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The Role of Retained Antigen as an Etiological Agent
in Rheumatoid Arthritis

by

Patricia Liccardi Rice
B.S., University of Dayton, 1973

Thesis

submitted in partial fulfillment of the requirements for the
Degree of Master of Science in the Department of
Microbiology at the Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia
May, 1981

This thesis by Patricia Liccardi Rice is accepted in its present form as satisfying the thesis requirement for the degree of Master of Science.

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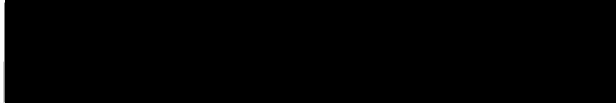
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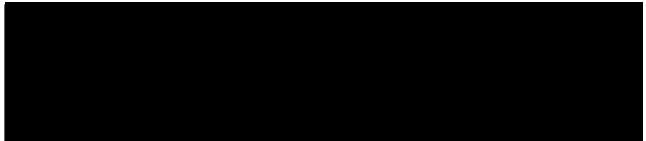


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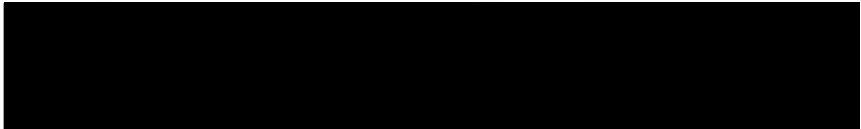
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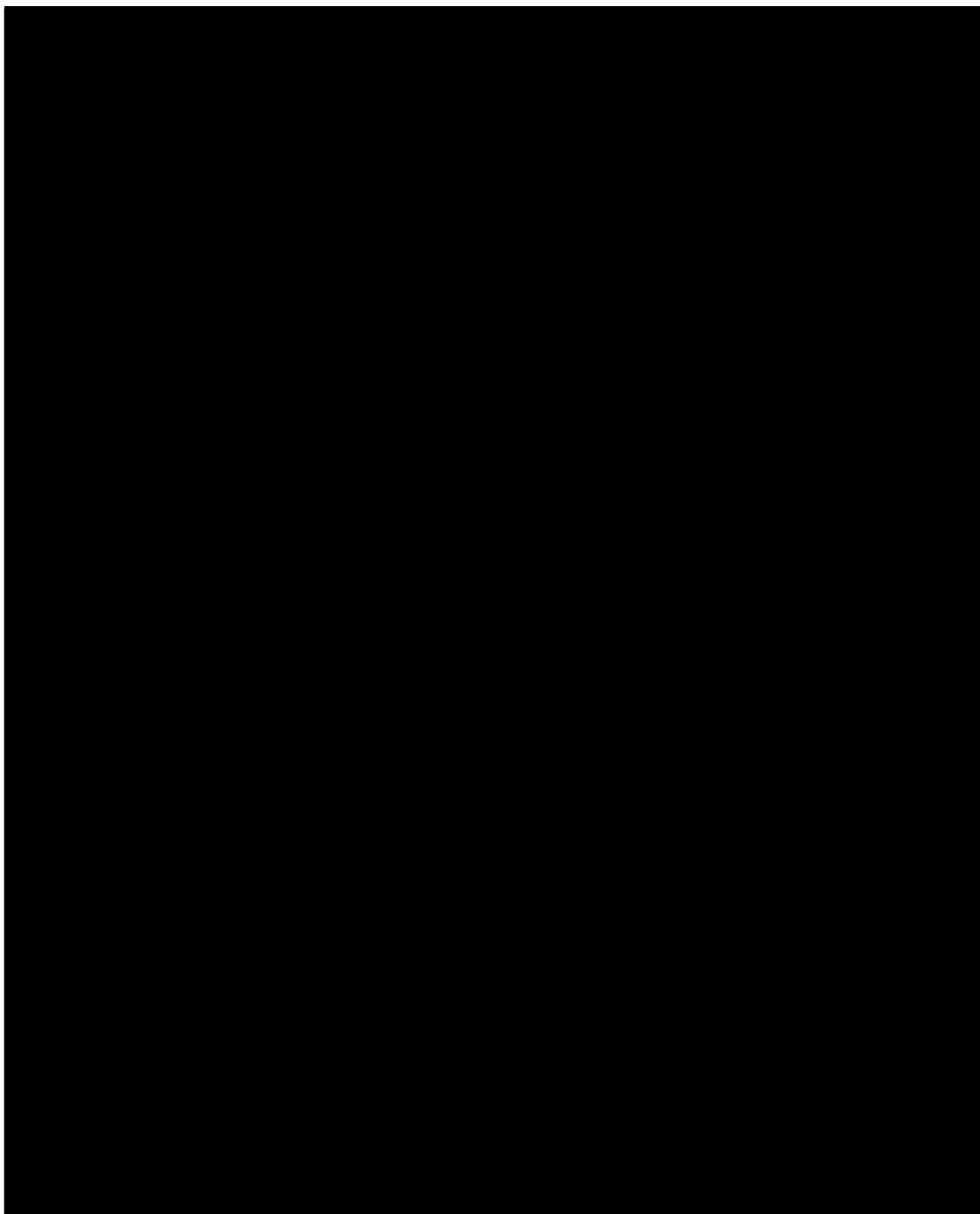


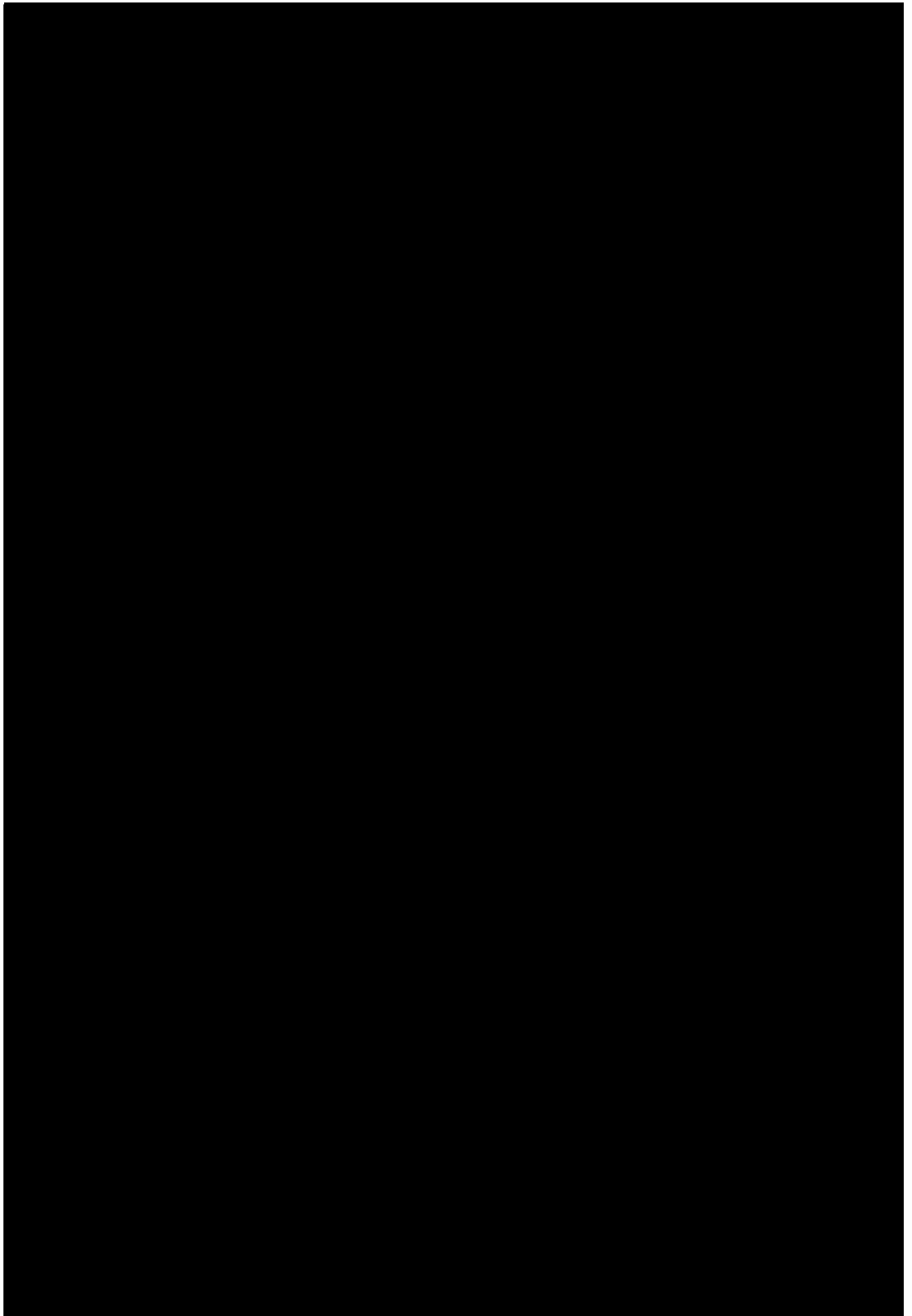
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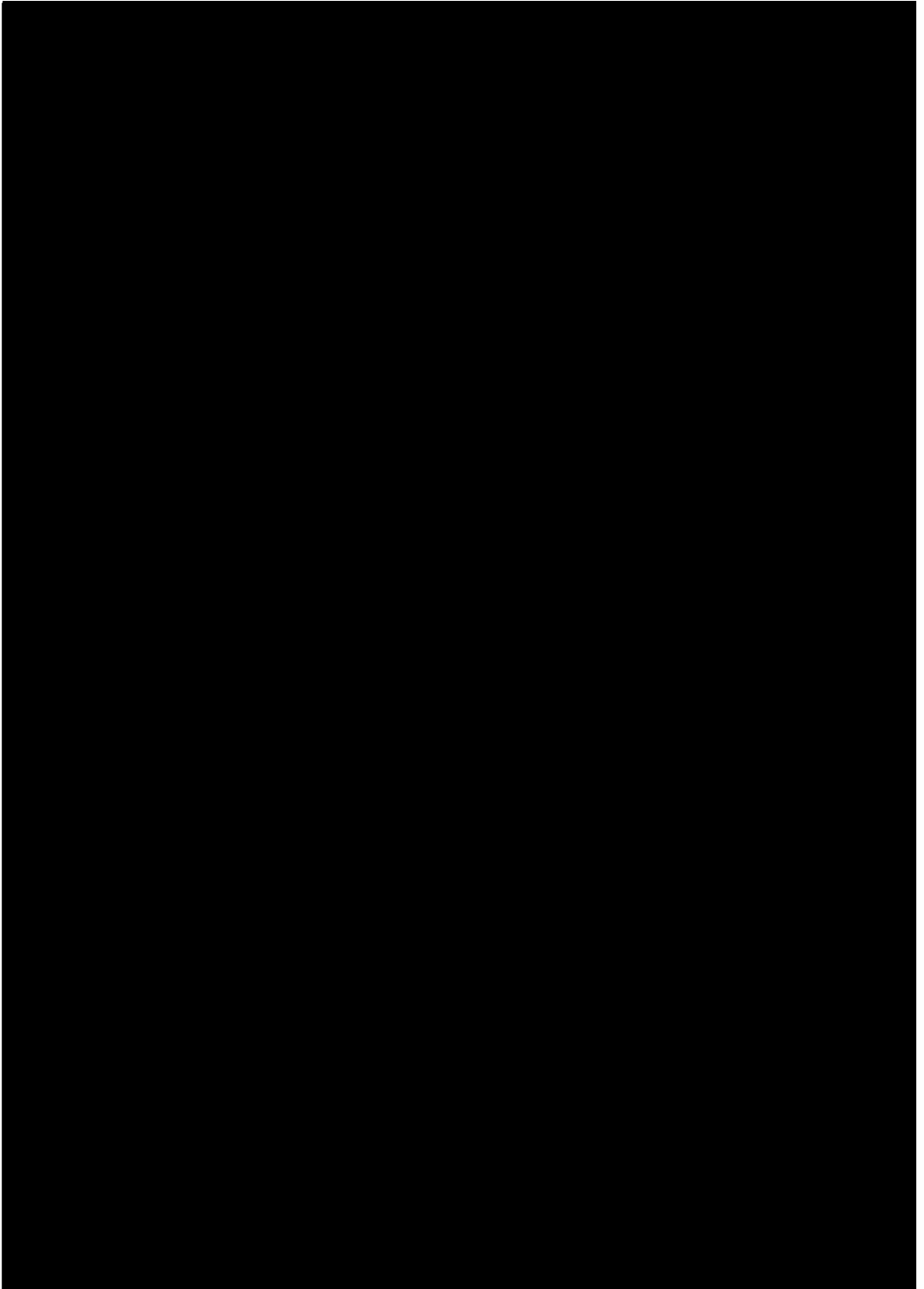


