Polyarthritis Associated with *Salmonella* Infection in Rats

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Rats infected intravenously with *Salmonella enteritidis* develop a chronic destructive polyarthritis. The joint lesions resemble those of human rheumatoid arthritis (RA), largely because of a lack of close overall correspondence between the syndromes in animals and man. Examples of discordant features include the presence of suppuration in or near the affected joints, lack of chronicity, and absence of one or more of the anatomical hallmarks of RA such as palpable subcutaneous nodules or microscopic lymphoid aggregates. In addition, immunochemical alterations typical of RA (such as hyperglobulinemia and so-called rheumatoid factor) have been notably absent from virtually all the experimental systems so far described.

Among the experimental arthritides that have received attention recently are those associated with certain infectious agents: *Erysipelothrix* in swine (4, 16), mycoplasma in a variety of species (1, 2, 11, 15, 19), and *Corynebacteria* in rats (12). Other systems which have been the subject of intensive study have been linked with certain forms of cell-mediated immunity. These include the so-called adjuvant arthritis in rats that can occur as a result of intradermal sensitization to mycobacterial antigens (14) and the joint lesions that can emerge during the course of graft-versus-host disease in rats (17).

We present in this paper observations on still another example of chronic polyarthritis in rats that first manifested itself during an investigation of the mechanism of acquired specific resistance to a *Salmonella* infection. Certain features of this syndrome, which we call *Salmonella*-associated arthritis (SAA), resemble human RA so closely as to warrant evaluation of the experimental disease as a potential model for studies relating to man.

**MATERIALS AND METHODS**

**Animals.** Young adult male (Lewis × BN)F<sub>1</sub> hybrid (LBN) rats (150 to 180 g), derived from highly inbred parents, were conventionally maintained on a standard commercial diet and permitted tap water ad lib.

**Infectious agents.** *Salmonella enteritidis* (NCTC 5694) was obtained from the National Type Culture Collection, Collindale, England. It was maintained on tryptose-soy-agar (TSA) slants kept at room temperature in the dark and subcultured at 3 month intervals. After four such transfers, the culture was discarded, and a fresh culture was prepared from lyophilized stock. Challenge inocula were grown in tryptose-soy-broth at 37°C for 6 h (late logarithmic growth phase), standardized turbidimetrically to approximately 10<sup>9</sup> organisms per ml, diluted suitably in saline, and immediately injected into the rats. The number of viable bacteria in the challenge inoculum was checked by plating suitable saline dilutions of the suspension on TSA and counting the number of colonies after overnight incubation at 37°C. Reinfection studies were carried out with the same organism or a streptomycin-resistant variant of *S. enteritidis* able to multiply in the presence of a concentration of 20 μg of drug per ml of agar. In the latter case, the two populations of bacteria were distinguished by double plating of organ homogenates on TSA with and without streptomycin (5, 10).

**Infection.** Rats were infected intravenously (i.v.) with 10<sup>9</sup> to 3 × 10<sup>9</sup> viable *S. enteritidis*. The mean lethal dose (LD<sub>50</sub>) in the LBN rat is about 5 × 10<sup>8</sup>. Reinfection by the same route was performed at selected intervals in certain experiments by using
streptomycin-sensitive or -resistant strains of *Salmonella enteritidis*.

**Bacterial enumeration.** Quantitative bacterial counts were carried out on randomly selected groups of eight rats at increasing time intervals after the i.v. injection of *Salmonella enteritidis*. Saline homogenates of the whole spleen and the axillary lymph nodes were diluted suitably in sterile saline and plated on nutrient agar, and the resulting colonies were counted after 24 h incubation at 37 C. Heart blood and synovial fluid were sampled aseptically from affected and apparently normal joint cavities and directly plated onto agar. The relative errors in this counting system were similar to those reported earlier for mice (6).

**Delayed-type hypersensitivity.** The level of delayed hypersensitivity was assessed by injecting 5 μg of a *Salmonella* test antigen (7) intradermally. The increase in skin fold thickness was determined after 24 h by using a dial gauge caliper (1 unit = 0.1 mm).

