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Cutaneous Inflammatory Reactions to Group A Streptococcal Cell Wall Fragments in Fisher and Lewis Inbred Rats

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Systemic administration of an aqueous suspension of group A streptococcal cell wall fragments induces severe, chronic erosive polyarthritis in LEW/N female rats, but rarely in F344/N female rats. In the present study, we attempted to exclude unresponsiveness to the cell walls as a mechanism for arthritis resistance in F344/N females. Cutaneous inflammatory reactions were assessed in both strains at various time points after direct injection of cell wall fragments of three different average molecular weights.

Fragments of all sizes induced an acute inflammatory reaction, with infiltration of polymorphonuclear leukocytes and a few mononuclear cells. Small fragments (~5 megadaltons) induced a transient response which resolved by day 14. Large fragments (~500 megadaltons) induced severe imflammation characterized by prominent mononuclear leukocyte infiltration, whereas the intermediate-sized fragments (~50 megadaltons) induced inflammation of intermediate intensity and duration. The intensity and severity of the lesions paralleled the persistence of cell wall antigens at the site of deposition. F344/N female rats responded acutely to the cell walls, with an intensity equal to or greater than that of LEW/N female rats, but the lesions tended to resolve more rapidly. These findings indicate that severity and chronicity of streptococcal cell wall-induced inflammation are dependent on the size of the fragment and provide evidence that arthritis resistance in F344/N female rats does not result from a completely unresponsive state to the proinflammatory effects of the cell walls.

Chronic symmetrical, erosive polyarthritis involving distal joints may be induced in selected rat strains by a single systemic administration of bacterial cell wall fragments in aqueous suspension (2,10). At present, cell wall fragments derived from streptococci groups A, B, and C (2) and selected strains of lactobacilli (T. J. Lehman, J. B. Allen, P. H. Plotz, and R. L. Wilder, Arth. Rheum., in press) are known to possess arthritogenic properties. In contrast to cell walls from non-arthritogenic bacteria, cell walls from these bacteria are relatively resistant to degradation by lysozyme and persist for prolonged periods in the tissues in which they are deposited (6, 8).

Although cell wall resistance to biodegradation appears essential for induction of arthritis, the pathogenic mechanisms remain controversial. We have been studying the role of host-related factors in an attempt to clarify important parameters of pathogenesis. In earlier studies, we demonstrated that susceptibility to the development of chronic arthritis after intraperitoneal injection of group A streptococcal cell walls is highly dependent on rat strain and hormonally mediated sex-linked effects (1, 10). For example, LEW/N female rats develop severe chronic polyarthritis, whereas LEW/N male and F344/N male and female rats are relatively resistant. Since an understanding of the mechanisms regulating susceptibility and resistance would probably provide insight into the dominant pathogenetic mechanisms of this model, we have concentrated on a comparative analysis of susceptible and resistant rat strains.

One possible mechanism which would explain arthritis resistance is failure to develop an inflammatory reaction to the cell walls, i.e., an unresponsive state. In the present study, we addressed this potential mechanism by comparing the cutaneous inflammatory reaction induced at the site of a group A streptococcal cell wall injection in F344/N and LEW/N female rats. We also studied the effect of cell wall fragment size, since it has been demonstrated that the kinetics and duration, as well as the severity, of the experimental arthritis are dependent on fragment size (4, 5). Our findings indicate that F344/N female rats, which are relatively resistant to induction of chronic arthritis,

clearly develop an acute inflammatory reaction which tends to resolve more rapidly than the cutaneous lesions in LEW/N female rats. Moreover, our findings indicate that the severity and duration of the cutaneous inflammatory reaction are dependent on cell wall fragment size.

MATERIALS AND METHODS

Animals. Specific pathogen-free inbred LEW/N and F344/N female rats were obtained from Harlan Sprague Dawley, Inc., Walkersville, Md. The animals weighed approximately 100 g at the initiation of each experiment.