Chairman, MCV Graduate Council, Dean, School of Basic Science

CURRICULUM VITAE







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I would also like to thank the members of my Committee, John Svirsky and Glenn Miller, for their advice and direction during the course of this project. Their help has made my tenure as a graduate student a pleasant learning experience.

It is my strong hope that this project, although only a small seed in the vast field of arthritis research, will blossom through the work of countless others into an ultimate cure for the millions of people who suffer daily pain from arthritis.

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LIST OF ABBREVIATIONS

Ag	antigen
BGG	bovine gamma globulin
BSA	bovine serum albumin
DTH	delayed type hypersensitivity
EA	egg albumin
FCA	Freund's complete adjuvant
FIA	Freund's incomplete adjuvant
HGG	human gamma globulin
HSA	human serum albumin
HSV-1	herpes simplex virus type 1
Ig	immunoglobulin
i.p.	intraperitoneal
LFP	left footpad
mBSA	methylated bovine serum albumin
MCV	Medical College of Virginia
mHGG	methylated human gamma globulin
N.D.	not determined
PFU	plaque forming unit
RFP	right footpad
S.E.	standard error of the mean
U.V.	ultraviolet

INTRODUCTION

History and Incidence

Rheumatism is derived from the Greek word rheumatismos, meaning mucus. It was believed that mucus, an evil humor, flowed from the brain to the joints and other portions of the body, producing pain. It is one of the oldest known diseases in history. Earliest records of its existence are preserved in fossils. Multiple sites of arthritis were seen in plateacarpus, large swimming reptiles dating back to 100,000,000 B.C. (Murphy, G. E., 1943). Chronic arthritis of the spine was noted in the Ape Man of the Pliocene period, 2,000,000 B.C., in the Java and Lansing Men of 500,000 B.C., and in the Egyptian mummies of 8,000 B.C. (Osgood, 1940). At the height of the Roman Empire, 300 A.D., citizens of the day were known to frequent the baths in an effort to cleanse themselves of the pain associated with rheumatism. It was not until the late 1500's that the term rheumatism came into use. The recognition of rheumatism as a clinical entity is generally attributed to Guillaume de Baillou (1538-1616). The term has evolved through the years. In present day medicine, any condition in which pain and stiffness of the musculoskeletal system is prominent is classified as a rheumatic disease. When joints are the major seat of the rheumatic disease, then the term arthritis is applied.

Over thirty million people in the United States suffer from some form of arthritis (Freese, 1978). The impact this crippling disease has on the national economy is reflected in health survey figures which show fifteen million lost workdays per year (Freese, 1978). There is no known cure or prevention for this disease.

Animal Arthritides

Arthritis occurs naturally in both wild and domesticated animals. Because of the economic interest to breeders, research has been done on the pathogenesis of arthritis in several of these naturally-occurring systems. The most notable example is arthritis of swine (Sikes, 1961). Mycoplasma hyorhinis, one of the etiologic agents of swine arthritis, was first recognized by Switzer (1953). Histopathology done on the involved joints of these animals showed chronic synovial membrane changes, cellular infiltration of the synovia, and pathologic bone changes similar to those seen in human arthritis patients (Roberts, Switzer, and Ramsey, 1963). Experimentally, injection of live mycoplasma are also known to produce polyarthritis in mice (Cole, Ward, Jones, and Cahill, 1971), rats (Ward and Jones, 1962), and rabbits (Cole, Griffiths, Eichwald, and Ward, 1977). The exact mechanism of synovial inflammation remains to be determined. With the use of proper cultural techniques, mycoplasma can be recultured from nearly all of these involved joints, even after lengthy intervals, implying an infectious etiology.

Erysipelas Arthritis of Swine

Another extremely interesting form of naturally-occurring arthritis of the swine which has also been induced experimentally is Erysipelas arthritis (Collins and Goldie, 1940). This is a polyarthritic condition characterized by lameness, weight loss, and swollen, deformed joints in the extremities. Histopathologically, the changes seen in these animals are similar to those found in human rheumatoid arthritis. Organisms can be recovered early in the infection when erythematous skin lesions are present, but cannot be isolated during the chronic arthritic stages of the disease. In some cases, the chronically arthritic animals also

manifest a vegetative endocarditis. The causative agent appears to be Erysipelothrix insidiosum, a highly adaptable microaerophilic organism. Some of the interesting aspects of this model experimentally include: (1) the ability to induce the arthritis with injections of non-viable E. insidiosum (Freeman, Segre, and Berman, 1964), (2) the ability to induce it with injections of sterile Erysipelothrix antigens (Freeman and Berman, 1964a), (3) the ability to induce synovitis by intra-articular injection of sterile Erysipelothrix antigen into animals receiving passive transfer of sterile immune serum (Freeman and Berman, 1964b), and (4) the increased severity seen in pigs vaccinated prior to injection of viable organisms (Freeman, 1964).

Clinical lesion resembling those seen in synovium of rheumatoid arthritis patients can also be induced in rabbits by intravenous injection of sterile cell-free aqueous extracts or heat-killed cultures of E. insidiosum (White, Puls, and Mirikitani, 1971). Using fluorescein-tagged anti-Erysipelothrix antisera, specific antigen was found localized in the synovium. It was found to persist at this site long enough to initiate an inflammatory response (White, Puls, and Mirikitani, 1971).

Streptococcal Arthritis

Another model system of considerable importance is the streptococcal cell wall system advanced by Schwab and co-workers. This model system has been established in several animal species. Intra-articular injection of cell wall fragments of Group A streptococci leads to arthritis in rabbits (Schwab, Cromartie, Ohanian, and Craddock, 1967). A single intraperitoneal injection of cell wall fragments of Group A streptococci in mice leads to pancarditis with rheumatic fever-like

cardiac lesions (Ohanian, Schwab, Cromartie, 1969). In rats, intraperitoneal injection of cell wall fragments in forty-eight hours develops into arthritis which undergoes periods of remission and exacerbation (Cromartie, Craddock, Schwab, Anderle, and Yang, 1977). Whole heat-killed streptococcal cells injected intraperitoneally also lead to arthritis in the rat, but with a much lower incidence that requires a longer latent period. Immunofluorescence of articular tissues of these rats showed C-polysaccharide antigen of Group A streptococci persisted up to 63 days after injection (Cromartie, Craddock, Schwab, Anderle, and Yang, 1977). The presence of this group specific carbohydrate side chain is required to induce arthritis (Schwab, Cromartie, and Roberson, 1959) and the chronicity of the lesion seems to correlate with the persistence of the peptidoglycan-polysaccharide complex in macrophages in situ (Ohanian and Schwab, 1967).

