

Histopathological and Serological Progression of Experimental *Staphylococcus aureus* Arthritis

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In a newly developed mouse model of *Staphylococcus aureus* arthritis the kinetics of joint destruction and serological manifestations as well as the clinical course of arthritis and osteitis were studied. Almost all mice developed histopathological signs of arthritis upon a single intravenous injection of 10^7 *S. aureus* LS-1 cells. There was rapid joint destruction, with synovial hypertrophy already visible, within 24 h after injection of the bacteria. Cartilage and/or bone erosions were seen in a majority of the mice within 72 h. Extra-articular manifestations, especially signs of bone infection, were also found soon after inoculation of the bacteria. Tail osteitis was frequent (50% of the mice) but appeared later than arthritis. Polymorphonuclear cells prevailed in the early joint lesions and were also common in the extra-articular manifestations. Within 3 days, mononuclear cells were also seen in the inflamed synovium, gaining a dominant position 3 weeks after the start of the disease. Serum interleukin-6 levels were already increased within 6 h after bacterial injection and remained elevated throughout the course of arthritis. Serum tumor necrosis factor levels were increased within 24 h. There was a tremendous induction of immunoglobulin production, especially of the immunoglobulin G1 isotype. This was paralleled by the production of specific antibodies to *S. aureus* (cell walls and toxin), as well as autoantibodies (rheumatoid factors and anti-single-stranded DNA antibodies), all predominantly of the immunoglobulin G isotype. The type and magnitude of the immunoglobulin G response together with the elevated interleukin-6 levels speak in favor of both antigen-specific and polyclonal B-cell activation during *S. aureus* arthritis. This study points out important similarities between our new model of *S. aureus* arthritis and human *S. aureus* arthritis. This resemblance will enable controlled studies of pathogenetic mechanisms of septic arthritis as well as therapeutic and prophylactic approaches.

Bacterial arthritis is a rapidly progressive and highly destructive joint disease in humans. Other destructive joint diseases, including inflammatory disorders, such as rheumatoid arthritis, are connected to an increased incidence of bacterial arthritis (14). Also, certain forms of surgical and medical therapy such as joint implants and immunosuppressive treatment, are connected to an increased frequency of bacterial arthritis (14). *Staphylococcus aureus* is the causative agent in about 60% of cases of nongonococcal bacterial arthritis (10). In patients with rheumatic diseases, this figure is even higher, approaching 75% (11).

Laboratory models of bacterial arthritis have been used previously (12, 17, 21, 24, 25, 28, 30, 31, 33-35). In most instances, bacteria have been injected intra-articularly, usually with rabbits as experimental animals. In contrast, human bacterial arthritis usually spreads in a hematogenous manner (14). We recently reported a spontaneous outbreak of *S. aureus* arthritis in our mouse colony (3) and described the clinical and microbiological features of a murine model of bacterial arthritis following injection of an *S. aureus* strain, LS-1, originating from a spontaneously arthritic mouse (4). This staphylococcal strain binds avidly to bone sialoprotein, a possible important homing mechanism. In this report, we describe the histopathological and serological characteristics of experimental *S. aureus* arthritis.

MATERIALS AND METHODS

Mice. Outbred, 4- to 6-week-old, male Swiss mice (original breeding stock; ALAB) were bred in the animal facility at

the Department of Clinical Immunology, University of Göteborg, Göteborg, Sweden. The mice were fed laboratory chow and water ad libitum under standard conditions of temperature and light.

Bacterial strain and culture conditions. *S. aureus* LS-1, used in the experiments described below, was originally isolated from a swollen joint of a spontaneously arthritic NZB/W mouse (3). This bacterial strain was proved to be catalase and coagulase positive and displayed the following phage type: 6/47/53/54/75/77/85/88+. Strain LS-1 is encapsulated by capsular polysaccharide type 5 (serotyping kindly performed by R. Schneerson and J. Robbins, National Institutes of Health, Bethesda, Md.) and produces large amounts of toxic shock syndrome toxin 1 (TSST-1) and traces, if any, of other toxins, as determined by S. Arvidson (Karolinska Institutet, Stockholm, Sweden). 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. A bacterial solution was prepared by use of McFarland nephelometer standard 8 (16) and then further diluted in physiological saline to the desired concentration.

