Rat Arthritis Due to Whole Group B Streptococci

Clinical and Histopathologic Features Compared With Groups A and D

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Heat-killed streptococci of Groups A, B, and D injected intraperitoneally into Sprague–Dawley rats induced arthritis. The histopathologic features of the arthritis were those of erosive synovitis. Early acute lesions were associated with deposits of streptococcal antigens. The serogroups and the physical state of the streptococci determined the incidence, the time of onset, the duration, and the severity of the disease, the severity being a blend of degree of inflammation, tendency to relapse, and occurrence of ankylosis. Whole Group A usually failed to induce arthritis. Group A disrupted with sonication regularly induced arthritis after a 24-hour latent period. The disease lasted over 60 days and caused ankylosis. Whole Group B regularly induced arthritis but only after a latent period of 6–8 days. The disease lasted over 40 days and caused ankylosed joints. With sonicated Group B a similar disease was induced, except that, as with sonicated Group A, the latent period was 24 hours. Whole Group D induced disease after a latent period of 48 hours. The arthritis lasted only 2 weeks and was transient. In contrast to its effects on Group A and B cocci, sonication of Group D abrogated its capacity to induce arthritis. It is postulated that for whole streptococci, in contrast to sonicated streptococci, arthritogenicity depends on the sensitivity of the cocci to initial processing *in vivo*. Processing may be partial digestion by enzymes of phagocytes. Cocci such as those of Group A that are insensitive to processing, injected whole, tend not to cause arthritis, but when they do cause disease, it is chronic. A coccus, such as one of Group D, that is very sensitive to processing produces a transient arthritis after a short latent period, while a coccus of intermediate sensitivity, such as one of Group B, induces disease only after a substantial latent period, and the disease is severe and chronic. The nature of processing remains to be determined. (Am J Pathol 1983, 112:37–47)

CERTAIN RHEUMATIC DISEASES are supposed to arise because of inflammatory events that happen in two phases.[1] The first phase is acute and primarily due to nonspecific inflammatory responses. The second phase is more chronic and may depend on immunologic responses. The first phase triggers the process. The second phase perpetuates it. The triggering event may depend on microbial infection.[2,3] The second phase may depend on continued presentation of microbial antigen displayed on host cell membranes, on host antigen cross-reactive with microbial antigens, or on other ill-defined mechanisms. Sonicated Group A streptococcal cell walls appear to provide the necessary trigger in experimental arthritis in rabbits and rats.[4–6] Muramyl dipeptide, a biochemically defined structure of microbial cell walls provides an arthritogenic component in adjuvant arthritis.[7] Collagen may act as the endogenous antigen[8] in experimental arthritis. It was our purpose to explore the mediation of arthritis in an experimental model based on triggering due to microbial antigen(s) in whole bacteria.

Properties of Group A cell wall fragments critical in induction of experimental inflammation and arthritis are physical size (ranges from 5.3×10^6 to 500×10^6 molecular weight), the inclusion of a complex of group carbohydrate and peptidoglycan,

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and the capacity of the complex to resist digestion by host enzymes or microbial enzymes.[4,9-12] The pathogenic requirements that Group A streptococci resist digestion and that at the same time the peptidoglycan–carbohydrate complexes be presented in fragments seem paradoxical, because under conditions of infection the enzymes of the host or of microbial cells would clearly have to create the fragments. This paradox raises questions concerning the attributes of a bacterial agent that would be likely to cause arthritis as a result of naturally occurring infection. Could it be both digestible and indigestible? The facts suggest that this agent would have cell walls at the same time partially sensitive and partially resistant to degradation by host enzymes. Moreover, the wall structure of such an organism would necessarily ensure that partial degradation produced the necessary particle sizes with essential arthritogenic moieties intact and suitably exposed to host cells and tissues. Organisms with this kind of partial digestibility, injected whole, would be expected to produce arthritis comparable to the arthritis due to sonicated Group A cell walls. With one exception, such an organism has not been investigated. Cromartie et al[6] showed that a typeless mutant of whole Group B streptococcus, heat-killed, and injected intraperitoneally readily induced arthritis in rats. Whole Group A streptococci did this only infrequently and with a latent period of several weeks.

In this paper we report that while streptococci of Groups A, B, and D all possess structures able to induce arthritis, they differ substantially in their capacity to do this when injected whole intraperitoneally into rats. We characterize here the arthritis induced by these cocci. With Group B we relate the onset of inflammation to deposition of antigens in the periarticular soft tissues.

Materials and Methods

Animals

Outbred Sprague–Dawley rats (100–150 g, female) were purchased from Zivic-Miller Laboratories, Inc. (Allison Park, Pa) and from Charles River Breeding Laboratories (Wilmington, Mass). Wistar rats were purchased from Harlan–Sprague-Dawley (Indianapolis, Ind). Fischer 344 rats were purchased from Charles River Breeding Laboratories.