Adjuvant Arthritis

Over the last 25 years much work has been done on adjuvant-induced arthritis. This experimentally induced arthritis model is only seen in rats and susceptibility differs in the various strains (Kohashi, Pearson, Beck, and Alexander, 1977). The classic method for inducing adjuvant arthritis is by a single injection of Freund's complete adjuvant administered intradermally (Pearson, 1956).

Freund's complete adjuvant is an emulsion of an aqueous suspension of killed mycobacteria and mineral oil. The arthritogenicity of this compound has been studied extensively. It appears that activity lies in Wax D, a complex peptidoglycolipid extracted from the mycobacteria cell wall. Further subfractionation shows that the amino acids of the peptide chain bear the activity (Wood, Pearson, and Tanaka, 1969).

Arthritis appears in the rat 12 to 14 days post-injection. The intensity of the reaction has been shown to wax and wane for weeks. The response may be passively transferred with immune, lymph node or spleen cells, but not with immune serum (Pearson and Wood, 1964). It has been hypothesized that adjuvant disease is the result of a delayed hypersensitivity reaction to disseminated antigenic constituents of the injected mycobacteria.

Conclusions from animal arthritides

Erysipelothrix arthritis, streptococcal cell wall induced arthritis, and adjuvant-induced arthritis are all laboratory models of chronic, recurrent synovitis. The histologic changes seen in the joints of these animals mimic those of the human disease. Scientists doing human and animal research on arthritis have long been searching for an infectious organism which would fulfill Koch's postulates, thus establishing a causal relationship between a specific microorganism and rheumatoid arthritis. That relationship is not seen in these animal models, and the literature to date on human research is equivocal.

It has been postulated that the bacterial factors involved in the etiology of rheumatoid arthritis may be the persistence of non-biodegradable microbial cell wall components (Hadler, 1976; Ginsburg, 1977). This postulate offers a plausible explanation for the inability to identify an infectious causative agent from arthritic sites. To evaluate this explanation more thoroughly, a close look at the literature on antigen retention in joints and on collagenous surfaces is warranted.

Fate of Injected Antigen

Some of the earliest animal work concerning the relationship between the introduction of simple protein antigens and the appearance

of antibody suggested that rapid antigen elimination from the blood immediately preceded the appearance of detectable antibody (Hawn and Janeway, 1947; Talmage, Dixon, Bukartz, and Cammin, 1951). As techniques improved, however, it was found that small amounts of catabolizable antigen could persist in tissue for long periods of time after the appearance of antibody (Campbell and Garvey, 1963; Nossal and Ada, 1971; Tew, Mandel and Burgess, 1979). These two concepts are now commonly referred to as immune elimination and immune retention. Recent work using a mouse model system reinforces these phenomena. When HSA-immune and non-immune mice were injected with ^{125}I -HSA, the radiolabel was cleared from the liver, lung, kidney, blood and urine of all mice (immune elimination). In contrast, the spleen, lymph nodes, and feet on only the immune mice retained significant radiolabel (immune retention) for long periods of time (Tew, Mandel, and Rice, 1980).

Retained radiolabel has been shown to be a reliable marker for intact antigen in several studies (Cooke, Hurd, Ziff, and Jasin, 1982; Hollister and Mannik, 1974; Tew, Mandel, and Rice, 1980). Using guanidine hydrochloride, the retained radiolabel was eluted from the tissues. The immunologic identity of the eluted radiolabel was then determined. It was found that 88-92% of the solubilized radiolabel could be specifically coprecipitated with antibody directed against the original antigen. The eluted radiolabel was further shown to actually represent intact antigen in its native form (Tew, Mandel, and Burgess, 1979). A portion of the solubilized radiolabel was used for gel filtration chromatography. The peak obtained from the eluted radiolabel was found to be indistinguishable from the peak of native HSA run under identical conditions.

Antigen Half-Life

Reports have been published dealing with antigen retention on collagenous tissues of several animal species. Rabbits, previously immunized with EA or BSA, were injected intra-articularly with the same antigen labeled with ^{125}I . At various time points post-injection, autoradiography was done on tissue sections from the excised joints. The radiolabel found in the synovial fraction decreased rapidly over a six-week period whereas the radiolabel in the collagenous tissue remained constant over the same period (Cooke, Hurd, Ziff, and Jasin, 1972). In later experiments, more precise elimination patterns of the radiolabel were obtained using an external probe. Consistent with the immune elimination theory, for the first five days immune animals eliminated radiolabelled antigen four times faster than normal animals or animals immunized with a heterologous antigen. However, for the duration of the experiment, while very little additional antigen was eliminated by immune animals, between days 10 and 14 control rabbits showed rapid antigen elimination, coinciding with the development of a primary response to the injected radiolabelled antigen. The half-life for intra-articular antigen in this system was calculated to be 20 days (Cooke and Jasin, 1972).

Another group, studying the duration of antigen injected into the knees of immune rabbits, determined the half-life of the injected antigen by counting the radiolabel found in the dissected tissue in an automatic well-type gamma counter. The half-life of the injected ^{125}I -HSA was as follows: meniscus, 141 days; articular cartilage, 31 days; ligaments, 17 days; synovium, 8 days; and synovial fluid, 4 days (Hollister and Mannik, 1974).

Antigen half-life studies were also done in mice by following the kinetics of clearance of ^{125}I -HSA injected in the immune animal. As in the rabbit system, a biphasic retention pattern was obtained. Initially, antigen was cleared rapidly from all sites by macrophages, $T_{1/2} = 2$ hours. However, in the second phase long-term retention of remaining antigen was seen in the lymph nodes and feet. Calculations based on radiolabel retained at the site during the second phase of retention show that the half-life of antigen in the lymph nodes was 8.1 weeks and in the foot was 6.1 weeks (Tew and Mandel, 1979).

Criteria for Antigen Retention in Collagenous Tissues

With reports appearing in the literature that simple protein antigens could be retained in immune animals for long periods of time, interest turned to the basis for this retention. Since it was known that antigen retention occurred only in immune animals, the nature of this immunity was the first area to be investigated. Normal rabbits were passively infused with purified rabbit anti-BSA antibody. Later, ^{125}I -BSA was injected in both the experimental animals and in the control animals. Tissues from the passively immunized rabbits retained significantly more antigen than the control animals (Jasin, 1975). In other passive transfer experiments, specific autologous immune plasma was administered intraperitoneally to normal mice. These mice were then injected in one foot with ^{125}I -HSA. Radiolabel was found in the foot and draining lymph node of the injected side. This is the same pattern of retention that was seen with the actively immunized mice. In addition, the level of radiolabel at these sites is again seen to remain stable for long periods of time (Tew, Mandel, and Miller, 1979). It appears from this work that antigen retention is mediated by antibody alone.

Confirmation of the role of humoral immunity came from studies conducted in the nude mouse. Hyperimmune serum was passively transferred to nu/nu mice. As in the previous experiment, these mice were injected with ^{125}I -HSA and monitored for the quantity of radiolabel retained. Antigen retention in the lymph nodes of these athymic mice was comparable with the levels seen in the passive transfer experiments conducted in normal mice. Interestingly, levels of antigen retention in the feet of nu/nu mice was greater than that seen in either actively or passively immunized normal mice (Tew, Mandel, and Miller, 1979). Thus, it would appear that antigen retention does not require the presence of T-cells or T-cell factors and is only dependent on the presence of antibody.

The nature of this antibody was the next area of major interest. Rabbits were twice immunized with HSA emulsified in FCA. Subsequently, the animals received ^{125}I -labelled HSA or EA intra-articularly to determine the specificity of antigen retention. Radiolabel was only found to persist in HSA-immune rabbits challenged with HSA intra-articularly. It appears, therefore, that the antibody must be directed against the challenging antigen for long-term retention of that antigen (Hollister and Mannik, 1974).

In later experiments, it was determined that antigen retention could take place in vivo (Tew, Mandel, and Miller, 1979) or in vitro (Hollister and Mannik, 1974; Teuscher and Donaldson, 1979), when only the $\text{F}(\text{ab}')_2$ portion of the antibody molecule was present. Consistent with these results, antigen retention was normal in actively or passively immunized mice which have been de complemented with cobra venom factor (Tew, Mandel, and Miller, 1979). It would seem, therefore, that

complement is not necessary to obtain antigen retention in collagenous tissues, although complement is required for binding and retention of antigen in the spleen (Papamichail, Gutierrez, Embling, Johnson, Holborrow, and Pepys, 1975; Klaus and Humphrey, 1977).

Mechanism of Antigen Retention in Collagenous Tissues

Antigen has been shown to be retained for long periods of time on several types of collagenous tissue including meniscus, articular cartilage and ligaments of the knee, and the extensor and flexor tendons of the feet. Electron microscopic radioautography of tendons retaining ^{125}I -HSA indicated that the persisting radiolabel was not cell-associated but was localized on the tendon and on the tendon sheath (Tew, Mandel, and Rice, 1980). Immunofluorescence studies on antigen retained on articular surfaces showed IgG and C3 in close association with the antigen. These results led to the hypothesis that retained antigen persist in the form of antigen-antibody complexes (Cooke, Hurd, Ziff, and Jasin, 1972).

The mechanism of deposition and formation of these immune complexes in collagenous tissues was subjected to further study. In one such extensive in vitro study normal rabbit collagenous tissue from diverse origins including the eye, meninges, kidney, vascular system, skin, muscle, and four types of cartilage were studied. Free antibody and free antigen were found to penetrate all tissues and form immune complexes. It did not matter whether antibody or antigen was presented first. Pre-formed immune complexes, however, are not retained except in the case of muscle (Teuscher and Donaldson, 1979).

