Immunopathological Features of Rat

*Staphylococcus aureus* Arthritis

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*Staphylococcus aureus* is the most common bacterial species found in nongonococcal bacterial arthritis in humans. We present the first description, to our knowledge, of an outbreak of spontaneous staphylococcal arthritis in a rat colony. In a group of 10 rats, 9 displayed arthritis. Clinically, the most obvious findings were arthritis of one or both hindpaws and malaise. Bacteriophage typing showed the common phage type 85 in isolates recovered from the joints, blood, and bedding of rats and from the nose and cheeks of one person from the staff of the animal facility. The *S. aureus* strain proved to produce staphylococcal enterotoxin A and exhibited strong binding to collagen types I and II and bone sialoprotein, which are potentially important virulence factors. When the recovered *S. aureus* strain was injected intravenously into healthy rats, severe septic arthritis was induced in almost all of the animals. The arthritic lesions were characterized by infiltration of phagocytic cells and T lymphocytes into the synovium. Many of the synovial cells strongly expressed major histocompatibility complex class II molecules. Increased levels of interleukin 6 in serum as well as a prominent polyclonal B-cell activation were noted throughout the disease course. Pretreatment of *S. aureus*-injected rats in vivo with an antibody to the αβ T-cell receptor significantly decreased the severity of the arthritis. Our results indicate that αβ+ T lymphocytes contribute to an erosive and persistent course of *S. aureus* arthritis.

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**MATERIALS AND METHODS**

Rats. Outbred male Sprague-Dawley rats obtained from ALAB (Stockholm, Sweden) were used in all experiments. They were housed in the animal facility at Sahlgren's University Hospital (experiment i) and at the Department of Clinical Immunology (experiments ii to v), University of Göteborg, Göteborg, Sweden, under standard conditions of light and temperature and fed standard laboratory chow and water ad libitum. The rats were kept in the animal house for 1 to 2 weeks before use. In experiment i, 5-month-old rats were used, housed one to two in each cage. In experiments ii to v, 6- to 9-week-old rats were housed two to six in each cage. In the immunohistochemical part of experiment iv, 8-month-old female rats were used.

Bacterial strain and culture conditions. The *S. aureus* strain AB-1, used in all experiments in this study, was originally isolated from a swollen talocrural joint of a Sprague-Dawley rat in experiment i. This bacterial strain proved to be catalase and coagulase positive and exhibited the phage type 85. *S. aureus* AB-1 enterotoxin production was evaluated by using a toxin detection kit (Oxoid Ltd., Basingstoke, United Kingdom). Before each experiment, bacteria were cultured on blood agar (5% human erythrocytes) for 24 h and then reincubated on blood agar for another 24 h.

Administration of bacteria. A bacterial suspension was prepared by using a McFarland no. 10 nephelometer (24) (experiments i to iii) and then further diluted in phosphate-buffered saline (PBS; 0.13 M NaCl, 10 mM sodium phosphate [pH 7.4]) to the desired concentration. Administration of bacteria was always performed by intravenous (i.v.) injection. In experiments iv and v, bacteria were frozen and viable counts were used to check the number of bacteria in the frozen aliquot as well as in the remaining bacterial suspension after i.v. injection had been performed.

Clinical evaluation. All rats were monitored individually.

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TABLE 1. Binding of 125I-labelled extracellular matrix proteins to live staphylococcal cells

<table>
<thead>
<tr>
<th>Matrix protein</th>
<th>Protein bound (ng/10^6 bacteria [mean ± SEM]) to strain:</th>
<th>AB-1</th>
<th>O24</th>
<th>7686</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vimentin</td>
<td>22.14 ± 1.95</td>
<td>22.16 ± 1.67</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Fibronecin</td>
<td>2.04 ± 0.08</td>
<td>0.62 ± 0.03</td>
<td>0.10 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Collagen type I</td>
<td>43.73 ± 5.67</td>
<td>44.06 ± 4.98</td>
<td>0.25 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Collagen type II</td>
<td>24.31 ± 1.76</td>
<td>23.24 ± 2.65</td>
<td>0.64 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Bone sialoprotein</td>
<td>2.33 ± 0.07</td>
<td>3.00 ± 0.09</td>
<td>0.01 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

* The total amounts of proteins added to 10^6 bacteria were as follows: vimentin, 31 ng; fibronecin, 12 ng; collagen types I and II, 65 ng each; bone sialoprotein, 25 ng.
* O24 is an S. aureus strain isolated from a patient suffering from osteomyelitis. The strain is used as a positive reference strain.
* 7686 is an S. epidermidis strain used as a bone sialoprotein-negative reference strain.