Bacteria

Streptococcus agalactiae (Group B streptococci) Strains 090R (typeless), 090 (Type Ia), H36b (Type Ib), A909 (Type Ic), 18RS21 (Type II), and D136c (Type III) were kindly provided by Dr. R. Lancefield (Rockefeller University). Group B Strains 509-80 (Type Ia), 1135-80 (Type Ia), 603-79 (Type III), and 606-79 (Type III) were provided by Dr. R. Facklam (Centers for Disease Control, Atlanta, Ga). *Streptococcus pyogenes* (Group A streptococcus) Strain D58 and *Streptococcus faecalis* (Group D streptococcus) Strain F24 were provided by Dr. J. Schwab (University of North Carolina). Group C *Streptococcus*, Strain 55957, was kindly provided by Dr. H. Wilkinson (Centers for Disease Control, Atlanta, Ga). Bacteria were cultured in Todd–Hewitt broth (BBL, Cockeysville, Md) for 18 hours at 37 C, harvested, and washed three times in phosphate-buffered saline (PBS). Bacterial suspensions (15% wt/vol in PBS) were heated at 60 C for 30 minutes. Heating was repeated until all bacteria were killed, as shown by no growth when inoculated onto sheep blood agar plates.

Sonicates of Whole Cells

These were produced as previously described.[6] Briefly, bacterial suspensions (15% wet wt/vol in PBS) were sonicated 90 minutes at a power setting of 8 on a Heat Systems Model 220F sonicator (Heat Systems Ultrasonics, Inc., Plainview, NY) in a sealed atmosphere chamber (Heat Systems). Cells were continuously cooled to 4 C. We centrifuged sonicated material at 3000*g* for 15 minutes to remove sonicator tip debris and any unfragmented bacterial material. Sonicated material that passed through a 0.45-μ filter (Millipore Corp., Bedford, Mass) provided whole cell sonicate (WCS).

Purification of Cell Wall Fragments

Purified cell walls were prepared from the 15% suspensions of bacteria by cell breakage with glass beads in a Braun Cell Homogenizer MSK (VWR Scientific, Atlanta, Ga).

Centrifugal separation of cell walls and sequential enzyme digestions (4 hours at 37 C each) of lyophilized crude cell walls with ribonuclease (0.025% wt/wt) and trypsin (0.025% wt/wt) were performed as described by Anderle et al.[13] Briefly, glass beads were removed at 1*g*, and the supernatant was centrifuged (10,000*g* for 30 minutes). The top layer of the sediment provided crude cell walls. Washed four times with PBS, this fraction was lyophilized and was the crude cell wall fraction. Crude cell walls (10 mg/ml) were further purified, as suggested by Dr. John Schwab (University of North Carolina), by treatment

with papain (Type IV, Sigma Chemical Co., St. Louis, Mo; 0.025 mg/mg cell wall) in 0.1 M phosphate buffer, pH 7.0, with 0.001 M cysteine and 0.001 M EDTA at 37 C for 4 hours. Cell walls were washed three times in distilled water and extracted three times for 30 minutes with chloroform–methanol (2:1). The cell wall pellet after centrifugation was washed six times in distilled water, and the lyophilized product was termed "purified cell walls." We conducted all procedures aseptically, and cell wall preparations were sonicated for 30 seconds (at a power setting of 6 with a microtip for a Model 220F sonicator) to assure complete dispersion of cell walls during enzyme treatments and washes. Purified cell wall fragments were prepared by suspension of purified cell walls in 40 ml PBS (10 mg/ml) followed by sonication as described for whole cells but for 70 minutes. Material passing through a sterile 0.45-μ filter was employed as purified cell wall fragments. All cell wall preparations failed to produce bacterial growth on sheep blood agar plates.

Rhamnose Assay

Doses of cell suspensions and cell wall fragments or whole cell sonicates were compared on the basis of their rhamnose content, assayed by the method of Dische and Shettles,[14] as established by Schwab et al.[9]

Scoring of Arthritis

Etherized rats were given intraperitoneal injections of sterile cell preparations. Rats were monitored daily for gross joint inflammation, and each extremity was scored from 0 to 4 for disease in joints distal to the knees and elbows on the basis of the scoring system for adjuvant arthritis in the rat developed by Wood et al.[15] The maximum score a rat could receive was 16, the sum of the maximum scores of 4 for each extremity.

Sources of Tissue for Histologic and Immunohistologic Examination

Doses of unsonicated, heat-killed Group B streptococci known to produce arthritis in approximately 50% of rats given injections were administered intraperitoneally. At selected intervals rats with clinically inflamed joints were sacrificed. An animal from the group given injections but without clinical inflammation was chosen at random (table of random numbers used) and sacrificed at the same time. In each experiment animals not given injections also provided similarly collected tissues as additional controls. In other experiments rats were given intraperitoneal injections of sonicated Type III Group B streptococci and then selected for sacrifice as described for rats given injections of whole streptococci.

Histologic Methods

Animals sacrificed were perfused via the heart with 50 ml PBS followed by 50 ml of 10% neutral buffered formalin. Rat limbs were removed with the skin intact and fixed along with selected soft tissues and organs for an additional 24 hours at 4 C in 10% neutral buffered formalin. All tissues were placed in PBS at 4 C for 48 hours. Tissues without bones were dehydrated and embedded in paraffin. Bony tissues were decalcified for 4 hours in RDO (DuPage Kinetic Laboratories, Naperville, Ill) and washed in PBS for 4 hours before embedding in paraffin. To assure sufficient infiltration of ankles by paraffin, we removed the skin from one side of the joint to be embedded. Embedded in paraffin blocks (Tissue-Tek II), the tissues were sectioned at 6 μ with a Leitz 1400 base sledge microtome. Sections were mounted on glass, deparaffinized by passage through xylene, absolute ethanol, and 95% ethanol, then rehydrated in deionized water prior to staining. Histologic methods were guided by personal communication from Dr. Peter Petrus of the Department of Anatomy of the University of North Carolina.