These results were verified in vivo by two other groups working with rabbits. Sequentially administering antibody and then antigen into

normal animals produced greater retention of antigen, and the bound antigen was more resistant to washing. Therefore, it appears that in immunized animals antibody first binds to joint cartilage, and then immune complexes form within the cartilage matrix (Hollister and Mannik, 1974; Jasin, 1975).

Consequences of Retained Antigen at Collagenous Sites

In 1962, Dumonde and Glynn reported that they had successfully produced arthritis in rabbits as a consequence of an immunologic reaction to fibrin. Rabbits were immunized with human or autologous fibrin in FCA, and then given a single injection of the same type fibrin intra-articularly. Histology of the knee was followed at various time points. The chronic changes seen in these animals bear a striking resemblance to the synovitis seen in rheumatoid arthritis (Dumonde and Glynn, 1962).

As an antigen, fibrin has several advantages. First, it is naturally-occurring denatured protein, and secondly it is insoluble and will therefore persist locally for a longer amount of time. However, the same general protocol for establishing experimentally-induced arthritis has been repeated in mice as well as in rabbits, using a variety of simple protein antigens such as BSA (Gall and Gall, 1980), mBSA (Brackertz, Mitchell, and Mackay, 1977), EA (Cooke and Jasin, 1972), HSA (Lowther, Sandy, Santer, and Brown, 1978), and BGG (Consden, Doble, Glynn, and Nind, 1971). It has provided researchers with a readily available, easily managed animal model for studying a human disease. Commonly referred to as antigen-induced arthritis, these animals show hyperplasia of the synovial lining, pannus formation, and infiltration of the joint with lymphocytes and plasma cells.

Criteria for Antigen-Induced Arthritis

Much work has been done on the conditions for the development of antigen-induced arthritis. In studies done in mice to determine the specificity of antigen-induced arthritis, animals were immunized with mBSA or mHGG in FCA and then injected intra-articularly with either mBSA or mHGG. Arthritic joint changes were only seen when the same antigen was administered both for immunization and induction. Thus, induction of arthritis in these animals does not appear to show any cross-reactivity (Brackertz, Mitchell, and Mackay, 1977).

In antigen-induced arthritis, animals are commonly immunized with an emulsion of antigen and FCA. In studies using antigen emulsified in FIA for immunization, upon challenge no synovitis developed: only acute inflammation (Menard and Dion, 1976; Fox and Glynn, 1977). However, if these same rabbits were reimmunized with antigen in FCA and then re-challenged, chronic arthritis was seen as usual (Fox and Glynn, 1977). The crucial difference between immunization with FCA as opposed to FIA is the development of delayed type hypersensitivity with FCA. It appears that delayed type hypersensitivity is necessary for subsequent development of chronic histopathology.

The role of cells, specifically T-cells, in the expression of antigen-induced arthritis was reinforced by results obtained in adoptive transfer experiments. Lymphoid cells from mBSA-immunized animals were injected into normal mice. These mice were later shown to express a potent delayed type hypersensitivity reaction as a consequence of the cellular transfer. When these mice were challenged with mBSA, a typical antigen-induced arthritis was seen. If the lymphoid cells to be adoptively transferred were first treated with anti-Thy-1 antibody to

destroy the T-cells no arthritis developed; but if the lymphoid cells were first passed through an anti-Ig column to enrich for T-cells, a much more severe arthritis ensued (Brackertz, Mitchell, Vadas, and Mackay, 1977). The development of antigen-induced arthritis would appear to be a T-cell dependent process.

Finally, it should be noted that antigen-induced arthritis cannot be established in all strains of inbred mice. Studying three strains of mice, it was determined that C57B1/6 and BALB/c are susceptible to arthritis whereas CBA are relatively resistant to arthritis as evidenced by histopathologic joint changes. Hybrid and backcross studies show arthritis to be a dominant trait: one gene linked to the b allele of the H-2 complex of C57B1/6 (Brackertz, Mitchell, Mackay, 1977).

RATIONALE

The cause(s) of arthritis and many other connective tissue diseases are largely unknown. An infectious agent, either bacterial or viral, has not been unequivocally isolated. Theories on the pathogenesis of arthritis abound. Many of these theories implicate the immune system as the problem.

The early information on animal arthritides implicates retained bacterial cell wall antigens as the basis of the arthritis seen. However, numerous types of antigens, both complex polymers and simple proteins, have been shown to persist in immune animals. The antigens persist in the lymph nodes and spleens playing a role in mediating antibody feedback mechanisms. Antigens also persist in collagenous tissues of the knees and feet of these animals. The role of retained antigen at these sites is unknown.

It is possible to induce arthritis in immune animals by administering antigen in the joint. Drawing from the information presented, I chose to explore the role of retained antigen on collagenous tissue in the pathogenesis of rheumatic diseases. This project is the continuation of work begun by J. G. Tew, T. E. Mandel, G. A. Miller, and A. W. Burgess. Through their research, the groundwork of antigen retention on the flexor and extensor tendons of immune mice was reported.

MATERIALS AND METHODS

Animals

Three different strains of mice, CBA/J, BALB/c, and C57B1/6, were used in this project. These strains were chosen for their varying susceptibility to develop antigen-induced arthritis (Brackertz, Mitchell, and Mackay, 1977). The resistant strain, CBA/J, was obtained from the Jackson Laboratories, Bar Harbor, Maine. BALB/c mice, a susceptible strain, were obtained from Flow Laboratories, Dublin, Virginia, and C57B1/6 mice, also a susceptible strain, were obtained from the National Institutes of Health in Bethesda, Maryland. All mice were housed in the central animal facilities at the Medical College of Virginia. They were fed Purina rodent chow and water ad libitum. Within a single experiment, the mice were matched for strain, age and/or weight, and sex.

Immunization

Footpad. Mice were injected with 50 μ l of a 1:1 emulsion of heat-aggregated, Fraction V, HSA (20 mg/ml, Sigma #A2386) and FCA (Difco #06-38-60-7), or, as the case in some experiments, FIA (Difco #06-39-60-6) per footpad. Two weeks later all mice received a second injection as before.

Intraperitoneal. Mice were injected with 100 μ l of a 1:1 emulsion of heat-aggregated HSA (20 mg/ml) and FCA in the peritoneal cavity. Two weeks later all mice received a second injection as before.

Subcutaneous. Mice were injected with 100 μ l of a 1:1 emulsion of heat-aggregated HSA (20 mg/ml) and FCA in the loose skin of the neck. Two weeks later all mice received a second injection as before. In some

experiments, to obtain antigen deposited on the tendon, the mice were injected a third time, two weeks later, in one foot with 10 µg HSA/50 µl saline and in the other foot with 50 µl of saline alone.

Virus. Mice were injected with 50 µl of a 1:1 emulsion of an HSV-1 vaccine preparation (Kitces, Morahan, Tew, and Murray, 1977) and FCA in one footpad. This dose of vaccine represented 10^9 PFU of HSV-1 before inactivation and approximately 1.5 mg of protein. Two weeks later all mice received a second injection of vaccine alone.

Footpad Measurement

The mice to be measured were gently restrained by an assistant to assure the measurer free and unencumbered access to both feet. A Starrett micrometer caliper was used to measure the breadth of each footpad at the widest point on the underside of the foot. Measurements were made in triplicate at the start of each experiment and at several time points during the course of the reaction, generally at 1, 6, 24, and 48 hours after the challenge dose of antigen was administered. The measurements were recorded to the nearest 0.01 mm.

Calculations and Statistics

The mean and standard error of the mean of the three measurements for each foot were calculated. When antigen was retained in both feet as a result of the initial immunization procedure, specific swelling, recorded in footpad units (1 FP unit = 0.01 mm), was calculated using the following formula:

$$\text{Footpad Units} = \frac{(\text{LFP}_{\text{post}} - \text{LFP}_{\text{pre}}) + (\text{RFP}_{\text{post}} - \text{RFP}_{\text{pre}})}{2}$$

To determine specific swelling when antigen was retained in only one foot as the result of the initial immunization procedure, then the following formula was used:

$$\text{Foot pad units} = (\text{FP+Ag}_{\text{post}} - \text{FP+Ag}_{\text{pre}}) - (\text{FP-Ag}_{\text{post}} - \text{FP-Ag}_{\text{pre}})$$

Histopathology

Samples were immediately placed in 50% neutral buffered formalin and stored at room temperature. When tissues were sufficiently fixed, the specific area of interest, usually the footpad, was excised and processed in an autotechnicon. Paraffin sections were stained with hematoxylin and eosin and then evaluated for the presence of inflammatory cells.

The processing, embedding, sectioning, and staining were done by Lerlene Taylor and Doris Wood, histology technicians in the Department of Oral Pathology. The sections were examined and the findings reported to the author by Dr. John A. Svirsky, Assistant Professor, Oral Pathology.

Proline Uptake

Mice immunized in one foot with HSA as described were challenged with 10 μg HSA/50 μl saline in both feet. Immediately following antigen challenge, the mice were injected intraperitoneally with 5 μCi ^{14}C -proline (New England Nuclear #NEC-285-E, specific activity 250 mCi/mmole). One week later the tendons from both feet were removed, dehydrated, weighed, and dissolved in Protosol, a tissue solubilizer (New England Nuclear, #NEF-935), and placed in Econofluor, a compatible scintillation cocktail (New England Nuclear, #NEF-941). The samples

were counted in a Beckman LS-350-T Beta Counter and the results standardized on the basis of weight.