Limbs were inspected visually by three observers (T.B., S.L., and A.T.) at regular intervals. Arthritis was defined as visible joint swelling or erythema of at least one joint. The overall condition was evaluated by assessment of weight, general appearance, alertness, and skin abnormalities. A clinical examination of arthritis was carried out by using a system in which macroscopic evaluation of arthritis yields a score of 0 to 3 for each paw. An arthritic index was constructed for each animal by addition of points for all four paws as described previously (2).

**Bacteriologic examination.** Bacterial sampling was performed with charcoaled sticks. The isolates from the animal house staff were obtained by using Rodac plates for surface sampling (experiment 1). All samples were transferred to blood agar with 5% human blood and incubated at 37°C for 48 h. Bacterial colonies were tested for catalase and coagulase activities. To avoid false-positive results due to contamination, a sample was considered positive when more than 20 colonies of S. aureus were present. Bacteriophage typing was performed for all positive isolates in experiments 1 to 5 by using an internationally standardized technique (51).

Binding of radiolabeled proteins to staphylococcal cells. Bone sialoprotein purified from bovine bone (15) was kindly provided by Dick Heinegård, Department of Medical and Physiological Chemistry, University of Lund, Lund, Sweden. Vimentin of human origin (52) was kindly provided by Bianca Tomasini Johansson, Uppsala, Sweden. Collagen type I from calf skin (Vitrogen 100) was purchased from Collagen Corp., Palo Alto, Calif. Collagen type II was purified from rat chondrosarcoma (44), kindly provided by Kristoffer Rubin, Uppsala, Sweden, and fibronecin was purified from human plasma (56). All proteins were radiolabeled with 125I (Radiochemical Centre, Amersham, United Kingdom) by using the Iodobead (Pierce Chemical Co., Rockford, III.) method (34). The estimated specific activities of the labeled proteins were in the range of 0.2 × 10^6 to 1.0 × 10^6 cpm/μg of protein.

Binding of radiolabeled proteins was performed as described previously (43). In all of the binding experiments, a positive control strain, S. aureus O24, and a negative control strain, Staphylococcus epidermidis 7686, were used.

**Serological analyses.** Serological analyses were performed in experiments iii and v. (i) **IL-6 assay.** The cell line B13.29, subclone B9, which is dependent on interleukin 6 (IL-6) for its growth, was used for IL-6 determinations (1, 22, 29). The samples were tested in twofold dilutions and compared with an IL-6 standard (5). The B9 cells were shown previously not to react with several recombinant cytokines, including IL-1α, IL-1β, IL-2, IL-3, IL-5, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor alpha, and gamma interferon. There was only a weak reactivity with IL-4 (22).

(ii) **Immunoglobulins.** Levels of total immunoglobulin G (IgG) in serum were measured by the radial immunodiffusion technique (33). The antisera and immunoglobulin standard specific for IgG were purchased from Cappel Laboratories (Cochranville, Pa.). Levels of total IgM in serum were measured by an enzyme-linked immunosorbent assay (ELISA) using rabbit anti-rat IgM as the antisera and immunoglobulin standard (Zymed Laboratories, San Francisco, Calif.).

(iii) **Anti-ssDNA antibodies.** Serum antibody levels to single-stranded DNA (ssDNA) were measured by an ELISA using methylated bovine serum albumin (BSA; 10 μg/ml) to precoat wells which were then coated with 50 μg of heat-denatured (boiled for 20 min and then cooled rapidly on ice) calf thymus DNA (Sigma) per ml as described previously (10a).

(iv) **Rheumatoid factors.** Levels of IgG and IgM rheumatoid factors in serum were measured by a diffusion-in-gel ELISA (13) as described previously (46).