Cell wall fragments. Group A streptococcal cell walls were isolated as previously described in detail (10). Cell wall fragments were generated by sonication for 70 min at maximum frequency with a Biosonic IV sonicator fitted with a 3/4-inch (1.9-cm) probe (VWR Scientific, Baltimore, Md). Fragments with mean molecular weights of approximately 500, 50, and 5 megadaltons (Md) were isolated by centrifugation as described by Fox et al. (5). Before injection, the cell walls were checked for sterility and suspended in phosphate-buffered saline (PBS), pH 7.4.

Animals studies. Cell wall fragments were injected subcutaneously with a 30-gauge needle in various doses (0.1 to 100 μg) in a volume of 0.1 ml into the shaved flanks of the rats. Control animals were injected with PBS. The animals were usually studied in groups of five. Induration was measured with calipers (Fisher Scientific Co., Silver Spring, Md.) and qualitatively scored (scale, 0 to ++++) daily for the first week after injection and three times weekly thereafter until termination of the experiment (usually at 1 month). Full-thickness skin biopsies were obtained from representative animals in each group at 1, 3, 7, 14, 21, and 28 days after injection. The biopsies were fixed in buffered Formalin, sectioned, and stained with hematoxylin and eosin.

Additional paraffin-embedded specimens were analyzed for group A streptococcal cell wall antigens by immunofluorescence techniques. In brief, the paraffin-embedded sections were rinsed for 24 h in xylene (Fisher Scientific) and then rehydrated by 10 min rinses in absolute, 95, 80, 70, and 50% alcohol in PBS. The final rinse was in PBS. The rehydrated sections were stained for cell wall antigens with fluorescein-conjugated rabbit and anti-group A streptococci globulin (Difco Laboratories, Detroit, Mich.). Controls included staining injected tissue with fluorescein-conjugated rabbit nonspecific globulin (Difco) and staining tissue from noninjected animals with specific antibody. The reagents had been cross-absorbed by the supplier to remove cross-reactivity to those antigens which are shared among streptococcal groups A, C, and G. Both preparations were also absorbed on rat liver powder (Sigma Chemical Co., St. Louis, Mo.). They were used at the same protein concentration (0.1 μg/ml) and had similar fluorescein to protein molar ratios (~3). Positive staining was indicated by granular intracellular apple-green fluorescence, which was absent in all controls. Specificity was also verified by absorption on group A streptococcal cell walls, which completely eliminated positive staining.

RESULTS

The reaction of F344/N and LEW/N female rats to cutaneous injection of 100 μg of group A streptococcal cell wall fragments of different mean molecular weights is summarized in Table 1 (average of five animals in each group). Over the 72 h after the injection, both inbred rat strains developed acute erythema and induration at the site of injection. The mean diameter of the lesions tended to be greater in the F344/N female rats, but the differences did not reach statistical significance ($P > 0.05$). At day 3, histological characterization indicated that polymorphonuclear leukocytes were the primary infiltrating cells. The 500- and 50-Md fragments induced more intense acute inflammation that the 5-Md fragments (Fig. 1).

By day 7 after injection, histological characterization revealed that the major infiltrating cells were mononuclear leukocytes (both lymphocytes and macrophages). At this point, the lesions tended to be more intense in LEW/N female rats, and the intensity paralleled fragment size. In fact, the lesions healed within 7 days after injection of the smallest cell wall fragments. By day 14, the lesions in F344/N female rats were almost resolved, and only low-grade mononuclear cell infiltration was evident at the site of injection of the 500-Md fragments. More prominent mononuclear infiltration was noted in LEW/N female rats at the site of injection of the 500-Md cell wall fragments. Moreover, low-grade mononuclear cell infiltration was noted at the site of injection of the 50-Md fragments, in contrast to F344/N female rats. Histological analysis at later time points revealed continued repair, with fibroblast infiltration and a return to normal histological appearance. Persistent inflammatory disease was not observed beyond 3 to 4 weeks.