Histologic Stains

Sections were routinely stained with hematoxylin and eosin (H&E).[16] In addition, stains were done with Masson's trichrome technique, Gram stains (Brown and Brenn), periodic acid–Schiff, and toluidine blue, according to standard methods.[16] In addition to sections of joints, sections of spleen, liver, heart, lung, thymus, brain, and lymph nodes (inguinal) were prepared.

Immunofluorescence

Deparaffinized tissues were treated with homologous antiserum prepared in rabbits against Type III Group B streptococcus. Both direct and indirect techniques were used. Immunoglobulins partially purified by precipitation with saturated ammonium sulfate at 4 C, followed by dialysis against PBS, and conjugated to fluorescein isothiocyanate with published procedures[17] provided group- and type-specific immunofluorescent reagents. Control sections were prepared by staining with immunoglobulin from preimmuniza-

tion bleedings or immunoglobulin absorbed with appropriate antigens. Sections were mounted in n-propylgallate in PBS–glycerol with 0.5% paraformaldehyde after staining for preservation of the section and reduction of quenching.[18,19] Sections were viewed and photographed with a Leitz Orthoplan microscope equipped with Ploem illumination and a Vario-Orthomat camera. The microscope was equipped with the following filters for fluorescein conjugates: KP490/TK510 and K515.

Immunoperoxidase staining was done by the method of Sternberger.[20] Briefly, immune serum or ammonium-sulfate–fractionated crude immunoglobulins were used as first antibodies. The second antibody was a peroxidase-conjugated IgG fraction of goat anti-rabbit immunoglobulin (anti-IgA + IgG + IgM) obtained from Cappel Laboratories, Cochrane, Pa. Appropriate controls verified the specificity of the reagents and showed that fixation and processing of tissues did not interfere with immunocytochemical reactions. The sections were photographed on Kodachrome with a Leitz Orthoplan microscope equipped with a 3400°K tungsten lamp and an Vario-Orthomat camera. Preliminary screening and study of all sections was done by an individual who had no knowledge of the prior treatment or clinical condition of individual animals.

Antiserum

A vaccine of Type III Group B *S agalactiae* provided the immunizing antigen. The organisms were adjusted to 15% (wt/vol) and killed by heating (60 C for 30 minutes). We injected 0.5 ml of this into a 2-kg female albino New Zealand rabbit after a preliminary bleeding (serum S-49-0) was made. Six weeks later the animal received a series of 1-ml intravenous injections until a series of 12 injections had been made. The rabbit was again bled 3 months (Serum S-49-3) after the initial bleeding. Crude immunoglobulins were prepared by precipitation with half-saturated ammonium sulfate at 4 C. Both pre- and postimmunization immunoglobulins were conjugated to fluorescein isothiocyanate. The resulting protein to fluorescein ratios were approximately 1:3. The conjugates were adjusted to a concentration of 2.0 mg protein/ml. The postimmunization immunoglobulin gave just detectable staining with homologous bacteria and bacteria of the same serotype at 0.04 mg protein/ml. Similar staining was obtained with spleen macrophages and granulocytes only at 2.0 mg/ml. Hence, the ratio of nonspecific (spleen macrophage/granulocyte) staining to specific (*S agalactiae* antigen) staining was 50. Specific absorption studies showed the serum contained antibodies to Group B antigen, Type III antigen, and chitin (poly-N-acetyl-glucosamine). For immunoperoxidase staining (see above) S-49-3 and the preimmunization bleeding were used as first antibody; S-49-3 detected antigen at dilutions in excess of 1:500.

Results

Induction of Arthritis With Whole Heat-Killed Streptococci: Comparison of Serologic Groups

We have reexamined the arthritogenic capacities of whole Group B streptococci and compared them with the capacities of whole Group A and whole Group D streptococci. We found (Table 1) that suspensions of whole, heat-killed (WHC) Group B and D streptococci readily induced joint inflammation following intraperitoneal injection. In contrast, Group A organisms produced only a low level of inflammation in 1 of 20 rats several weeks after injection. Whole Group B streptococci induced a dose-related arthritis with a delayed onset (mean latent period, 7 days). With the optimum dose the incidence was usually at least 80%. Doses of 80 μg rhamnose/g rat often produced a somewhat lower incidence of arthritis than did the optimum, 60 μg rhamnose/g rat. The reasons for this are under investigation. In contrast to Serogroups B and A, whole Serogroup D streptococci produced a high incidence of early-onset inflammation (mean latent period, 48 hours), but the response was also of much shorter duration (Figure 1C and D) than responses to B (Figure 1A and B) or A (results are not shown because they have been published previously[6]). Whereas the maximum severity of acute inflammation was comparable between groups of rats given injections of Group B or Group D streptococci, prolonged joint damage with ankylosis commonly ensued only in rats given injections of Group B streptococci.

Comparison of Group B Serotypes

Because evidence suggests that the causative agent of experimental streptococcal inflammation is a complex of carbohydrate and peptidoglycan,[12] we compared the arthritogenicity of different serotypes within Group B. The five serotypes are known to have different pathogenic potential.[21] Arthritogenicity of whole heat-killed Group B streptococci was most readily demonstrated with Serotype III strains (Table 2). The typeless strain, 090R, was also arthritogenic, as previously reported,[6] suggesting that the type antigen was unnecessary for arthritogenicity.