**Monoclonal antibodies.** The monoclonal antibodies to CD5 (OX19 [3, 11]) and to αβ T-cell receptor (TCR) (R73 [25]) were used in vivo experiments (experiments i and v, respectively). In the immunohistochemical examination (experiment iv), monoclonal antibodies to major histocompatibility complex (MHC) class II (OX6 [35]), CD8 (OX8 [9]), CD4 (W3/25 [50]), macrophage surface protein (ED2 [12]), and IL-2 receptor (OX39 [39]) were used.

**Histopathologic examination.** The histopathological process included routine fixation, decalcification, plastic embedding, and staining with a mixture of basic fuchsin-methylene blue-Azur II (experiments i to iii) or paraffin embedding and staining with hematoxylin and eosin (experiments iv and v). In all experiments, tissue sections from knees, ankles, tarsal bones, toes, elbows, wrists, carpal bones, and fingers were studied, except for experiment v, in which the knees and elbows were used for bacteriologic examination.

**Immunohistochemistry.** After the rats were sacrificed, their limbs were removed and demineralized by a procedure detailed in an earlier report (26). The demineralized specimens were mounted on cryostat chucks, frozen in isopentane prechilled by liquid nitrogen, and kept at −70°C until they were cryosectioned. Six-micrometer-thick sections were cut sagittally. All sections were fixed in cold acetone for 5 min and washed in PBS. After additional washes in PBS, the sections were incubated overnight in a humid atmosphere at 4°C with 50-μl portions of unlabeled mouse monoclonal antibodies or rat monoclonal antibodies diluted in PBS containing 1% BSA. Biotin-labeled rabbit anti-rat immunoglobulins diluted in PBS-BSA were used as secondary antibodies. Depletion of endogenous peroxidase was performed with 0.3% H2O2 for 5 min. Binding of secondary antibodies was detected by stepwise incubation with avidin-biotin-peroxidase complexes (24) and 3-aminoo-9-ethyl-carbazole containing H2O2 (27). All sections were counterstained with Mayer’s hematoxylin. The proportion of a given cell phenotype was expressed as the percentage of stained cells in relation to all nucleated cells within a defined area of synovial tissue.

**Induction and registration of delayed-type hypersensitivity.** Rats were sensitized by epicutaneous application of 450 μl of a mixture of absolute ethanol and acetone (3:1) containing 15% 4-ethoxymethylene-2-phenylxazolone (OXA; BDH Chemicals, Poole, United Kingdom) on the shaved abdomen skin. Eleven days after sensitization, all rats were administered 0.5 mg of the monoclonal antibody to the αβ TCR and
challenged by topical application of 40 μl of 10% OXA dissolved in olive oil on both sides of the left ear. The right ear was exposed to olive oil only. The thickness of each ear was measured before and 24 h after challenge by using an Oditest spring caliper (Kröplin, Hessen, Germany) as described previously (10). All challenges and measurements were performed under light ether anesthesia. The intensities of the delayed-type hypersensitivity reactions were expressed as (ear thickness at 24 h − ear thickness at 0 h) × 10^{-3} cm.

**Statistics.** Statistical comparisons were made by Student’s two-tailed t test, except for comparisons regarding experiment v, where Wilcoxon’s signed rank test was used. All values are reported as means ± standard errors of the means (SEM) if not stated otherwise.

**Experimental protocols.** (i) **Outbreak of spontaneous S. aureus arthritis.** Male outbred Sprague-Dawley rats, aged 5 months and weighing 400 to 500 g, were injected intraperitoneally, as a part of another study, with mouse anti-rat monoclonal antibody specific for CD 5 (OX19). Three different rats received 250, 500, and 1,000 μg of the monoclonal antibody, respectively. Two control rats were given PBS, while another three controls were treated with polyclonal mouse IgG in doses of 250, 500, and 1,000 μg, respectively. Finally, two rats were not injected at all. Blood samples were collected by making a
longitudinal incision in the proximal part of the tail vein on days 3 and 8 after the injection. One rat died on day 8, one was sacrificed on day 10 to obtain samples for bacteriological and histopathological examination, and the remaining eight rats were sacrificed on day 14 of the experiment. At the time of sacrifice, bacterial samples were obtained from the joints, blood, and skin of each rat as well as from the bedding. Also, bacterial isolates were obtained from the nose, cheek, abdomen, groin, and clothes of three animal house staff technicians working with these rats. Bacterial isolates were also obtained from the skin of three healthy unmanipulated female Wistar rats housed in another room at the animal facility as well as from one male Wistar rat and one female Sprague-Dawley rat housed in the same room as the arthritic rats. Bacterial samples were also obtained from other places at the animal facility as well as from unopened bedding material.