RESULTS

Swelling Response Following Distal Challenge

Animals were immunized in one foot with HSA in FCA, and in the other foot with saline. This has previously been shown to result in antigen retention on flexor and extensor tendons of the injected feet (Tew and Mandel, 1979). The mice were then challenged with 10 μ g HSA in saline administered intraperitoneally. The feet of these mice were measurably swollen within one hour (Figure 1). The swelling response peaked at 6 hours and then began to decline, although at 48 hours, when the experiment was terminated, the feet were still significantly swollen. Control mice challenged with saline or a heterologous antigen did not swell.

In a second experiment, footpad immunized mice were again challenged with antigen administered at a site distal to the site of antigen retention. In this instance a special curved gavage needle with a rounded, bulbous end was passed down the esophagus into the stomach. The antigen was then delivered directly into the stomach and the needle withdrawn. Footpad swelling was monitored as before. Significant swelling, as compared to control animals, was seen 6 hours after antigen challenge (Figure 2). Although the experiment was again ended after 48 hours, the swelling in this instance had not appreciably lessened by that time.

In both experiments, footpad swelling was found to be specifically induced only by challenge with the same antigen used for immunization. Heterologous antigen, EA, when administered in an identical manner, elicited no swelling response. Footpad swelling was also only seen in

Figure 1. The specific induction of footpad swelling by antigen administered intraperitoneally. Three groups of mice immunized in both feet with 1 mg HSA in adjuvant (6 animals/group) were studied: one group (●—●) was challenged with 100 μg HSA in saline i.p.; the second group (□—□) was challenged with 100 μg EA in saline i.p.; and the last group (○—○) received only saline i.p.

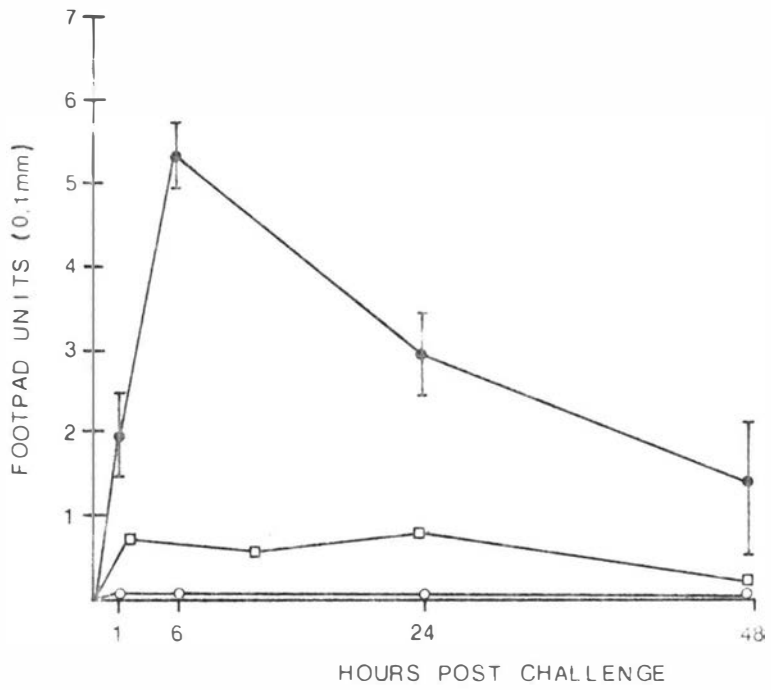
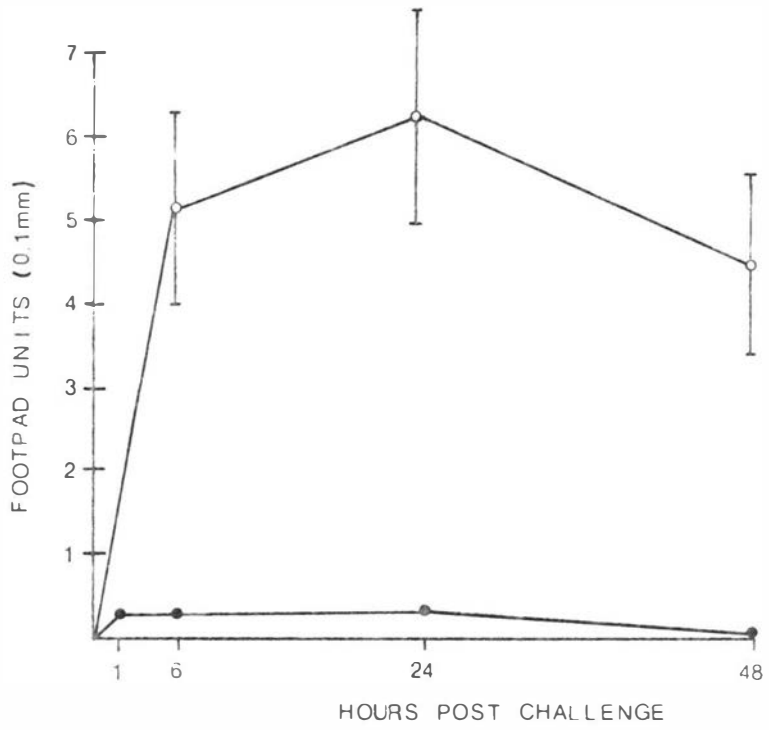


Figure 2. The specific induction of footpad swelling by antigen administered by gavage. Two groups of mice immunized in both feet with 1 mg HSA in adjuvant (6 animals/group) were studied: one group (○—○) was challenged with 100 μg HSA in saline by gavage; the second group (●—●) was challenged with 100 μg EA in saline by gavage.



animals that had antigen retained in the foot from initial immunizations. When normal mice or mice immunized intraperitoneally or subcutaneously with antigen were challenged intraperitoneally with the same antigen, no footpad swelling response was detectable (Figure 3).

Hypersensitivity of Foot Retaining Antigen

Mice were immunized in one foot with HSA as before. A seven-month interval between immunization and challenge was allowed to elapse to reinforce the long-term nature of antigen retention. At the end of this period the mice were injected in both feet with a challenging dose of HSA (10 ng, 100 ng, or 1 μ g). Footpad measurements were taken at 7 and 26 hours post-challenge. At the 10 ng and 100 ng dose, the foot containing persisting antigen swelled measurably; this was apparent at 7 hours post-challenge (Figure 4). In contrast, these low doses either failed to induce swelling or swelling was minimal in the foot primed with saline. At the higher 1 μ g dose, both feet swelled indicating that the unimmunized foot will swell when challenged with a sufficiently large dose of antigen. It appears that antigen retained in the foot serves to make the foot hypersensitive. Newly administered antigen acts additively with the antigen retained at the site to induce an inflammatory reaction in the immune mouse.

Histological Evaluation of Hypersensitive Feet

Early attempts at delineating the histology of footpad swelling ran into problems. It had previously been reported by Fox and Glynn (1977) that animals must exhibit potent DTH to elicit antigen-induced arthritis. The same phenomenon was observed in this mouse model; footpad swelling was only seen when mice were immunized with antigen emulsified in FCA. Freund's complete adjuvant, a mixture of oils and

Figure 3. Absence of induction of footpad swelling in mice lacking antigen retained in the foot. Three groups of mice were studied (6 animals/group): (●—●) immunized twice with 1 mg HSA in adjuvant i.p., challenged with 10 μ g HSA i.p.; (◻—◻) immunized twice with 1 mg HSA in adjuvant subcutaneously, challenged with 10 μ g HSA i.p.; (○—○) non-immune animals, challenged with 10 μ g HSA i.p.

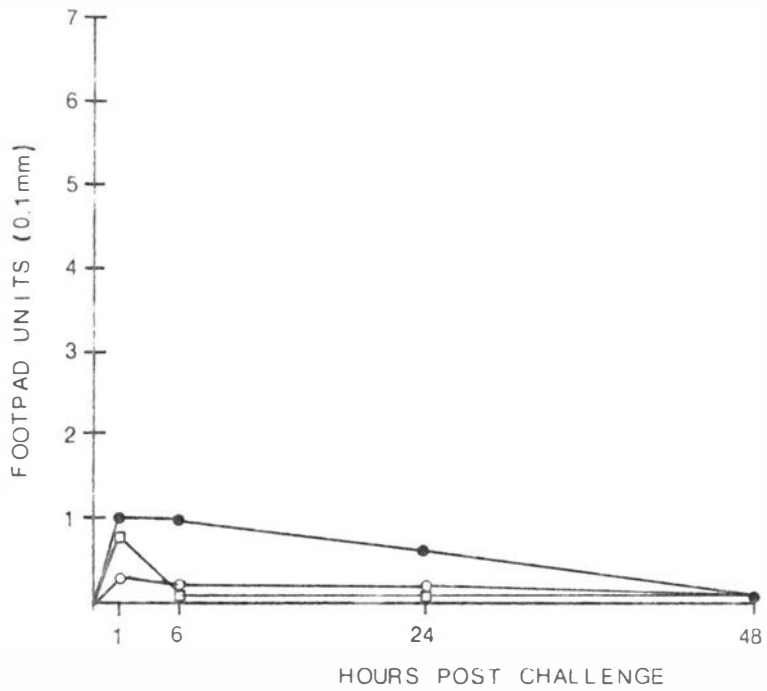
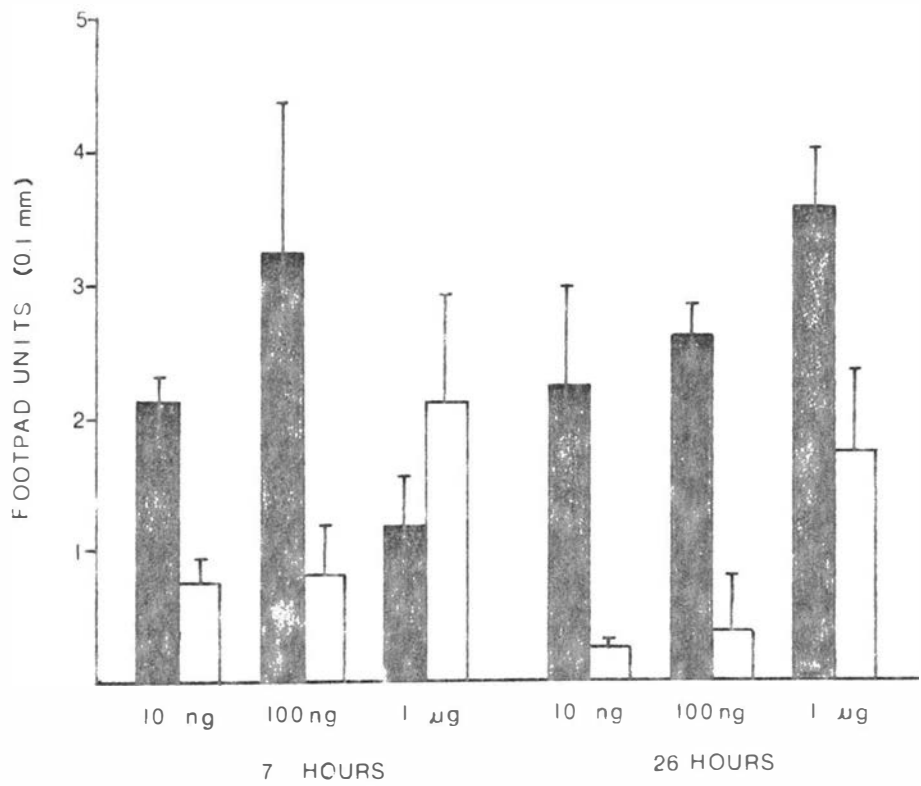