Table 1—Arthritis Induced With Whole Heat-Killed Streptococci of Different Serogroups*

Serogroup	Dose†	Arthritis incidence‡	Mean day§ onset	Mean severity‖	Presence of ankylosis (±)¶
A	80	1/5	29	2	–
	60	0/15	–	–	–
B	60	8/12	6	5	+
	40	15/28	8	7	+
	30	3/9	6	5	+
D	80	3/4	3	4	+
	60	4/5	2	7	–
	30	4/5	2	8	–

* Group A, Strain D58; Group B, Strain 603-79; Group D, Strain F24.

† Micrograms coccal rhamnose injected intraperitoneally per gram rat. Rhamnose values for Group A 7 μg/mg cells (wet wt); Group B, 6 μg/mg wet wt; Group D, 2.5 μg/mg cells wet wt.

‡ Arthritis incidence per rats injected.

§ Mean of the first days of arthritis for all rats. The experiment was maintained for 50 days.

‖ Mean severity calculated as the arithmetic mean of the highest scores of each positive rat.

¶ Presence of ankylosis in any rat following injection and lasting until the rat was sacrificed. Ankylosis was judged as 75% loss of joint movement in the absence of active inflammation.

The Type Ib strain (H36b) produced arthritis but only at high doses of bacteria. The lethal toxicity associated with serotypes Ia, Ic, and II strains interfered with determinations of their arthritogenic potential in rats. Whereas some strains (603-79) exhibited both toxicity and arthritogenicity, strains 090R and D136c were nontoxic, as compared with the other strains, but were still highly arthritogenic. Thus toxicity and arthritogenicity were dissociable properties. Although the precise contribution of serotype antigens to inflammatory potential is unclear, our data suggested that significant differences in arthritogenicity existed between strains and perhaps between serotypes of Group B streptococci.

Kinetics of Appearance and Duration of Group B and D Streptococcus-Induced Experimental Arthritis

The onset of Group B streptococcus-induced arthritis occurred about 7 days after the streptococci

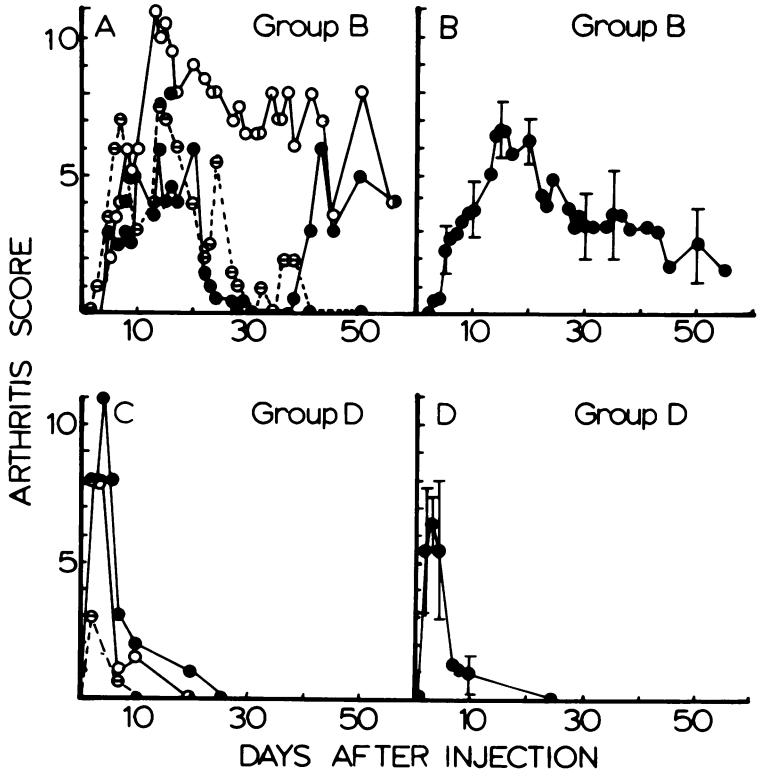

Figure 1—Time course of clinical symptoms in rats given injections of whole heat-killed Group B or Group D streptococci. **A**—Scores of 3 individual rats given 60 μg rhamnose (Group B, Strain D136c) per gram rat are plotted to show individual variation, delayed onset of disease, and occurrence of exacerbations of disease. **B**—Mean scores ± SEM of 6 rats given 60 μg rhamnose (Group B, Strain D136c) per gram rat are plotted to show average responses. **C**—Scores of 3 individual rats given 60 μg rhamnose (Group D, Strain F24) per gram rat are plotted to show individual variation, acute response, and rapid disappearance of inflammation. **D**—Mean scores ± SEM of 4 rats given 60 μg rhamnose (Group D, Strain F24) per gram rat are plotted to show the average response.

Table 2—Induction of Arthritis With Whole Heat-Killed Group B Streptococci of Different Serotypes

Serotype	Strain	Dose*	Arthritis incidence†	Severity‡
Ia	1135-80	80	0/2	–
		40	1/8	1
Ib	H36b	80	3/5	4
		60	0/9	–
Ic	A909	80	NE§	–
		60	NE§	–
II	18RS21	80	NE	–
		60	NE	–
III	D136c	80	4/5	4
		60	12/14	6
		40	6/12	6
		20	2/5	1
		10	0/5	–
	603-79	60	8/12	5
		40	15/28	7
		10	0/10	–
Typeless	090R	60	5/8	7
		60	6/9	6

* Micrograms coccal rhamnose injected intraperitoneally per gram rat. The rhamnose content in the different preparations ranged from 5.5 to 6.0 μg/mg cells by wet weight.