(ii) induction of S. aureus arthritis. Twenty 6-week-old outbred male Sprague-Dawley rats were used to study induction of arthritis. The rats were challenged with a single 1-ml i.v. injection containing 4 × 10⁹ S. aureus bacteria per rat (n = 6), 1 × 10⁹ S. aureus bacteria per rat (n = 6), 1 × 10⁶ S. aureus bacteria per rat (n = 6), and PBS (n = 2), respectively. One arthritic rat was sacrificed after 6 days for histopathological examination, and two rats were sacrificed after 10 days for bacteriological examination of the joints, liver, heart, spleen, and blood. Fourteen rats were sacrificed 16 days after i.v. injection of S. aureus. The remaining three rats died during the experiment.

(iii) Bacterial growth and serological findings in rats with S. aureus-induced arthritis. Thirty 7- to 9-week-old Sprague-Dawley rats were used to study bacterial growth and serological pattern after i.v. inoculation of S. aureus. Twenty-four rats were challenged with a single 1-ml i.v. injection containing 2 × 10⁹ S. aureus AB-1 bacteria. Six control rats received 1 ml of PBS i.v. The 30 rats were examined repeatedly for signs of joint swelling, nodose tail, and general appearance during 10 weeks. Blood samples were obtained from the tail at 2, 4, and 10 weeks (at the time of sacrifice) after bacterial inoculation. The blood samples were centrifuged and stored at −20°C until used. All sera were tested individually. The results for the
PBS-injected controls are described in the day 0 column in all tables and figures. Bacterial isolates were obtained from the talocural and radiocarpal joints bilaterally as well as from the tail, liver, kidney, spleen, brain, lung, and heart of 10 rats.

(iv) **Microscopic features of S. aureus-induced arthritis.** Histopathological examination was performed in 4 control rats, on day 5 after bacterial inoculation in 10 rats administered $5 \times 10^7$ S. aureus bacteria, day 11 after inoculation in 5 rats administered $5 \times 10^8$ S. aureus bacteria, and on day 15 in 9 rats administered $1 \times 10^9$ S. aureus bacteria. Immunohistochemical examination of joints and spleens was performed on day 15 after bacterial inoculation in two rats administered $2 \times 10^8$ S. aureus bacteria and in two rats inoculated with $2 \times 10^9$ S. aureus bacteria.

(v) **Anti-T-cell treatment.** Twenty 6-week-old rats were pretreated intraperitoneally with 0.5 mg of αβ TCR monoclonal antibody R73 (n = 10) or 0.5 ml of PBS (n = 10) 24 and 6 h before and 9 days after i.v. inoculation of $10^8$ CFU of S. aureus per rat. Clinical evaluation was performed until the time of sacrifice 15 days later when blood and serum samples were collected from all of the animals. Bacterial samples were collected from the knees, elbows, and tail, and histologic examination was performed on the wrists, carpal and finger joints, ankles, tarsal and toe joints, and the tail. To evaluate the in vivo T-cell suppressive effect of R73, three noninfected rats were subjected to a test of the delayed-type hypersensitivity reaction.

### RESULTS

**Spontaneous outbreak of infectious arthritis.** (i) **Clinical course.** The investigation was initiated after the finding of arthritis in a group of 10 Sprague-Dawley rats undergoing T-cell depletion as part of experiments concerning the regulation of cell proliferation after vascular injury (19) (experiment i). Eight days after the start of the experiment, the rats displayed atypical behavior and malaise. One rat died after blood samples were taken. The next day, arthritis was noted in the remaining nine rats. Hindpaws but not forepaws were affected. Lameness was found frequently. The symptoms occurred irrespective of treatment, i.e., even in control animals not injected at day 0, and had never been accounted before in similar experiments. No other arthritic rats were found in the animal facility, either in the same room or in any other place within the house.