Table 1 also shows a qualitative measurement of the amount of group A streptococcal cell wall antigen present at the site of injection for each the variables studied, i.e., rat strain, time, and fragment size. It shows that the elimination of cell wall antigens tended to parallel the intensity of the cellular infiltration. The smaller fragments were eliminated most rapidly from the sites of injection, whereas the largest fragments were cleared more slowly. Significant differences between F344/N and LEW/N female rats in the rate of cell wall antigen clearance were not documented.

The inflammatory reaction of the two rat strains to lower doses of cell wall fragments paralleled the results described above for 100-μg doses, except that the area of induration and the intensity of cellular infiltration were less at the smaller doses. Injections of PBS did not induce a

TABLE 1. Cutaneous inflammatory reaction of F344/N and LEW/N female rats induced by injection of streptococcal cell wall fragments of different average molecular weights[a]

Mean cell wall fragment size (Md) and rat strain	Day 3[b]				Day 7				Day 14			
	IND	PMNL	MonL	AG	IND	PMNL	MonL	AG	IND	PMNL	MonL	AG
500												
LEW/N	+++	++++	+/−	++++	++	+	+++	++	+	+/−	++	+
F344/N	++++	++++	+	+++	+	+	++	++	0	0	+	+
50												
LEW/N	+++	+++	+/−	+++	+	+/−	++	++	+/−	0	+	+
F344/N	++++	++++	+	++	+	+	+	++	0	0	0	+
5												
LEW/N	++	++	+/−	++	0	+/−	0	+	0	0	0	0
F344/N	++	++	+/−	+	0	0	0	+	0	0	0	0

[a] One hundred micrograms was injected intracutaneously. The rats weighed approximately 100 g at the initiation of each experiment. Skin induration (IND) is indicated on a qualitative scale of 0 to ++++. The type of cells infiltrating the skin lesions are indicated by PMNL (polymorphonuclear leukocytes) or MonL (mononuclear leukocytes); the intensity of cellular infiltration is qualitatively represented on a scale of 0 to ++++. AG, streptococcal cell wall antigen; the amount is qualitatively represented on a scale of 0 to ++++.

[b] For day 3, the mean diameters of the lesions (IND) are as follows. 500 Md: LEW/N, 6.7 mm; F344/N, 9.0 mm. 50 Md: LEW/N, 5.5 mm; F344/N, 10.5 mm. 5 Md: LEW/N, 3.0 mm; F344/N, 7.5 mm. The diameters of the lesions could not be reliably measured with calipers after day 3 becuase the margins were not well demarcated from the surrounding tissue.