† In several instances the injected streptococci proved to be toxic, and the rats died within 24 hours of injection. Toxicity thus precluded evaluation of the arthritogenicity of these strains (Ic and II). The toxicity is being described in a separate publication. The important point here is that toxicity and arthritogenicity appeared uncorrelated.

‡ Severity was calculated as for Table 1.

§ Not evaluated.

had been injected into the rats (Figures 1A and B). Initial inflammation involved primarily the ankles of both feet. Eventually the wrists and distal joints of all limbs were affected. No lesions occurred on the tail, skin, or ears. Grossly detectable inflammation reached its maximum 2 weeks after injection of streptococci. Arthritic scores gradually decreased over a period of 50–60 days. Relapses of active inflammation appeared most often in the digits of the forepaws 30–40 days after injection. Individual rats varied considerably in the time of onset, in the severity of inflammation, and in the course of the disease (Figure 1A). Some rats showed severe prolonged periarticular edema and erythema; whereas in other rats erythema, edema, and pain subsided by 4 weeks, leaving marked ankylosis and loss of joint function, primarily in the ankle joint.

The onset of arthritis following injection of whole Group D streptococci (Figures 1C and D) was more rapid (latent period, 48 hours) than the onset after Group B, and inflammation was evident for only a short time (1–2 weeks). Permanent joint damage failed to appear except in a few rats given injections of the highest doses of cocci we employed. Disease due to Group D thus was brief and did not recur.

Induction of Arthritis With Sonicates of Group B Streptococci

The differences in time of onset and duration of arthritic response to whole Group A, B, and D streptococci may have reflected the relatively low initial rates with which they could activate complement *in vivo*[22] and the capacity of host enzymes such as lysozyme to enhance and eventually to abrogate these interactions by digesting the cocci, first partially and then later totally.[23–28] To examine the possibility that host enzymes were activating the cocci by partially digesting them, we gave the rats injections of bacterial suspensions that had been subjected to sonication to see whether this would reduce the lag period for induction and to see whether, weight for weight, the preparations would be made more active or less active. Injection of sonicated whole Group B streptococci or isolated, purified, and sonicated Group B streptococcal cell walls induced an acute, severe polyarthritis (Table 3) in 100% of the rats even with doses equivalent to 5 μg rhamnose/g body weight. Sonicates therefore induced disease at doses of total dry weight of bacterial substance one-tenth those required with whole cells, indicating that, once fragmented, Group B streptococci were far more arthritogenic. The isolated cell wall fraction of whole cells was the only fraction with the capacity to induce prolonged joint disease. With purified sonicated cell walls, 30 μg rhamnose equivalent was required to induce arthritis in 100% of the rats, as compared with 1–5 μg rhamnose of whole cell sonicates.

The patterns of arthritis induced in rats with sonicated Group B streptococcal cell walls (Figure 2,

Table 3—Induction of Arthritis With Whole Cells, Sonicated Whole Cells, and Sonicated Purified Cell Walls of Group B Type III Streptococci

Material injected	Dose*	Arthritis incidence	Onset†	Severity‡
Whole cells	80§	4/5	3	4
	40	3/5	3	5
	10	–	–	–
Whole-cell sonicate	15§	5/5	1	7
	5	5/5	1	5
	1	3/5	1	1
Sonicated purified	30§	5/5	1	8
Cell walls	15	2/5	1	9

* Micrograms coccal rhamnose injected intraperitoneally per gram rat. The rhamnose content for whole cells and cell sonicates, 25 μg/mg dry cell weight, and for sonicated cell walls, 200 μg/mg dry weight.

† Mean of the first days of arthritis for all rats. The experiments were monitored for 30 days.

‡ Mean severity was calculated as the arithmetic mean of the highest score of each rat and only in "positive" rats.

§ Strain D136c. Strain 603-79 was equally effective in inducing arthritis (results not shown).