(ii) **Bacteriological findings.** In the spontaneous outbreak of arthritis (experiment i), S. aureus was isolated from a Sprague-Dawley rat talocural joint. Phage typing revealed S. aureus with phage type 85. Moreover, the identical S. aureus strain was also found in blood isolates from two other rats as well as from the bedding of the rats. S. aureus was also found on the skin of five of the rats. Phage typing was, however, not done on these isolates. Skin isolates from three Wistar rats in another room and one Wistar rat and one Sprague-Dawley rat in the same room as that of the arthritic rats did not exhibit growth of S. aureus.

Bacterial isolates obtained from the three persons handling the rats in the animal facility showed that one of them carried the identical S. aureus strain in the nose and on the cheek. S. aureus AB-1 exerts strong binding capacity to vitronectin, fibronectin, collagen types I and II, and bone sialoprotein (Table 1). It is encapsulated by capsular polysaccharide type 8 (serotyping kindly performed by R. Schneerson and J. Robbins, National Institutes of Health, Bethesda, Md.) and produces staphylococcal enterotoxin A.

(iii) **Histopathology.** Histopathologic analysis of a swollen joint from a rat in experiment i with arthritis of 1 to 3 days duration indicated the presence of marked hypertrophy and proliferation of synovial tissue. Most of the inflammatory cells had the appearance of polymorphonuclear cells (PMN). Notably, bacterium-like particles were observed along the synovial cartilage (Fig. 1).

### Establishment of an experimental model of S. aureus arthritis in rats

The S. aureus strain, recovered from a swollen joint of one rat, was injected i.v. into 18 Sprague-Dawley rats (experiment ii). The most noticeable effect was recorded for the rats receiving $4 \times 10^8$ bacteria. The first clinical signs of arthritis were noticed at 3 days of arthritis were observed in all rats receiving $10^9$ bacteria. No clinical signs of arthritis were observed in rats receiving $2 \times 10^8$ bacteria.

### Table 3. Histopathological and clinical findings in joints of Sprague-Dawley rats after a single i.v. injection of S. aureus AB-1

<table>
<thead>
<tr>
<th>Time after injection (days)</th>
<th>No. of rats</th>
<th>Dose of bacteria</th>
<th>Arthritis index (mean ± SEM)</th>
<th>No. of rats with synovial hypertrophy</th>
<th>Infiltrating cell types in synovia</th>
<th>Pannus formation</th>
<th>Cartilage destruction</th>
<th>Bone destruction</th>
<th>Extraarticular manifestation</th>
<th>No. of rats with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0.2 ± 0.2</td>
<td>2</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>$5 \times 10^7$</td>
<td>0</td>
<td>2</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>$5 \times 10^8$</td>
<td>1.3 ± 0.4</td>
<td>9</td>
<td>++++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The numbers of infiltrating inflammatory cells in synovia are estimated as follows: 0, no infiltrating cells; +, minimal infiltration; ++, mild infiltration; ++++, moderate infiltration; ++++, massive infiltration.
FIG. 5. (A) Micrograph showing an arthritic ankle joint in a male 6- to 9-week-old Sprague-Dawley rat sacrificed 11 days after bacterial inoculation. There is synovial hypertrophy (S), pannus formation (P), bone erosion (BE) at the cartilage-synovial junction and infiltration of PMNCs and MNCs. (B) For comparison, a normal joint is shown. The joint cavity is marked with an asterisk (*). Magnification, ×64.
FIG. 6. Immunoperoxidase-stained frozen section from the tarsal joints of a female 8-month-old Sprague-Dawley rat injected i.v. 15 days earlier with $2 \times 10^9$ CFU of S. aureus AB-1. Micrographs show the presence of CD4$^+$ T lymphocytes (A) and MHC class II-expressing cells (B) by staining with monoclonal antibodies to the CD4 and MHC class II molecules, respectively. Magnification, $\times 64$. 
TABLE 4. Pattern of immunohistochemical staining of arthritic joints in Sprague-Dawley rats 15 days after i.v. injection of S. aureus AB-1

<table>
<thead>
<tr>
<th>Cell phenotype</th>
<th>No arthritis (n = 4)</th>
<th>Mild arthritis&lt;sup&gt;a&lt;/sup&gt; (n = 2)</th>
<th>Severe arthritis&lt;sup&gt;b&lt;/sup&gt; (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>IL-2 receptor</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>MHC class II</td>
<td>2</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>Macrophages</td>
<td>3</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>

<sup>a</sup> n denotes number of rats examined.