Administration of bacteria. Intravenous (i.v.) injections of 0.2 ml of an *S. aureus* suspension (5×10^7 /ml) in physiological saline were administered to the tails of mice on day 0. Control mice were injected in the same way with 0.2 ml of physiological saline.

Evaluation of arthritis and osteitis. All mice were labelled and monitored individually. Limbs were inspected visually by 2 observers (T.B. and A.T.) at regular intervals (6 h and 1, 2, 3, 8, 15, 22, and 29 days after inoculation). Arthritis was defined as visible joint swelling and/or erythema of at least

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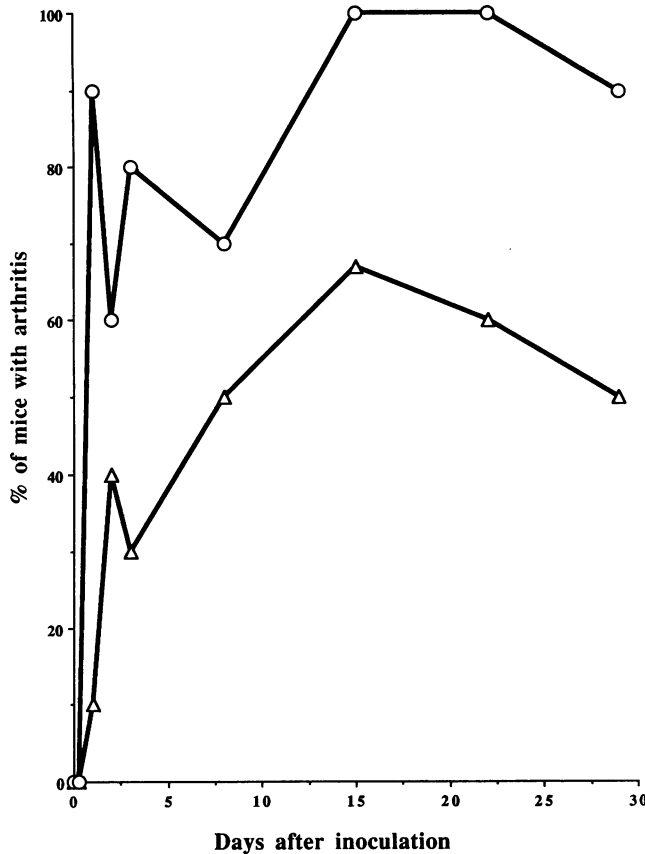


FIG. 1. Prevalence of arthritis at sacrifice in 9 or 10 Swiss mice after a single i.v. injection with 10^7 *S. aureus* cells. Note that histopathological (○) and clinical (△) evaluations were parallel, with the former being more sensitive.

one joint. A nodose tail was reported when there was visible and palpable localized swelling of the tail. The overall condition was evaluated by assessing the weight, general appearance, alertness, and skin abnormalities of each mouse.

Experimental protocol. Ninety-six 4- to 6-week-old male Swiss mice were housed 10 to a cage. Eighty mice were each given 10^7 *S. aureus* cells i.v., while 16 control mice were injected i.v. with physiological saline. The mice were weighed on one to three occasions during the experiments. Groups of 10 mice were sacrificed at selected intervals, i.e., after 6, 24, 48, and 72 h and 8, 15, 22, and 29 days. The 16 control mice were sacrificed after 24 h (5 mice), 8 days (5 mice), and 29 days (6 mice). After the sacrifice, the forepaws, the hindpaws, and part of the tail were immersed and fixed in 4% formalin for further histopathological examination. Blood samples were also taken after the sacrifice, and the sera were stored at -20°C until analysis. All sera were tested individually. The results for the saline-injected control mice are given as the day-0 data in the table and in all the figures.