Figure 4. Footpad swelling elicited with nanogram levels of antigen in feet retaining antigen. Mice were primed and boosted with HSA in one hind footpad and were challenged 7 months later by injecting HSA in both hind footpads. Different groups of mice received 10 ng and 1 μ g of HSA in 50 μ l saline. Footpad swelling was measured at 7 and 26 hours post-challenge. The filled bars represent the response in feet used for priming and open bars represent the unprimed contralateral feet.



mycobacterium, was used for its well-documented ability to induce a strong DTH reaction when studying prepared histology sections of swollen tissue; however, it was extremely difficult to clearly distinguish between cells present in the tissue as a result of the DTH reaction and those present as a result of a specific inflammatory response to the inducing antigen.

To circumvent this problem, mice were immunized at a site far removed from the feet. Therefore, in these experiments the antigen-adjuvant emulsion was injected in the loose skin of the neck. A granulomatous nodule indicative of a potent DTH response could be felt at the site after the immunization procedure was complete. Two weeks later HSA in saline was injected in one foot to deposit antigen on the tendons; the other foot, which served as control, received saline. After another two-week period the mice were injected with 10 μ g HSA in both feet. Footpad measurements were taken at 1, 6, 24, and 48 hours (Table 1). By comparison, histology appears to be a more sensitive means of analysis. Paraffin sections of the excised pad, stained with hematoxylin and eosin, showed that the foot retaining antigen progressed from an acute inflammatory response, indicated by the presence of polymorphonuclear neutrophils at 6 hours, to a chronic inflammation characterized by the predominance of lymphocytes and blast cells still present at 48 hours post-challenge. In contrast, the control foot without retained antigen showed only slight edema at 1 hour post-challenge. This was probably the result of trauma incurred as a consequence of the injection. No inflammatory cells were seen in this foot and the edema quickly resolved.

TABLE 1
 Histologic Characterization of Inflammation Seen in the
 Foot as a Result of Direct Challenge with Antigen

Foot with retained Ag ^a	Footpad units	Major cell type ^c	Other cell type	Inflammatory response
1 hr ^b	4.8	none	none	none (some edema)
6 hr	3.8	PMN	some lymphs	acute
24 hr	0.6	lymphs	necrotic PMNs	mixed
48 hr	0.6	lymphs	none	chronic
2 weeks	N.D.	none	few	none

^a Sixteen mice were immunized twice, over a 14-day period, with 1 mg HSA in FCA subcutaneously in the loose skin of the neck. Two weeks later one foot was injected with 10 μ g HSA in saline (foot with antigen retained on tendon); the other foot received saline alone.

^b All animals were challenged with 10 μ g HSA in saline in both feet at start of experiment. Footpad measurements were taken at 1, 6, 24, 48, and 336 hours post challenge. At each time point two mice were killed by cervical dislocation and their feet processed for histology.

^c Paraffin sections of the feet were cut at each time point and stained with hematoxylin and eosin.

Indications of Collagen Remodeling

Other researchers in the field of antigen-induced arthritis have found a difference in susceptibility to the development of the disease in different mouse strains (Brackertz, Mitchell, Vadas, Mackay, and Miller, 1977; Brackertz, Mitchell, and Mackay, 1977). They found C57B1/6 and BALB/c mice were susceptible to antigen-induced arthritis whereas CBA mice were resistant.

In our experience, all three mouse strains (CBA, BALB/c, and C57B1/6) showed the development of an inflammatory state after antigen challenge as a consequence of retained antigen in the foot. However, visual examination of tendons from the CBA mice, even at the peak of the inflammatory response (24 to 48 hours after antigen challenge), did not reveal significant damage. In contrast, at the height of the response the tendons of BALB/c mice and C57B1/6 mice had lost their normal density and organized structure. To assess collagen damage and subsequent repair, proline turnover in the tendons was monitored in both strains of mice. Footpad-immunized CBA and C57B1/6 mice were challenged with antigen only in the left hind foot. The right foot remained as a control. At this time the mice were also injected with ^{14}C -proline intraperitoneally. One week later the tendons were removed, dried, weighed, dissolved, and the ^{14}C present in the tendon was measured. As indicated in Table 2, the ratio of ^{14}C -proline uptake in the left and right tendons of CBA mice was one. This is compatible with the lack of damage seen in the tendon. In contrast, the C57B1/6 mice had twice as much ^{14}C -proline in the inflamed tendon which is compatible with collagen remodeling.

TABLE 2

Effect of Footpad Swelling on ^{14}C -proline Incorporation in
Tendons of CBA/J vs. C57B1/6 Mice

Mouse strain ^a	Ratio of $\frac{^{14}\text{C}\text{-proline in tendon of left foot}^b}{^{14}\text{C}\text{-proline in tendon of right foot}}$
CBA/J	1.00 \pm 0.07
C57B1/6	2.04 \pm 0.05

^a Mice (4/group) were immunized twice, at 14-day intervals, with 50 μg of human serum albumin mixed with Freund's complete adjuvant. Immunizations were in the left foot only. Two weeks later both feet were challenged with 10 μg human serum albumin.

^b Five μCi ^{14}C -proline was injected intraperitoneally at the time of antigen challenge. The flexor tendons were removed one week later.

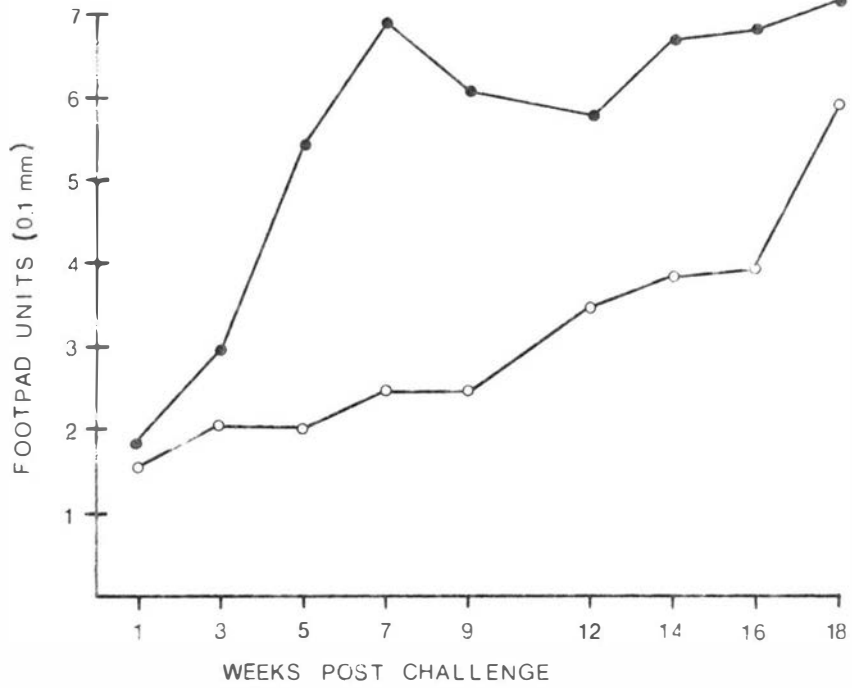
Effect of Repeated Distal Challenge

It appears that tendons which retain antigen are damaged and undergo repair as either the result of the direct interaction of newly administered antigen with antigen already retained in the foot or as the result of the ensuing inflammatory response. Therefore, the effect on the foot of repeated episodes of inflammation, in this case triggered by distally administered antigen was studied.

BALB/c mice were immunized in one foot with HSA in FCA, while the other foot received saline. The mice were challenged biweekly with 100 µg HSA intraperitoneally beginning at week 1. Footpad measurements of both feet were taken immediately prior to administering each antigenic challenge. The foot with retained antigen swelled following the initial antigen challenge (Figure 5) and continued to swell even further following the second and third challenge (weeks 3 and 5). Following subsequent antigen challenge this foot did not swell any further, but remained at this maximal level through week 18. It is very exciting to note that this foot with retained antigen is beginning to show deformative arthritic-like changes at the gross level. The foot itself is puffy; the extensor tendon is visibly taut and has pulled the toes at odd, unnatural angles. The ankle also appears to be involved. Although the joint is still fluid, the ankle is extremely red and swollen. Furthermore, any movement of the ankle seems to cause some discomfort to the animal.