<sup>b</sup> Rats with mild arthritis received 2 × 10<sup>6</sup> CFU of S. aureus.

<sup>c</sup> Rats with severe arthritis received 2 × 10<sup>7</sup> CFU of S. aureus.

arthritus appeared already within 24 h. In this group, five of six rats developed arthritis within 3 days. One rat also displayed a nodose tail. However, three of six rats, injected with 4 × 10<sup>6</sup> bacteria, died within 4 days. In the group receiving 10<sup>6</sup> bacteria, three of six rats displayed transient arthritis, whereas in the groups receiving either 10<sup>7</sup> CFU of bacteria (n = 6) or PBS (n = 2), no signs of arthritis were detected. In the three latter groups, no mortality was found (Table 2).

In experiment ii, 24 rats were injected with 2 × 10<sup>6</sup> S. aureus AB-1 bacteria, whereas 6 control rats received PBS. Greater than 90% of the rats injected with S. aureus AB-1 developed arthritis within a week (Fig. 2). However, also in this experiment, about 50% (13 of 24) of the rats died (Table 2). No arthritis or malaise was noted among the controls. One control rat died because of anesthesia overdose during blood sampling. Death in rats injected with S. aureus occurred between 5 and 12 days after inoculation. The surviving rats displayed all signs of arthritis, and all but one remained arthritic during the 10-week course of the experiment. Arthritis started generally during the first week after inoculation and was preceded by malaise. Already during the first week of infection, the S. aureus-injected rats lost weight significantly compared with the PBS-injected controls (211 ± 7 g versus 290 ± 8 g; P < 0.001). Similar differences, although of a lower magnitude, were noted later in the course of the disease (data not shown).

(i) Bacteriological findings during the course of infectious arthritis. Bacterial isolates were obtained from two arthritic rats inoculated 2 weeks prior to sacrifice in experiment ii. In one rat, S. aureus phage type 85 was recovered from the liver, heart, blood, and spleen as well as from both talarocrural and radiocarpal joints. In another rat, S. aureus phage type 85 isolates were obtained from the blood, tail, and the right radiocarpal joint as well as from both talarocrural, knee, and hip joints (Table 2). In experiment iii, bacterial isolates were obtained from 10 animals at 10 weeks after inoculation. Nine rats displayed growth of S. aureus in joints, and all of the isolates displayed the original phage type 85. In contrast to the results of experiment ii, positive isolates were not found in organs other than joints (Table 2).

(ii) Serological manifestations during S. aureus arthritis. (a) Immunoglobulins. During the course of S. aureus arthritis, a marked increase in serum immunoglobulin levels was noted. Thus, within 10 weeks after inoculation, serum IgG levels increased sixfold, with the highest values occurring after 10 weeks, and IgM levels increased fourfold, with peak values occurring in the second week. Control rats injected only with PBS showed low and constant immunoglobulin levels (Fig. 3).

(b) Autoantibodies. The obvious increase in immunoglobulin production prompted us to analyze other signs of polyclonal B-cell activation such as the production of autoantibodies. Serum IgG antibodies to ssDNA increased twofold and IgM anti-ssDNA antibodies increased fourfold within 2 weeks in rats injected with S. aureus compared with that in PBS-injected controls. Rheumatoid factor levels peaked 2 weeks after bacterial inoculation for both IgG (10.0 ± 1.0 mM [mean ± SEM]) and IgM (7.0 ± 0.5 mM) and persisted elevated throughout the disease course of 10 weeks. PBS-injected controls displayed background levels of rheumatoid factors in serum.

(c) IL-6. One of the most potent cytokines regulating growth and differentiation of B cells and, hence, immunoglobulin production, is IL-6. Levels of IL-6 in serum peaked 2 weeks after bacterial inoculation and remained elevated even at the time of sacrifice 10 weeks after inoculation. Control rats did not exhibit any significant IL-6 production (Fig. 4).