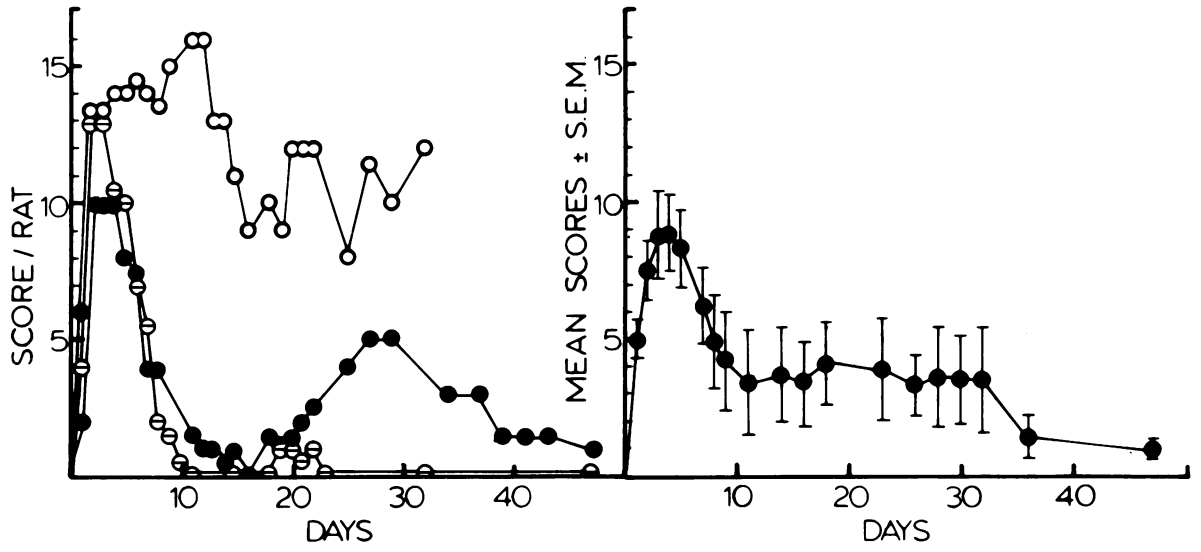

Figure 2—Time course of arthritis induced in rats with purified, sonicated cell walls of Type III Group B streptococci. Arthritis scores for individual Sprague-Dawley rats (*left panel*) ae plotted versus time and may be compared with mean arthritis scores for 10 rats (*right panel*). The rats received 15 μg/g of rhamnose from Strain 603-79. *Vertical bars*, SEM. Noteworthy are the onset within 24 hours of symptoms in these rats, the individual variation in the clinical course, and the tendency of some animals experience a relapse.

left and right panels) resembled those described for rats given injections of sonicated cell walls of Group A streptococci[6] and included the three variations seen in the left-hand panel of Figure 2. The period of induction was shortened (latent period, 24 hours, compared with 7 days for whole Group B). Severe inflammation appeared, primarily in the ankles, by 2 days and gradually subsided over 2–3 weeks, leaving knotty, ankylosed joints. Active inflammation primarily in the carpal joints reappeared after 3–4 weeks and again gradually subsided, to leave enlarged joints with little edema or erythema. Little further change was seen when these animals were examined for up to 60 days. Individual rats usually exhibited one of three clinical courses of arthritis in response to sonicated Group B cell walls (Figure 2A, left panel). All three patterns showed an acute response that, in a typical experiment, was prolonged in 3 of 11 animals (27%), short-lived but recurrent in 5 of 11 (45%), or short-lived and nonrecurrent (3 of 11, 27%).

Sonicates of Group D streptococci have been reported to lack the capacity to induce chronic arthritis[6] in rats. We confirmed this finding (data not shown); however, Group D sonicates induced a diffuse acute erythema and edema of the legs, which subsided in most animals in 1 week or less after injection.

Gross Pathology and Histopathology of Group B Streptococcus-Induced Arthritis

Arthritis was scored according to the severity of edema and erythema in the affected joints. Both whole bacterial cells and sonicates induced bright red inflamed joints (Figures 3B and C; Figure 3A was of a control). Inflammation due to whole streptococci (Figure 3B) lacked the severe edema often associated with the acute responses to sonicates (Figure 3C). The gross appearance of the arthritis in rats receiving either of these treatments was otherwise similar. Rats with active inflammation were passive and avoided putting pressure on their ankles by lying with their feet thrust to one side.

Tissue sections of the involved joints in rats given injections of sonicated cell walls of Group B streptococci revealed synovitis 24 hours after injection. Polymorphonuclear leukocytes and mononuclear cells infiltrated the subsynovium and periarticular connective tissue. Tenosynovitis was especially severe. At 24–48 hours homologous streptococcal antigens were associated with the inflammatory cells but not found in uninflamed sites, according to studies both with immunofluorescence and with immunoperoxidase. Antigen was also found in the bone marrow, especially in marrow just adjacent to the joint cartilage. No evidence of inflammation was seen in the bone about such marrow. At 1 week pannus formation was evident over cartilage surfaces in the ankles. At 2 weeks advanced erosive synovitis was seen. Hypertrophy and hyperplasia of the synovial cells developed, with an intense inflammatory infiltrate of macrophages and lymphocytes. Inflammatory cells were visible in the synovial space along with deposits of fibrin. Similar pathologic conditions were evident in rats receiving whole streptococci; however, the inflammatory process was delayed in proportion to