The foot without retained antigen did not swell following the first four challenges (Figure 5). However, at week 12, this foot also began to swell and by week 18 swelled almost as much as the foot with retained antigen. In view of the deformative changes seen in the foot

Figure 5. Continued increases in footpad swelling seen in mice challenged repeatedly with antigen. Twenty BALB/c mice were immunized twice, at 14-day intervals, with 10 μ g HSA emulsified in FCA (LFP, ●—●). The control foot received saline (RFP, ○—○). All mice were challenged biweekly with 100 μ g HSA intraperitoneally. Prior to challenge, footpad measurements were taken. The swelling, in footpad units, was calculated for each foot, and the mean for the group was plotted versus time.

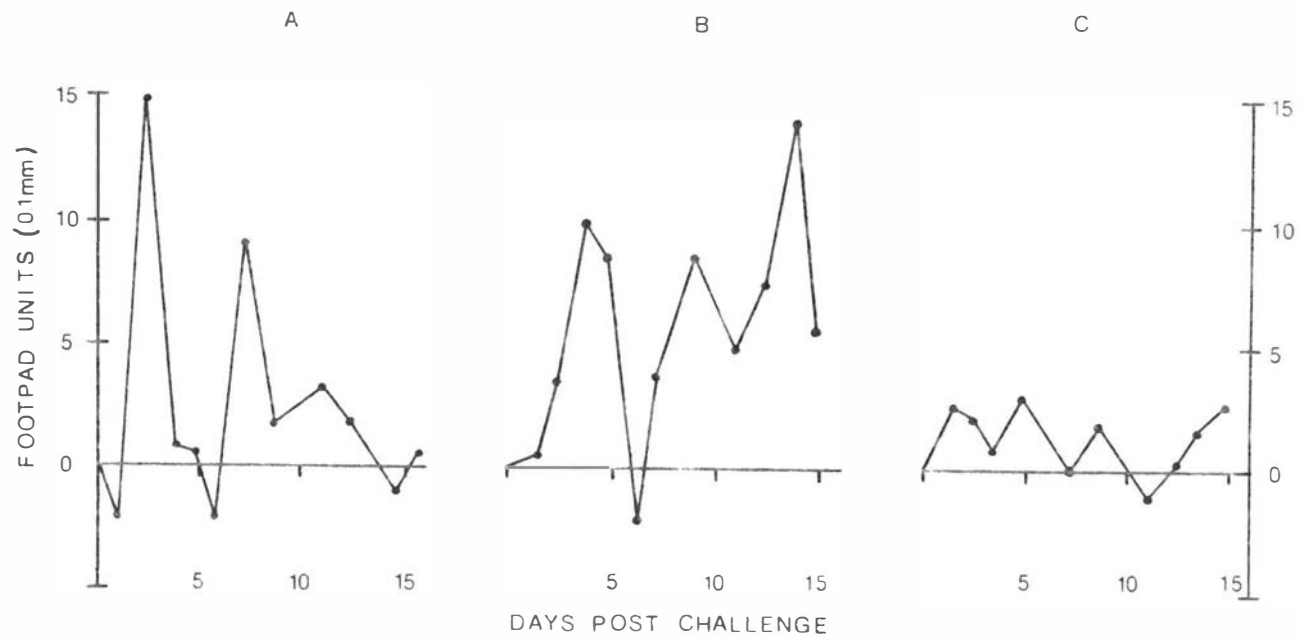


with retained antigen, it would seem entirely possible that, as with human arthritis, the condition is secondarily manifesting itself in the contralateral joint.

Herpes Simplex Virus Model

In this work, mice were routinely immunized twice in one foot with a HSV-1 vaccine that was free of all detectable nucleic acid (Kitces, Morahan, Tew, and Murray, 1977). Ten days after immunization the mice were challenged with live virus on the lip. The main thrust of this work, conducted by Terry Thomson, was directed toward assessing the protective capacity of different vaccine preparations. During the time frame of the experimental protocol it was noticed that the feet of these mice swelled following labial challenge with live virus. Footpad swelling assays, similar to those done in the HSA model but followed over a period of 20 days were therefore carried out. Three general patterns of response emerged with these mice (Figure 6). Thirty-two percent of the mice gave a response similar to that shown in panel A. Unlike the HSA model where swelling occurred within 1 hour, detectable footpad swelling in the HSV system did not become apparent until two to four days after challenge. This corresponds to the peak period of viral replication. After a period of 8 days, the swelling appeared to subside. Another group of mice, 26%, represented by panel B also showed an initial response two to four days after challenge, but these animals seemed to undergo several cyclic recurrences of inflammation which could be due to shedding of live virus from the ganglia. Finally, as can be seen in panel C, there were always some mice, 42%, that did not respond significantly to labial challenge.

Figure 6. Three patterns of footpad swelling obtained in immune mice following lip infection with herpes simplex virus. Mice were immunized twice in one foot, once with a HSV-1 vaccine preparation and adjuvant, and once with the vaccine alone. Ten days later they were challenged with 10^6 - 10^7 PFU of live virus on the lip. Footpad swelling was followed over a 16-day period. Specific footpad swelling calculated and the mean \pm S.E. is shown in footpad units.



The swelling response was found to be associated with labial challenge with live virus. If vaccine-immunized mice were challenged with U.V.-inactivated virus, no appreciable response was seen (Figure 7). Likewise, no swelling was seen when mice immunized with vaccine were challenged with HEp-2 cells, the cell line used to grow the virus for vaccine preparation (Figure 8). Footpad swelling was also found to be associated only with animals that had been immunized with vaccine and presumably had antigen on the tendon since mice immunized with saline and adjuvant and subsequently challenged with live virus showed no swelling (Figure 9). The inflamed feet of representative mice were checked for the ability to grow live virus and none was found. It would seem that the inflammation was not due to an infection in the foot, but was the result of a local hypersensitivity reaction.

Some of the mice in Group B, those undergoing several cycles of inflammation, were observed over a four-month period. At the end of that period, the feet of these mice had undergone gross deformative changes similar to those seen in arthritic patients (Figure 10). Radiographs of the feet, taken with a small X-ray unit in the dental school, were interpreted by Dr. Ann Brower, a faculty member in the Radiology Department at MCV, as showing definite shrinking of the joint space, an early change often seen in arthritics.

Figure 7. Footpad measurements obtained in immune mice following labial challenge with U.V.-inactivated virus. Mice were immunized twice in one foot, once with a HSV-1 vaccine preparation and adjuvant, and once with vaccine alone. Ten days later they were challenged with 10^6 - 10^7 PFU of U.V.-inactivated virus on the lip. Footpad swelling was followed over a 16-day period. Specific footpad swelling was calculated and the mean \pm S.E. is shown in footpad units.

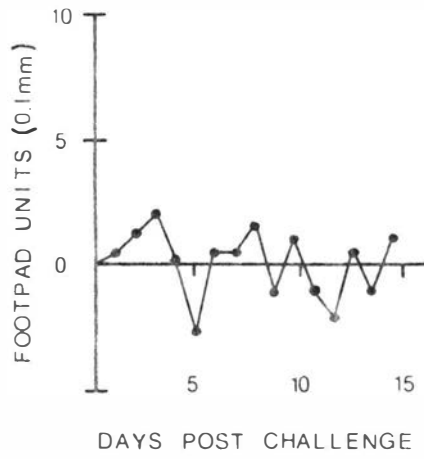


Figure 8. Footpad measurements obtained in immune mice following labial challenge with cell antigen. Mice were immunized twice in one foot, once with a HSV-1 vaccine preparation and adjuvant, and once with vaccine alone. Ten days later they were challenged on the lip with HEp-2 cells, the cell line used in the vaccine preparation. Footpad swelling was followed over a 16-day period. Specific footpad swelling was calculated and the mean \pm S.E. is shown in footpad units.

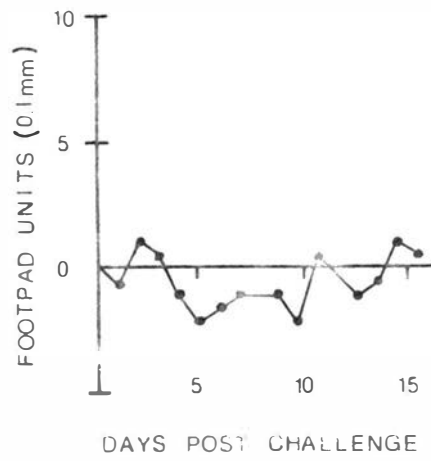


Figure 9. Lack of footpad swelling obtained in mice receiving only adjuvant prior to lip infection with herpes simplex virus. Mice were injected once with saline and Freund's complete adjuvant. Ten days later they were challenged with 10^6 - 10^7 PFU of infectious virus on the lip. Footpad swelling was followed over a 10-day period. Specific footpad swelling was calculated and the mean \pm S.E. is shown in footpad units.

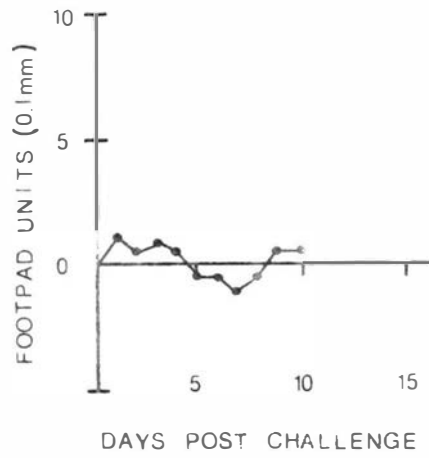


Figure 10. Gross examination of deformative changes seen in a group of mice over a four-month period as a result of repetitive cycles of inflammation. Mice in this group were immunized twice in one foot, once with a HSV-1 vaccine preparation and adjuvant and once with vaccine alone. Ten days later they were challenged on the lip with 10^6 - 10^7 PFU of live virus.