(iii) Histopathology. Joint sections displayed erosive arthritis with pannus formation and cartilage and bone erosions in the majority of rats given more than 5 × 10<sup>6</sup> CFU of S. aureus AB-1 (Table 3). Infiltration of PMNCs and mononuclear cells (MNCs) was noted; PMNCs occurred frequently during the first 11 days, whereas MNCs prevailed in the later stages of arthritis after bacterial inoculation (Table 3; Fig. 5). Extra-articular manifestations, mainly osteitis, were noted in one-third of the rats. A joint section from one rat with arthritis of 10 weeks duration still showed marked hypertrophy and proliferation of synovial tissues, pannus formation, and destruction of cartilage and subchondral bone. Within the bone tissue, granuloma formations were seen.

FIG. 7. Severity of arthritis in 20 male 6-week-old Sprague-Dawley rats after a single i.v. injection of 10<sup>7</sup> S. aureus AB-1 bacteria per rat. Ten of these rats were treated with an anti-μβ-TCR antibody intraperitoneally on days −1, 0, and 9, and the other 10 rats (controls) were given PBS. Clinical evaluation was carried out by using a system in which macroscopic evaluation of arthritis yields a score of 0 to 3 points for each paw (0, normal appearance; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; 3, marked swelling and erythema). An arthritis index was constructed for each animal by addition of points for all four paws. Statistical evaluation (Wilcoxon signed rank test) refers to comparisons between arthritis indexes of anti-μβ-TCR antibody-treated rats and PBS-treated controls. Both groups were challenged with identical doses of S. aureus. Comparisons between the groups were done with regard to arthritis index (NS, not significant; *, P < 0.05).
Role of T lymphocytes in S. aureus arthritis, (i) Immunohistochemical evidence of T-cell participation in S. aureus arthritis. Immunohistochemically, the arthritis was characterized by infiltration of T lymphocytes, mainly of the CD4+ phenotype (12% of infiltrating cells) (Fig. 6). Twenty-five percent of these expressed IL-2 receptor, indicating that many of the T cells were activated. There was a high prevalence of macrophages (9 to 16% of infiltrating cells), and in rats with severe arthritis, more than 30% of all cells expressed MHC class II, indicating an ongoing immunologic process in the joint lesions (Table 4).

Antii-αβ TCR treatment. The rats treated with the monoclonal antibody R73 (anti-αβ TCR) and the PBS-injected controls exhibited similar disease courses during the first 5 days after inoculation of S. aureus. However, 9 days after inoculation, only 50% of the R73-treated rats displayed arthritis compared with 90% of the PBS-injected controls, and after another 5 days, the frequencies of arthritis were 25 and 67%, respectively (Fig. 7). Histopathologic examination of joints showed that rats treated with anti-TCR antibody displayed a milder course of arthritis compared with that of the control group (data not shown). Serum levels of immunoglobulins, autoantibodies, and IL-6 were not significantly different between PBS controls and rats treated with anti-TCR antibodies.

To assess the functional properties of the anti-TCR antibody, we have challenged healthy, noninfected rats with the simultaneous administration of the anti-TCR antibody and the challenge with OXA. The delayed-type hypersensitivity response to OXA, measured as an increase in ear thickness, was \((42.0 \pm 3.0) \times 10^{-3}\) cm for the control rats in comparison with \((40.3 \pm 2.2) \times 10^{-3}\) cm \((P < 0.0001)\) for the rats receiving R73, indicating an almost total T-cell suppression by the αβ TCR-specific antibody.

