rabbit and one immune rabbit were given 1.0 ml intravenously. These two rabbits were sacrificed after 4 and 2 months, respectively.
At necropsy, injected and noninjected knee joints were opened by a suprapatellar tendon incision followed by downward reflection of the transected soft tissue. This approach allowed a uniform exposure of most of the anterior and lateral synovial membrane and permitted repetitive sampling and comparison of the same synovial regions, namely, the subpatellar pouch as well as lateral margins. Both knees were cultured on serum-supplemented beef infusion broth and PPLO medium. The tissues were then immediately fixed in neutral buffered Formalin. Transverse sections of the fixed tissues at various levels were then examined with the use of hematoxylin and eosin staining. In addition, the heart, kidneys, and bilateral proximal tibia, fibula, and distal femur were also collected and examined microscopically after similar fixation and staining. Decalcification of bone specimens was carried out in 10% Sequestrene (Geigy Chemical Co.).

Pretreatment and terminal blood was obtained by heart puncture, and the serum was examined for precipitating antibody by immuneelectrophoresis.

RESULTS

Gross examination of injected and noninjected knees revealed no detectable swelling or other outward signs of inflammatory reaction, and caliper measurements were abandoned as valueless.

Histopathology of rabbits injected with crude extract. In normal rabbits that received fewer than five injections of crude extract, inflammation was acute and mild to moderate in degree. In normal rabbits treated for 16 and 28 days, the reaction became marked and assumed the characteristics of a chronic proliferative synovitis, i.e., proliferation of synovial cells, stroma vasculature, and fibrous tissue. Villi hypertrophied and contained nodular aggregates somewhat resembling Allison-Ghormley bodies composed predominantly of large numbers of lymphocytes and plasma cells (Fig. 1). Fibrinoid material was present amid synovial cells and in the underlying stroma.

When hyperimmunized rabbits were similarly injected with crude extract, inflammation in the short-term rabbits was acute and mild. The immune rabbits treated for 16 and 28 days exhibited pronounced changes characterized by moderate to marked proliferation of synovial cells, stromal vasculature, and fibroblastic elements (Fig. 2). The cellular response was primarily mononuclear (macrophages, lymphocytes, and plasma cells), but appeared neither as exuberant nor as early as that observed in the similarly treated nonimmune animals. The macrophage was the predominant mononuclear cell seen early; a few lymphocytes and plasma cells occurred later. Focal fibrin or fibrinoid material (or both) was present amid synovial cells or in the synovial stroma of the rabbits treated for 16 and 28 days.

Histopathology of rabbits injected with crude extract and challenged at 28 days. The normal rabbit which had been injected with crude extract and challenged at 28 days was sacrificed at 126 days. Synovial tissues from both knees revealed a moderate chronic synovitis. The proliferative and exudative changes were similar to those seen in the rabbits treated with crude extract for 16 days.

The hyperimmunized rabbit treated with crude extract and challenged at 28 days was sacrificed at 91 days. Synovial tissues from both knees showed severe chronic synovitis similar to

Fig. 1. Normal rabbits injected with crude extract, 28 days: synovial cell hyperplasia, proliferation of stromal components and marked mononuclear cell infiltration. The mononuclear cell configuration is similar to the Allison-Ghormley body. Hematoxylin and eosin stain. (A) X 120. (B) X 300.
that resulting from the 28-day treatment, and in addition exhibited a more pronounced mononuclear cell response. The cellular exudative reaction appeared nearly as marked as that seen in Fig. 1. The articular surfaces showed bilateral early pannus formation.

Histopathology of normal rabbits injected with the carrier buffer alone. Inflammation in the short-term rabbits was acute and mild. The long-term rabbits showed a more moderate focal reaction consisting of focal synovial cell hyperplasia, stromal fat necrosis, fibrous tissue proliferation, and accumulations of mononuclear cells which were primarily macrophages.

Histopathology of normal rabbits injected with spleen homogenate. Inflammation in the short-term rabbits was acute and mild. A marked preponderance of polymorphonuclear inflammatory cells over mononuclear inflammatory cells was noted at all synovial stromal layers. Proliferative changes were observed in the synovial cells, stromal vasculature, and fibroblastic elements. The rabbits treated for 28 days showed a dramatic diminution of the inflammatory process described above, with the only residua being multifocal areas of mild synovial cell hyperplasia, stromal fibrosis, and deep stromal accumulations of a few mononuclear cells.

With the exception of the previously described changes seen in the challenged animals, the opposite knee joints and synovial membranes examined on all experimental animals and controls generally showed no changes.

Gross and histopathological examination of heart tissue was uninterpretable because of the heart puncture used for obtaining blood. Similar examinations of kidneys and osseous material were interpreted as negative.

Serology. Precipitating antibody was first detected in the rabbits sacrificed at 16 days. The level and pattern of precipitation was entirely analogous to immune serum prepared by more conventional methods by use of whole killed cells with or without adjuvant. Five distinct bands of precipitation were uniformly present and persisted throughout the exposure period. Serum from each of the immune animals showed the same five bands of precipitation at the time of the first knee injection.

The normal rabbits injected with the nonantigenic, high-protein material remained negative to spleen extract as well as to crude extract.

**DISCUSSION**

These initial studies were designed to determine whether certain components of *Erysipelothrix* could elicit a rheumatoid arthritis-like response in the knees of rabbits, and whether such responses were alterable by specific antibody. An additional objective was to consider the jet injection method as a means of reducing damage to the integrity of the joint capsule.

Since the crude extract contains many types of material and the total of each dose was only 200 μg of protein, it can safely be assumed that if a single factor is specifically responsible for the reaction it is present in minute quantities. Similarly, such a factor must be considered potent because known active agents used by others (7, 9) were injected at somewhat higher levels. The physical-chemical description of the array of antigens which make up the crude extract is known (3, 4, 10); hence, we are presently examining the ability of each fraction to cause the same reaction produced by the crude extract. At the same time, specific antibody is being used to alter the reaction.
Failure of the nonantigenic, high-protein material to initiate a similar inflammatory response indicates the significance of the host immune response in the development of synovitis. Nonantigenic material containing a level of protein similar to that of the crude extract did not call forth the cells associated with an immune response and did not elicit pathology analogous to that produced by the crude extract.

A thorough investigation by Shuman et al. (8) showed that whole organisms, dead or alive, without reference to serological type, induce arthritis in swine only if, as part of the experimental regimen, the swine are exposed to viable Erysipelothrix.

After 28 days, the inflammatory response of the immunized rabbits appeared pathologically quite similar to that of the nonsensitized rabbits, and actually showed considerably less exuberance at 1 and 7 days. This indicates that performed antibody probably does not enhance the early inflammatory response as has been proposed (1), although an antibody level concurrent with septicemia may initiate a detrimental reaction.

Our data indicate that production of monarticular chronic arthritis in the knee joint of a rabbit can be elicited by crude extracts of E. rhusiopathiae without experimental exposure to live organisms; however, polyarticular chronic arthritis was induced by the intravenous injection of live Erysipelothrix cells in all rabbits treated with crude extract. We propose that during the period of exposure to Erysipelothrix an isolated soluble constituent is bound by a mechanism unique to joint tissues, and the incipient host response predisposes the joint to subsequent infection with degenerative results. The durability of a toxic factor such as that described by Schwab (7), who used Streptococcus in rabbit joints, is a significant observation.

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