**RESULTS**

Resistance to reinfection and the development of delayed hypersensitivity were among the factors being assessed in a study of acquired immunity to *Salmonella* infection in rats. Unexpectedly, most of the reinjected animals developed swelling and erythema in a number of joints in the paws within 10 to 14 days of challenge. None of the singly infected rats developed such lesions during several months of observation. For a time, arthropathy remained dependent upon reinfection; but when a newly constituted culture of *Salmonella enteritidis* was prepared from lyophilized stock, the disease appeared in the course of the primary infection. In some experiments, as few as 10⁶ viable organisms elicited joint lesions in a high proportion of the rats. The period required for the emergence of SAA was less regular than after challenge; but using 30 days as an arbitrary interval for assessment, the overall frequency with which one or more joints became grossly affected after a single injection of organisms has been 72% (112/156). In these rats, *Salmonella enteritidis* was isolated from 40% of the involved joints but never from uninvolved joints (Table 1). To evaluate the response of the host and the fate of the infecting organism, a variety of organs and tissues was cultured at intervals after infection and reinfection. The data of Fig. 1 show growth patterns of *Salmonella enteritidis* in liver and spleen. The more effective inhibition after challenge indicates a substantial degree of resistance to reinfection. Rats infected with *Salmonella enteritidis* also develop a high level of cutaneous delayed-type hypersensitivity (Fig. 1), which is not unexpected in view of comparable results obtained in mice (7, 18) and certain suggestive observations in rats (8).

During the period when a challenge was necessary to elicit SAA, a streptomycin-resistant strain of *Salmonella enteritidis* was used in one experiment to establish the secondary infection. This made it possible to determine whether the primary or secondary infections were responsible for persisting foci of infection. The results of this qualitative analysis are given in Table 2. Only the primary infecting agent was present in sufficient numbers to be detected by culture. A quantitative study is clearly needed to assess

![Fig. 1. Growth curves for *Salmonella enteritidis* in the spleen and liver of LBN rats infected and reinfected intravenously with 3 × 10⁶ viable bacilli. The histograms represent the average increase in skin thickness in mm 24 h after injection of 5 μg of skin test antigen into shaven flank skin.](http://iai.asm.org/)

**Table 1.** Frequency of gross joint pathology and positive joint cultures following the intravenous injection of 3 × 10⁶ living *Salmonella enteritidis* into male (Lewis × BN)F₁ hybrid rats

<table>
<thead>
<tr>
<th>Determination</th>
<th>Day of sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Positive gross joint pathology</td>
<td>0/4</td>
</tr>
<tr>
<td>Positive joint cultures*</td>
<td>0/4</td>
</tr>
<tr>
<td>Positive cultures/positive pathology*</td>
<td>0</td>
</tr>
</tbody>
</table>

* Proportion of positives with respect to number of rats sacrificed at each interval.
* Grossly normal joints did not in any instances yield positive cultures.
fully the incidence, distribution, and size of the persisting microbial populations. Nevertheless, the results indicate that the host is systemically immune, but that the primary infection may persist focally for at least 28 days. There appeared to be no correlation between the presence of organisms in joints of the forelimbs and in the draining lymph nodes. The foci of infection in the kidney were recognizable both macroscopically and histologically as granulomas. They were found in some experiments after 3, 6, and 9 months in animals showing no evidence of the infecting organism at other sites, including diseased joints. The relationship of the persistent infectious foci within the kidney to the sustained arthropathy requires further evaluation.

The distribution of visible lesions in SAA is generally bilateral and often roughly symmetrical. Forepaws and hindpaws are involved with equal frequency. Spontaneous regression at one focus with subsequent flare-ups at other joints imparts the illusion of a migratory process to the clinical course of the disease. As in human RA, however, one or more lesions eventually persist and result in localized deformity. Figure 2A to C shows some examples of the distribution of gross lesions involving the forepaws. The joints that are most often affected in SAA are the radiocarpal, tibiotalar, interphalangeal, metacarpophalangeal, carpometacarpal, tarsometatarsal and, occasionally, the proximal portion of the tail.

Microscopically, the inflammatory process is localized predominantly to the synovial tissues of periarticular and articular structures, including the tendon sheaths. Most of the histological features observed are illustrated and described in Fig. 2D to F. The cellular exudate consists largely of a variety of mononuclear leukocytes, including lymphocytes and focal concentrations of plasma cells, but neither granulomas nor lymphoid nodules have as yet been seen in this location. The hyalinized small blood vessels occasionally found in the inflamed synovium (Fig. 2E) are taken to be evidence of vasculitis. Granulocytes, mononuclear phagocytes, and precipitated material resembling fibrin can also be seen within the joint spaces.

The possibility was considered that the Salmonella infection might not be directly responsible for the joint lesions. For example, S. enteritidis may have activated a latent mycobacteria, some species of which are known to be arthritogenic (1, 2, 11, 15, 19). Cultures of synovial fluid from affected joints, however, have been negative for this agent. Moreover, it should be emphasized that no spontaneous occurrences of joint disease have been observed to date by anyone in the Trudeau Institute who has used the LBN strain of rat, although it has been bred here for about 4 years and some 14,000 have been raised to varying levels of physical maturity.