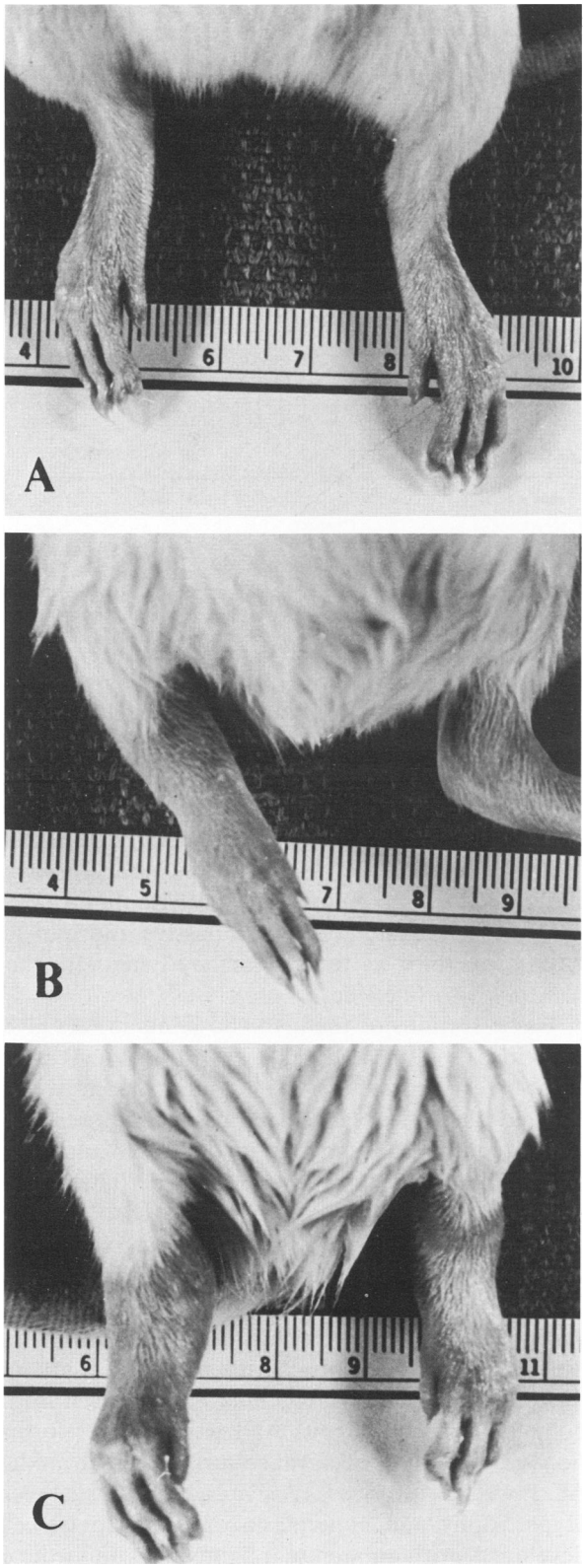

Figure 3—Gross inflammation of ankles and feet of rats given whole heat-killed Group B streptococci or whole cell sonicates. **A**—Normal Sprague-Dawley rat. **B**—Sprague-Dawley rat 7 days after injection of whole heat-killed Group B streptococci (Strain 603-79, 40 μg/g). **C**—Sprague-Dawley rat 4 days after injection of whole cell sonicate of Group B streptococci (Strain 603-79, 40 μg/g).

the delay in clinical onset. This finding correlated well with the gross observations of the course of the disease. Destruction of the joints progressed rapidly, with loss of cartilage, invasion by pannus, and proliferation of granulation tissue in the joints. New bone formation was marked, and ankylosis with fibrosis was seen at 2 weeks or more. Involvement of soft tissues surrounding the joints was extensive and severe. Tendonitis and fasciitis were commonly present, even when synovial space and cartilage appeared essentially normal.

Induction of Arthritis With Whole Group B Streptococci in Different Rat Strains

We were concerned that the Group B disease might only affect certain rat strains, perhaps because of possible differences in the microbial flora to which the animals might have been exposed in the facilities of the different suppliers or in our own animal quarters. We found that whole Group B streptococci induced arthritis in a variety of rat strains from several commercial sources, indicating that the disease described was not a specialized response of one rat strain. In general, the responsiveness of outbred Sprague-Dawley rats was similar regardless of the commercial source of the animals (Table 4). Sprague-Dawley rats from one supplier seemed an exception to this rule, but Wistars from the same supplier were not an exception. To control for possible influences of microbial infection on the arthritis studied, the rats were screened (courtesy of Dr. Gail Cassell, University of Alabama in Birmingham) for serum antibody to *Mycoplasma*. No significant antibody (ELISA assay) to *Mycoplasma arthritidis* was detectable in rats from any source. *Mycoplasma pulmonis* infection, common in conventionally housed rats, did not appear to contribute to the arthritogenic effects of streptococci, because barrier-sustained mycoplasma-free rats exhibited arthritis responses similar to those of mycoplasma infected rats. The data supporting this assertion are shown in Table 4. The Charles River rats noted were barrier-sustained, and many of the rats noted in Tables 1, 2, and 3 were barrier-sustained.

Discussion

With Group B streptococci the latent period to onset of arthritis averaged 7 days, the incidence was 80%, and the duration averaged 40 days. With Group A the latent period was 30 days, the incidence was less than 20%, and the duration was in excess of 60 days. Group D streptococci induced arthritis with

Table 4—Induction of Arthritis With Whole Heat-Killed Group B Streptococci in Different Strains of Rats

Rat strain	Source	Bacterial strain	Dose*	Arthritis† incidence	Mean day‡ of onset	Mean§ severity
Sprague-Dawley	Zivic-Miller	D136c‖	40	3/7	4	8
	Harlan	D136c	40	1/5	4	2
	Charles River	D136c	40	3/5	3	4
Wistar	Harlan	D136c	40	4/5	2	3
Fischer 344	Charles River	D136c	40	3/5	4	3

* Micrograms of cell rhamnose per gram rat.
† Arthritis incidence per rats given injections.
‡ Mean of first days of arthritis for all rats. The experiments were maintained for 50 days.
§ As in Table 1.
‖ Similar results were produced with Strain 603-79 (results not shown).

a latent period of 2 days or less, with duration of less than 10 days and little or no irreversible arthropathy.