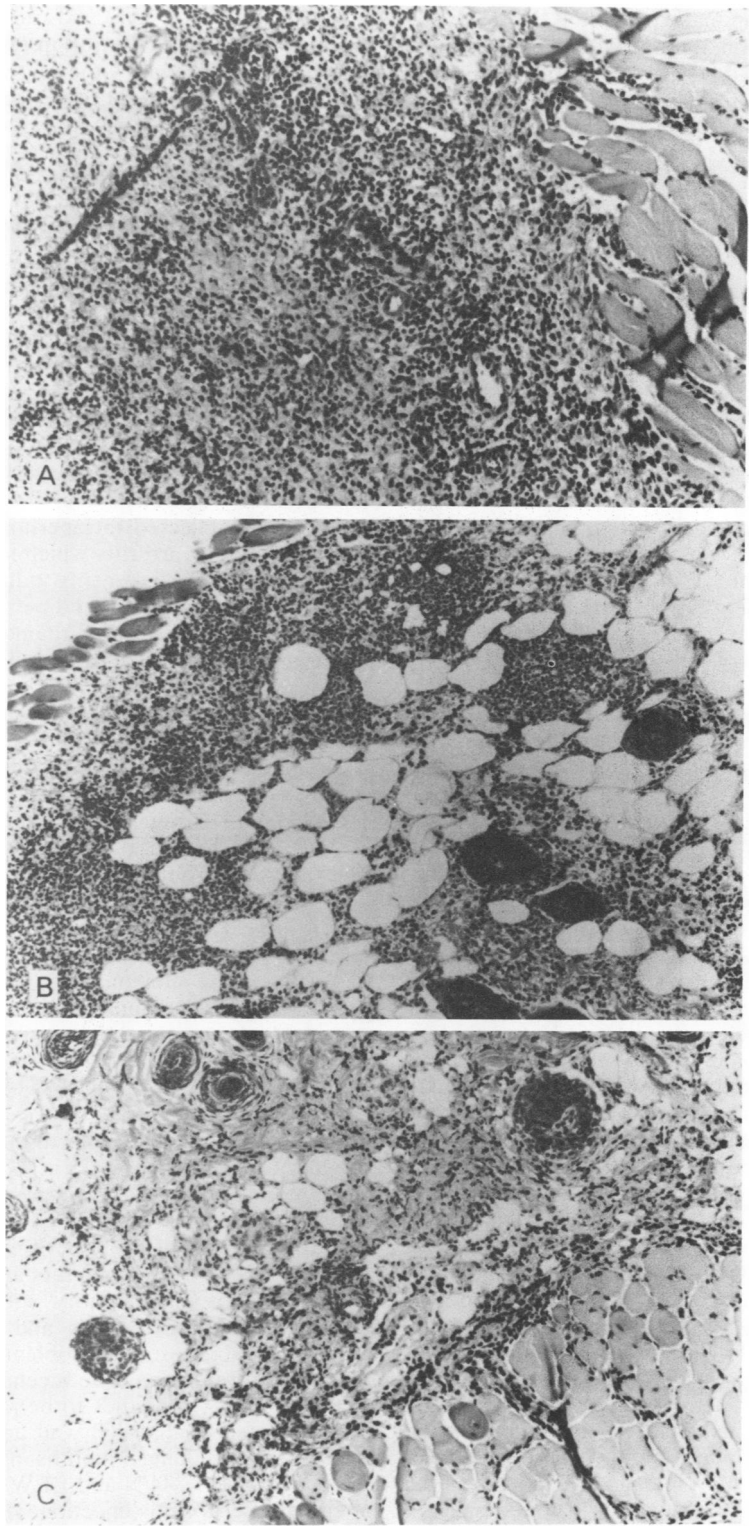

FIG. 1. Cutaneous inflammatory reaction in F344/N female rats 3 days after subcutaneous injection of 100 μg of cell wall fragments with different average molecular weights. Panels A, B, and C show the reaction induced by fragments with average molecular weights of 500, 50, and 5 Md, respectively. (×130).

measurable inflammatory reaction. Notably, significant induration and acute cellular infiltration were observed 3 days after injection into F344/N female rats of 1 μg of cell wall fragments of all size ranges, but a measurable reaction in LEW/N female rats required injection of at least 10 μg of the cell wall fragments. This difference was statistically significant ($P < 0.05$). By day 7, the lesions were almost resolved in both rat strains, and differences were not documented.

DISCUSSION

This study demonstrated that (i) F344/N female rats developed acute cutaneous inflammatory responses equal to or greater than those observed in LEW/N female rats; (ii) the acute reaction tended to resolve more rapidly in F344/N female rats than in LEW/N female rats; (iii) the intensity of the inflammatory reaction tended to parallel cell wall fragment size and persistence; and (iv) the rate of antigen clearance from the site of injection was not measurably different between F344/N and LEW/N female rats. What is the significance of these findings?

Previous studies have shown that LEW/N female rats develop severe acute arthritis followed by chronic erosive polyarthritis of distal joints after intraperitoneal injection of an aqueous suspension of group A streptococcal cell wall framents (10). Acute arthritis generally develops within 18 h after cell wall injection, is maximally severe at day 3, and usually resolves by day 8. The chronic phase of the arthritis begins as early as day 8, but may not develop for 2 to 4 weeks. It persists for more than 3 months and ultimately destroys the more severely affected joints. In addition to arthritis, LEW/N female rats develop clinical and histopathological abnormalities in other tissues. For example, splenic hypertrophy with mononuclear leukocyte hyperplasia, and noncaseating hepatic granulomas are consistent findings. In contrast, F344/N female rats do not develop chronic arthritis but do occasionally develop mild acute arthritis which resolves within 8 days. Other tissues are generally normal, except for the spleen, which typically exhibits mild hypertrophy and hyperplasia. At the usual doses used to induce arthritis, hepatic granulomas do not develop, although low-grade infiltration of the liver with mononuclear cells is occasionally noted (10).