Histopathological examination. Histopathological examination was performed after routine fixation, decalcification, and paraffin embedding. Tissue sections from upper extremities (elbow, wrist, carpal bones, fingers, and occasionally shoulder) and lower extremities (knee, ankle, tarsal bones, and toes) were prepared. A segment of the tail of each animal

was also studied. Van Gieson staining and staining with hematoxylin and eosin were used. The joints were studied with regard to synovial hypertrophy, defined as a synovial membrane thickness of more than two cell layers (13), pannus formation (synovial tissue overlaying joint cartilage), and cartilage and subchondral bone destruction. In addition, infiltration of inflammatory cells to the extra-articular space and types of invading cells were evaluated.

Serological analysis. (i) IL-6 assay. Cell line B13.29, which is dependent on interleukin-6 (IL-6) for growth, has been previously described (19). For IL-6 determinations, the more sensitive subclone B9 was used (1, 15). B9 cells were harvested from tissue culture flasks, seeded into microtiter plates (Nunc, Roskilde, Denmark) at a concentration of 5,000 cells per well, and cultured in Iscove's medium supplemented with 5×10^{-5} M 2-mercaptoethanol, 5% fetal calf serum (Seralab, Sussex, United Kingdom), penicillin (100 U/ml), and streptomycin (100 $\mu\text{g}/\text{ml}$), and serum samples were added. [^3H]thymidine was added after 68 h of culturing, and the cells were harvested 4 h later. The samples were tested in twofold dilutions and compared with an IL-6 standard (2). One unit of IL-6 is the concentration required for half-maximal proliferation of B9 cells. B9 cells were previously shown not to react with several recombinant cytokines, including IL-1 α , IL-1 β , IL-2, IL-3, IL-5, granu-

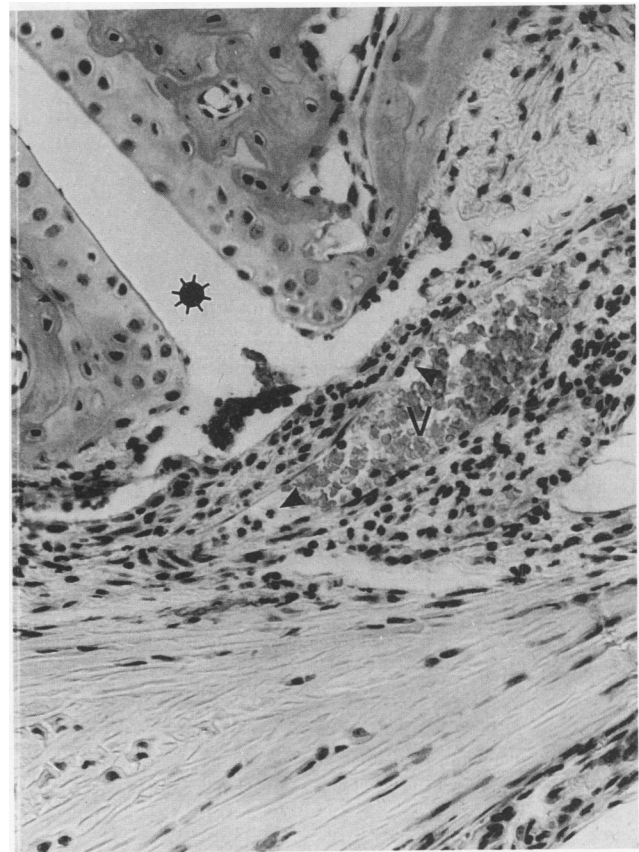


FIG. 2. Micrograph showing a tarsal joint of a 5-week-old male Swiss mouse 24 h after injection of *S. aureus*. Mild synovial hypertrophy is visible; within the synovium is a distended venule (V) with PMNCs adhering to the vessel wall (arrowheads). There are also PMNCs in the joint cavity, which is marked with an asterisk. Magnification, $\times 80$.