DISCUSSION

This is the first description of a spontaneous outbreak of S. aureus arthritis in rats. The course of the disease in rats has many similarities to the human counterpart since it gives rise to severe clinical illness and eventually death (18, 21). We demonstrate that a single S. aureus strain, AB-1, with the same phage typing pattern, was recovered from the joints, blood, and skin of the rats as well as from rat bedding. The fact that the identical \((42.0 \pm 3.0) \times 10^{-3}\) strain was also found with assistant working in the animal facility is most probably the result of bacterial transmission. We believe that S. aureus AB-1 entered the rat bloodstream during the blood sampling since, after this procedure, the tail showed local signs of infection with redness and swelling corresponding to the place of incision. Our assumption fits well with the observation that only rats participating in this particular experiment displayed arthritis. The i.v. injection of \(10^6\) S. aureus AB-1 cells induced rapidly appearing, erosive, and persistent arthritis in almost all rats. In preliminary experiments, we were able to induce infectious arthritis with S. aureus strains producing other staphylococcal enterotoxins, such as B (strain NCTC10654), C (strain NCTC10655), and D (strain NCTC10656), and TSST-1 (strain LS-1). The persistence of arthritis is also seen in mice with experimental infectious arthritis (8) and constitutes a hallmark of untreated human S. aureus arthritis (21). The histopathological appearance of joints with acute synovial inflammation characterized by an abundance of PMNCs and a very rapid destruction of bone and cartilage is also coherent with bacterial arthritis in humans (17, 29). In isolates obtained within 2 weeks after the start of infection (experiments i and ii), growth of S. aureus was noted not only in the joints but also in the blood, liver, spleen, and heart. In contrast, in the isolates obtained 10 weeks after inoculation, growth of bacteria was observed only in the joints. These differences may be due to a deficient clearance of bacteria in the joints in comparison with that in other organs. Indeed, phagocytosis of bacteria by PMNCs has been shown to be lower when bacteria are cultured on monolayers of synovial fibroblasts than when cultured on endothelial cells or embryonic fibroblasts (49). Alternatively, constituents of joint cartilage, bone matrix, or synovia may specifically interact with S. aureus, thereby facilitating the colonization of the joint. Indeed, we have recently described that binding of S. aureus to bone sialoprotein might be such a colonization factor (8). In this respect, the AB-1 strain displays strong in vitro binding to several extracellular matrix components such as bone sialoprotein, fibronectin, and perhaps most importantly collagen type II. Our ex vivo finding of bacterium-like cells lining the synovial cartilage (Fig. 1) fits well with the S. aureus AB-1 in vitro binding properties to collagen type II. The putative role of a collagen receptor on S. aureus strains in the bacterial homing process has been stressed in in vitro studies of S. aureus isolates of human origin (45). Moreover, our in vivo study, using a mouse model for S. aureus arthritis, supports the concept that the S. aureus collagen receptor is an important virulence determinant (40).

Immunohistochemical analysis of arthritic rat joints displayed an abundance of T cells. More importantly, on the basis of our in vivo depletion data, it appears likely that T lymphocytes play a pathogenic role, directly or indirectly, in the arthritic process. As inferred from the results shown in Fig. 8, deletion of T cells downregulates the intensity of S. aureus arthritis. It is for the moment unclear what the driving force of synovial T cells could be. Clearly, cartilage destruction with exposure to neoepitopes could have induced activation of autoreactive T cells. However, a very short time span between the cartilage degradation and the potential sensitization of autoantigen-specific T cells precludes this possibility. Alternatively, bacterial products, such as cell wall constituents (e.g., protein A) or exoproducts, might contribute to the T-cell activation. In this respect, it is of importance to stress that S. aureus AB-1 produces enterotoxin A, an exotoxin with superantigenic properties. We have recently shown that toxic shock syndrome toxin 1, another staphylococcal superantigen, leads to clonal expansion of T cells in joints and aggravates the course of arthritis in a mouse model of staphylococcal arthritis (2a). The serological pattern of S. aureus arthritis in rats is compatible with polyclonal B-cell activation. Thus, total IgG and IgM levels in serum as well as autoantibodies such as rheumatoid factors and anti-ssDNA showed pronounced increases. The long-standing, probably IL-6-induced, polyclonal B-cell activation may be caused by the persistence of S. aureus AB-1 and its secretory products in the joints.

On the basis of our observations from experimental models, it is tempting to postulate a three-step pathogenic process in S. aureus arthritis. (i) During the first hours of infection, bacteria home and bind selectively to different target organs by using their receptors for extracellular matrix components. (ii) Once in the joint, an unspecific inflammatory process takes place during the first 3 days, primarily with phagocytosis of bacteria by PMNCs and macrophages. The bacterial load and host phagocytic properties are important for progression of disease at this stage. (iii) If infection is not stopped, a third stage characterized by an influx of T cells leads to increased inflammation. At this stage, bacterial exotoxin production could exert superantigenic effects leading to T-cell proliferation and differentiation including induction of cytokine production.

The rat model of S. aureus arthritis displays striking similarities to its human counterpart. This model provides an oppor-
tunity to study the virulence determinants of *S. aureus* such as the influence of cell wall components and toxins on invasive-ness and arthritogenicity of *S. aureus* as well as the interactions between the bacteria and their target tissue.

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REFERENCES