Despite these differences, the microscopic changes in the joints of rats given injections of whole Group B streptococci resembled those in rats that received sonicated A or B streptococci. In rats given injections of Group D streptococci acute disease developed, but ankylosis and other chronic changes failed to occur. The distribution of the polyarthritis, its tendency for remission and relapse, the occurrence of chronic arthropathy, and in other instances the tendency for healing with scarring resembled the syndrome of human rheumatoid arthritis.[13,25] The present results confirm and extend the findings of Cromartie et al.[6]

Although streptococcal arthritis, like adjuvant arthritis, was bilaterally symmetrical and involved the feet, ankles, hands, and wrists,[30] several differences were evident. Latent periods were modulated by the physical state of the antigens and were short compared with latent periods in adjuvant arthritis. Streptococcal arthritis relapsed, whereas adjuvant arthritis usually does not. It failed to produce skin lesions and did not involve the spine or tail joints, as does adjuvant arthritis. Both B- and T-cell responses have been implicated in the pathogenesis of adjuvant arthritis.[31] The role of the immune response in streptococcal arthritis, however, remains to be defined. It is noteworthy that evidence has been obtained that the complement-activating capacity of peptidoglycan may be critical, at least in the inductive phase of the disease.[22]

Two technical problems arose. Toxic deaths occurred among rats given injections of several of the Group B streptococcal strains. These rats died in less than 24 hours after bacterial injection. This toxicity will be described (Warejcka et al, to be submitted). Toxicity and arthritogenicity appeared unrelated, because Strains 090R (typeless) and D136c (Type III) both were arthritogenic but not toxic in the doses used.

The other problem involved the hygienic conditions of available rats. We were concerned that the toxicity due to the Group B streptococci might reflect hypersensitivity in rats due to infection or colonization with cross-reactive bacteria. It also seemed possible that the arthritis might be due to *M arthritidis* (reviewed in Cassell et al[32]) or be a reflection of cross-reactive immunity between the normal microbial flora of the animals and streptococcal antigens. *M arthritidis* infection was ruled out serologically. Moreover, rats whelped and raised in a barrier-maintained colony free of *M arthritidis* and *M pulmonis* responded with comparable arthritis.

As Table 1 and Figures 1 and 2 show, heat-killed, whole Group B streptococci induced arthritis in rats, unlike whole Group A, which only induced arthritis readily when sonicated. Moreover, disease due to Group B, either whole or sonicated, although it became chronic (lasted more than 30 days) and tended to relapse, did not continue active as long as the disease due to sonicated Group A. Group D streptococci injected whole and heat-killed into rats caused a rapid onset of transient inflammation, with edema and erythema about the joints and without lasting sequelae. Sonicated Group D streptococci were not arthritogenic.

These variations were all consistent with the hypothesis that processing (partial microbial degradation in the host) is important to the characteristic induction of arthritis due to these streptococci.[9,10,11,23-25] The present results do not rule out the possibility that autolytic streptococcal enzymes participate in these degradative events. However, autoclaved bacteria were able to produce disease (experiments not shown), suggesting that autolytic microbial enzymes that could survive 30 minutes at 60 C but could not survive autoclaving were not involved. The biochemical moieties resistant to degradation, in order to mediate inflammation, need to retain the capacity to fix complement,[22] to activate enzyme secretion by macrophages,[33] to activate enzyme secretion by synovial cells, or to induce some combination of these

processes.[34] In further support of the role of partial microbial resistance is the fact that the resistance of these bacteria to lysozyme is ordered, Group D < Group B < Group A,[26,27,29] as is their arthritogenicity as whole cocci. Recently we verified this ordered resistance to lysozyme and showed that mammalian lysozyme enhances the capacity of Group B Type III streptococci to activate the alternate complement pathway but has no effect on the capacity of Group A streptococci to do this.[29] Group D streptococcal capacity to activate the alternate pathway also correlates with its arthritogenic capacity. In contrast to Groups A and B, the capacity of Group D to activate the alternate pathway was transiently enhanced and then destroyed by lysozyme.[29]

Group B antigen was intimately associated with the acute inflammatory lesions due to sonicated Group B streptococci. The situation thus resembled that found by Dalldorf et al in arthritis induced by Group A cell wall.[5] It was associated in inflammatory lesions with mononuclear as well as granulocytic phagocytes. The lesions were in the dermis, in the connective tissue, about tendons, about muscle, and in both subsynovia. There was vasculitis, apparently in small venules. Antigen also appeared in phagocytes of the marrow, but inflammatory response was absent in the bone around this marrow.

We postulate that arthritogenic moieties of Group B were, in whole cocci, masked, that is, incapable of causing disease. *In vivo* they became unmasked, actively pathogenic, probably because of host enzymes (processing). Microbial structures were, however, sufficiently refractory to host enzymes to resist or delay irreversible deactivation of the arthritogenic moieties. In contrast, whole Group A organisms were highly resistant. Their arthritogenic moieties were masked and not readily revealed by host enzymes. Sonication revealed these moieties and rendered them pathogenic. The greater persistence of activity in Group-A-induced disease was seen as a further extension of the extraordinary resistance of Group A cocci[24] and failure of the host enzymes to degrade even sonicated fragments to a nontoxic state.

Group D were evidently cleaved and arthritogenic moieties revealed more readily than with either A or B. Degradation was, moreover, rapid and complete enough to eliminate them and prevent a chronic process. Sonicated Groups A, B, and D produced effects that were consistent with this interpretation. A and B sonicates produced disease quickly, within 1–2 days. Group D sonicates hardly produced any disease at all, and sonicates of Group A produced a longer lived disease than did Group B sonicates.

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