**DISCUSSION**

The present experiments show unequivocally that rats infected with S. enteritidis develop a chronic polyarthritis. The high proportion of joint cultures positive for the infecting agent suggests that all involved joints are transiently infected at some point in the experimental disease and that this event plays a critical inciting role in SAA. For several reasons, however, we do not believe that these active joint lesions represent a septic arthritis. First, the histology of the joints is inconsistent with this notion. Second, the high proportion of negative cultures from active lesions further opposes such a conclusion. In other experiments, apparently active joint lesions were invariably sterile many months after infection. Indeed, exceedingly few organisms could be isolated from many qualitatively positive cultures. Moreover, there appeared to be no dependence of positive lymph node cultures upon the presence of infecting organisms in the joints of the region drained by these nodes. We argue, on the contrary, that SAA is a consequence of a complex immune response triggered by the infec-

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**Table 2. Frequency of positive cultures obtained from various organs at intervals following primary and secondary intravenous infections in male (Lewis x BN)F1 hybrid rats with streptomycin-sensitive and streptomycin-resistant strains of Salmonella enteritidis**

<table>
<thead>
<tr>
<th>Source</th>
<th>Day of primary infection (3 x 10^6 streptomycin-sensitive S. enteritidis)</th>
<th>Day of secondary infection (4 x 10^6 streptomycin-resistant S. enteritidis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  4  8  14</td>
<td>1  4  8  14</td>
</tr>
<tr>
<td>Joint</td>
<td>0  0  0  2</td>
<td>2  1  3  1</td>
</tr>
<tr>
<td>Blood</td>
<td>4  3  4  0</td>
<td>0  0  0  0</td>
</tr>
<tr>
<td>Spleen</td>
<td>4  3  4  2</td>
<td>4  3  0  0</td>
</tr>
<tr>
<td>Lymph node*</td>
<td>2  4  4  4</td>
<td>—  4  2  2</td>
</tr>
<tr>
<td>Peyer's patch</td>
<td>0  1  3  3</td>
<td>2  4  0  0</td>
</tr>
<tr>
<td>Kidney</td>
<td>—  —  —  —</td>
<td>—  —  4  4</td>
</tr>
</tbody>
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* This dose was used to offset the lower degree of virulence of the streptomycin-resistant organism.

* All positive cultures proved to be streptomycin sensitive.

* Frequency of positive cultures per four rats sacrificed at each interval.

* Axillary.

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**Infect. Immunity**

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tious agent during its sojourn within the joint. This view is reinforced by other evidence for a pronounced immunological response: the development of a high degree of resistance to reinfection and the rapid onset of specific delayed hypersensitivity (Fig. 1). In this context, we can only speculate about the role of humoral or cellular mechanisms in promoting what is evidently an immunologically generated injury. In support of humoral mediation, one could hypothesize that the initial presence of infecting organisms in certain joints establishes antigenic reservoirs that later react with rising levels of circulating antibody to form immune complexes of an inflammatory nature. Such a mechanism is in fact believed to play a contributory role in the pathogenesis of human RA (20). Continued activity in the joints in SAA may thus be due to a continuing antigenic stimulus arising from living organisms persisting elsewhere in the body. Organisms such as were found in lymph nodes and kidney (Table 2) would thus provide a chronic source of antigens to sustain an immune response. With respect to cell-mediated injury, it is possible that a class of specifically reactive lymphocytes may have been induced and that they in turn are directly responsible for the local tissue damage, as is
thought to be the case in adjuvant arthritis. However, attempts to transfer joint lesions of SAA adoptively with up to $4 \times 10^6$ cells from thoracic duct lymph, lymph nodes, spleen, or peritoneal exudates of arthritic donors have been unsuccessful to date.

It is premature to do more than speculate on the value of SAA as a model of human RA, but its potential for this purpose is clear. As described in this report, the most striking similarities between SAA and RA are the anatomic distribution of the lesions, chronicity, and histopathological characteristics. Two other hallmarks of human RA, gross subcutaneous nodules and microscopic lymphoid aggregates in synovial or periarticular tissues, have not been seen. In addition, we do not yet know whether rats with SAA develop immunochimical alterations corresponding to those often present in RA, but this aspect is currently under investigation.

The fact that SAA arises during the course of an infection focuses on a controversial point with respect to RA (1, 3). An infectious etiology of human rheumatoid arthritis has never been conclusively established despite repeated investigations. However, the possibility that the sequence of events resulting in the presently recognized immunological and immunochimical alterations of RA is in fact triggered by infection remains a viable theory. We find it provocative that the initiating agent of SAA is a common enteric organism frequently associated with human disease.

ACKNOWLEDGMENTS

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LITERATURE CITED