We hypothesized that the differences in the severity of induced abnormalities between F344/N and LEW/N female rats reflect one or more of the following factors: (i) differences in the rate of degradation or detoxification of the cell walls, (ii) differences in the amount of the cell wall fragments which disseminate and deposit in various tissues, and (iii) differences in the type, intensity, or duration of the inflammatory reaction induced by the cell wall fragments. Our study addressed the latter mechanism (are F344/N female rats unresponsive to group A streptococcal cell walls?) by studying the inflammatory reaction induced by local injection into the skin.

Our results indicate that F344/N female rats clearly respond acutely to group A streptococcal cell walls. As we intuitively expected, the intensity of the inflammatory reaction tended to parallel fragment size. Thus, the smallest fragments were mobilized from the site of injection more rapidly than the larger fragments, and the lesions healed more rapidly. More rapid mobilization and clearance of small fragments is consistent with the recent study of Fox et al. (5). These investigators demonstrated that small fragments, when injected intraperitoneally, induce severe acute polyarthritis which subsides rapidly, whereas larger fragments induce mild acute arthritis but more severe and persistent chronic arthritis. Thus, cell wall fragment size is an important parameter of potential biological effects.

Of greater potential interest was the observation that F344/N female rats tended to resolve the cutaneous lesions more rapidly that LEW/N female rats. This finding supports the view that LEW/N female rats are defective, compared with F344/N female rats, in their capacity to neutralize the proinflammatory properties of the cell walls. That is, the chronic inflammatory lesions in LEW/N female rats may reflect a chronic persistent proinflammatory effect of the cell walls at the sites of tissue deposition. This hypothesis is also consistent with the recent report which compared susceptibility to pulmonary mycoplasmosis in F344/N and LEW/N rats (3). The authors noted that F344/N rats resolve the pulmonary lesions more rapidly than LEW/N female rats. Similar to our hypotheses, the authors suggested that LEW/N female rats were more susceptible to mycoplasma-induced nonspecific lymphocyte activation or an imbalance in regulation of lymphocyte proliferation or both (3). If LEW/N female rats are defective in neutralizing proinflammatory substances such as streptococcal cell walls and mycoplasma, then a critical unresolved problem is the mechanism of neutralization. The mechanism is likely to be complex. In studies to be published elsewhere, we show that cell wall antigens persist for periods exceeding 5 months in the livers and spleens in both F344/N and LEW/N female rats after intraperitoneal injection (R. L. Wilder, J. B. Allen, L. M. Wahl, G. B. Calandva, and S. M. Wahl, Arthritis Rheum., in press). At the usual doses of cell wall, LEW/N female rats

develop granulomas in these organs, but F344/N females do not.

Further emphasizing the potential complexity is the observation that arthritis development in LEW/N female rats follows a biphasic course. Our understanding of the pathogenesis of the model must reconcile these features. We suspect that the acute inflammatory lesions are probably a direct cell wall-induced proinflammatory effect which develops at sites of cell wall tissue deposition. However, the fact that the acute lesions substantially subside before the onset of severe chronic arthritis suggests that more than a simple "toxic" effect may be involved in the chronic lesion. We raise the possibility that the chronic lesions may in part represent the development of a chronic hypersensitivity reaction to the cell walls. Another possibility, although we believe it to be less likely, is that the chronic articular lesions reflect the development of a chronic hypersensitivity reaction to articular autoantigens, as proposed for classical oil-based adjuvant-induced arthritis (7, 9). These hypotheses will require additional investigation to be substantiated or refuted.

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