A: Changes after two months post-infection.

B: Changes after four months post-infection. Foot on left side of photograph received injection of vaccine.



DISCUSSION

The results presented in this thesis are an extension of earlier work done by Tew and co-workers on antigen retention. They reported that antigen is retained in the germinal centers of lymph nodes on follicular antigen-binding dendritic cells (Tew and Mandel, 1979; Mandel, Phipps, Abbot, and Tew, 1981). Antigen retained at this site plays a role with specific antibody in a regulatory feedback system which maintains antibody levels (Tew, Self, Harold, and Stavitsky, 1973; Tew and Stavitsky, 1974; Greene, Tew, and Stavitsky, 1975; Tew and Mandel, 1978).

It was also reported by this group that antigen is retained in the feet of animals immunized via footpad injections (Tew, Mandel, and Rice, 1980). The major site of antigen localization is the long flexor tendon near the site of injection. However, the extensor tendon, which is separated from the injection site by the metatarsal bones, also retains significant amounts of antigen.

The role played by antigen retained on tendons and other collagenous surfaces is still being questioned. It has been suggested that the chronicity associated with rheumatoid arthritis is attributable to the persistence of antigen as immune complexes on collagenous surfaces (Cooke, Hurd, Ziff, and Jasin, 1972; Jasin and Cooke, 1978). Immunofluorescence studies conducted on fresh biopsies of hyaline articular cartilage and menisci from patients with classic rheumatoid arthritis show positive staining for human immunoglobulin and complement in 92% of the samples (Cooke, Hurd, Jasin, Bienenstock, and Ziff, 1975). Thus, arthritis could be considered an immune complex disease. The

consequences of this disease is damage at the site of antigen retention and subsequent complex deposition, namely collagenous tissue.

Some researchers feel that bacterial cell wall antigens are retained in the animal as a consequence of a primary infection. They believe that these bacterial cell wall fragments, which contain peptidoglycan, may be perpetuating chronic arthritis (Hadler, 1976; Ginsburg, 1977; Bennett, 1978). Indeed experimentally, chronic arthritis was induced in rats following systemic injection of streptococcal cell wall fragments (Cromartie, Craddock, Schwab, Anderle, and Yang, 1977) and in rabbits following intra-articular injection of streptococcal cell wall (Schwab, Cromartie, Ohanian, and Craddock, 1967). The persistence of bacterial cell wall fragments offers an attractive answer to questions concerning the etiology of rheumatoid arthritis. Considering retained bacterial antigens as the etiologic agent of rheumatoid arthritis not only encompasses the long-lived suspicion of a bacterial origin, but also explains the inability to routinely isolate infectious organisms from arthritic lesions. The incidence of arthritis in swine as a consequence of naturally-occurring E. insidiosum infection when coupled with the experimental data generated using this model firmly reinforces this theory (Introduction: Erysipelas arthritis of swine).

Other researchers, however, question whether persisting antigen of any type can solely account for the chronicity of arthritis. It has been shown that antigen is retained in the immune animal regardless of the adjuvant used for immunization. However, chronic synovitis following antigen challenge only develops in animals showing DTH as a result of immunization with antigen in FCA (Menard and Dion, 1976; Fox

and Glynn, 1977). Glynn (1972), therefore, suggests that classic rheumatoid arthritis is a two-phase disease. Phase one (6 to 12 months) results from antigen retention in the joint. As a result of a local immune response to the retained antigen an inflammatory reaction is seen. He believes that only those patients with a genetic predisposition progress to phase two. Phase two (past 12 months) is believed to result from the development of autoantibodies to determinants exposed during the initial inflammatory phase (Glynn, 1972).

In the system presented in this thesis, histologic examination of the tendons and surrounding tissues from footpad immunized mice did not indicate that retained antigen was mediating any detectable damage. This would be consistent with the view that retained antigen alone is not sufficient to induce arthritis. The experimental data presented, however, indicates that retained antigen has rendered the local site hypersensitive, and therefore subject to episodes of swelling upon challenge with minute quantities of specific antigen (Figure 4). The data further showed that the antigenic challenge could arrive at the site of retention via the circulation from a remote area of the body and still cause a specific inflammatory response (Figures 1 and 2). If animals are repeatedly challenged over a series of months with distally administered antigen, the foot with antigen retained on the tendon swells drastically and remains swollen without signs of significant reduction (Figure 5). The control foot without retained antigen shows little inflammation for the first nine weeks, but then begins to swell. By week eighteen, the response seen in this foot approaches the amount of swelling seen in the foot with retained antigen. Perhaps like the human arthritic condition, our animal model also leads to involvement of the contralateral joint.

The ability to induce inflammation in the foot by antigen administered intraperitoneally or by gavage is a very important finding. This is especially true in light of the number of human diseases of gastrointestinal origin (ten) which are associated with arthritis as a commonly occurring secondary complication (Bennett, 1978). Microbial products from a gastrointestinal, respiratory, or other type of infection could, therefore, serve as a source of specific antigen. Re-infection or even re-exposure to the same organism could then provide the antigenic stimulus to trigger swelling at the site of antigen retention. This sequence of events also explains how episodes of arthritis could occur in the absence of an infection at the inflamed site.

Data has also been presented for what appears to a model for antigen retention and induction of local hypersensitivity with an infectious agent (Figure 6). Challenging mice on the lip with HSV-1 causes inflammation of the feet of those mice which were previously immunized in the feet with a viral vaccine. Admittedly, not all vaccine immunized mice showed an inflammatory response when challenged with HSV-1. However, it is significant that 58% of the mice showed hypersensitivity as measured by footpad swelling. It is also extremely important to note that 54% of the mice showing inflammation of the foot later exhibited joint deformities detectable visually (Figure 10) and on radiographs.

Collagen layers may present a non-phagocytosable surface which protects antigen from phagocytic cells and promotes long-term antigen retention. Although antigen retained as immune complexes bound to collagen are not subject to phagocytosis, they do stimulate

degranulation of neutrophils and subsequent release of bioactive substances (Hawkins, 1971; Ugai, Ziff, and Jasin, 1979). The substances released include the lysozymal enzymes, alkaline phosphatase, β -glucuronidase and lactate dehydrogenase (Henson, 1971).

When antigen is introduced into the feet of animals retaining antigen, the first cellular response seen histologically is the infiltration of PMNs (Table 1). These PMNs could account for the twofold response seen in the foot. It is highly likely that lymphoid cells which appear at the site by 24 hours are in response to chemoattractants released by the PMNs during degranulation. It is also possible that collagen damage and the related proline turnover seen in collagen repair (Table 2) is the result of the action of PMNs or their released products, on antigen retained at that site.

In the HSV-1 studies, looking at the extended effect of antigen retention and repeated cycles of inflammation, arthritic joint damage is seen to develop in the foot (Figure 10). In the HSA system, when the long-term study on the effect of repeated distal challenges is completed, I believe that histology and radiology performed on the feet of these mice will document arthritic pathology. Additionally, serum will be collected from these animals and tested for anti-collagen antibody to determine if the foot without retained antigen became involved as a result of an autoimmune response. It is possible that as a result of sites on the collagen surface repeatedly exposed to the immune system, anti-collagen antibodies are generated. It has been shown in rats (Trentham, Townes, and Kang, 1977) and in mice (Courtenay, Dallman, Dayan, Martin, and Mosedale, 1980) that immunization with heterologous collagen induces arthritis. This correlates nicely with

studies done on patients with rheumatoid arthritis. Ninety-seven percent of the patients exhibited antibody to native and denatured collagen (Andriopoulos, Mestecky, Miller, and Bradley, 1976).

It would seem a reasonable conclusion from the data presented that antigen retained in the foot on collagenous surfaces plays a role in the etiology of rheumatoid arthritis. The antigen can be an easily degradable protein, or a complex antigen of viral origin or bacterial origin. The antigen is most likely absorbed through the gastrointestinal tract as a result of a primary infection at some distal site. Upon re-exposure to the same antigen, a local immune response occurs. The site becomes inflamed, collagen remodeling takes place, and then the response subsides. Following repeated episodes of inflammation, susceptible subjects begin to show damage to the involved joint. Presumably, the damage is the result of an autoimmune response against collagen. So, although retained antigen alone does not appear to cause any damage, it does seem to play a key role in the chain of events leading to the chronic joint degeneration in rheumatoid arthritis.

SUMMARY

The pathogenetic mechanisms responsible for the initiation of rheumatoid arthritis remain obscure. The data presented support a pathogenetic mechanism which could explain many of the peculiar features of rheumatic disease. Antigen retained in collagenous tissue of immune animals renders the surrounding local site specifically hypersensitive. It is likely that the quantity of antigen persisting in the tissue approaches the concentration necessary to trigger an inflammatory response. A minute amount of additional antigen, acting in conjunction with the retained antigen, has been shown to elicit inflammation. The quantity of additional antigen appears to be so small that adequate amounts may be released into the circulation from the gastrointestinal tract or from an infectious process at a distal site in the body. This proposed mechanism could explain recurrent episodes of acute arthritis in the absence of physical trauma or an infectious process at the afflicted site. Furthermore, recurrent episodes of acute inflammation could lead to the development of antibody to host collagen, thereby inducing a chronic degenerative autoimmune disease.

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