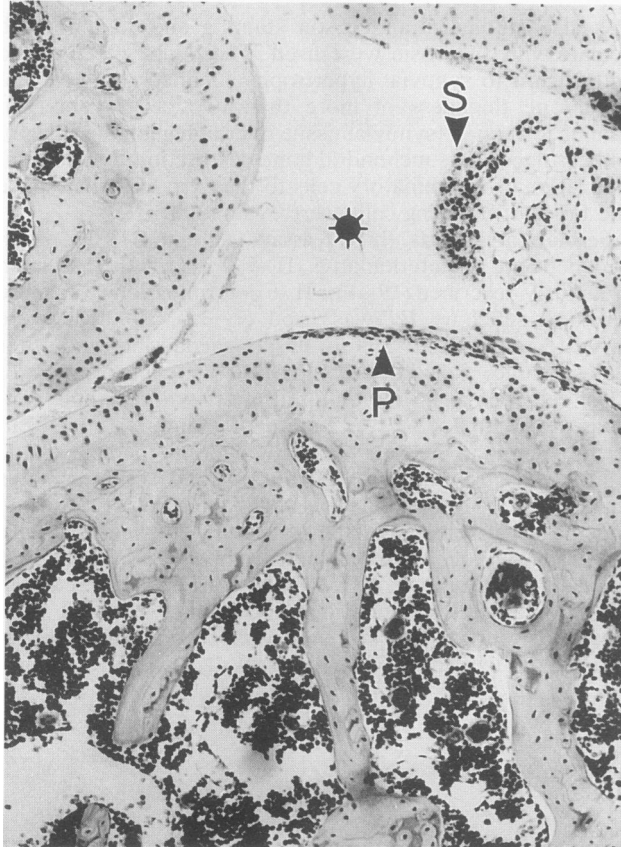


FIG. 3. Micrograph showing an elbow of a 5-week-old male Swiss mouse 48 h after injection of *S. aureus*. Synovial hypertrophy (S) is visible, as is commencing pannus formation (P). Magnification, $\times 32$.

locyte-macrophage colony-stimulating factor, tumor necrosis factor (TNF) alpha, and gamma interferon. There was only weak reactivity with IL-4 (15).

(ii) **TNF assay.** The MTT tetrazolium (Sigma Chemical Co., St. Louis, Mo.) cytotoxicity assay was used to measure levels in serum of TNF with clone 13 of the WEHI 164 cell line as target cells (8). In brief, target cells were seeded in complete medium in flat-bottom microtiter plates at a concentration of 2×10^4 cells per well. Different dilutions of

serum were added to the wells. After 20 h of incubation at 37°C , $10 \mu\text{l}$ of MTT tetrazolium at a concentration of 5 mg/ml in phosphate-buffered saline (PBS) was added, and further incubation for 4 h at 37°C was performed. After aspiration of $100 \mu\text{l}$ of serum from the wells, $100 \mu\text{l}$ of 95% ethanol was added to the wells, and incubation was performed for 10 min. The A_{570} was registered on a Dynatech microtiter plate reader. The test was performed in triplicate, and the results were extrapolated from the linear part of the standard curve. Sera were analyzed 6, 24, and 48 h and 15 days after inoculation with *S. aureus* or saline.

(iii) **Immunoglobulins.** Levels in serum of total immunoglobulin G1 (IgG1), IgG2a, IgG3, and IgM were measured by the radial immunodiffusion technique (26). Antisera and immunoglobulin standards specific for IgG1, IgG2a, IgG3, and IgM were purchased from Sigma.

(iv) **RF.** Levels in serum of IgG and IgM rheumatoid factors (RF) were measured by a diffusion-in-gel enzyme-linked immunosorbent assay (ELISA) (7) as previously described (32). In brief, the inner surfaces of polystyrene petri dishes were coated with 100 mg of aggregated rabbit IgG per liter. Melted agar was then poured into the dishes. Wells (3 mm in diameter) were punched in the gel and filled with serum samples. After incubation at room temperature for 66 h in a moist atmosphere, the agar was removed and the pertinent biotin-conjugated F(ab')_2 fractions of anti-mouse IgG and IgM (Jackson Laboratories), diluted 1:750 in PBS containing 0.05% Tween 20, were poured into the dishes. After 2 h of incubation at room temperature, the dishes were rinsed, exposed to avidin-horseradish peroxidase, and rinsed again, and melted 1% agar containing *p*-phenylenediamine and 0.01% hydrogen peroxide was poured into the dishes. Positive reactions were recognized as gradually developing, brownish, circular areas. After 15 min, the dishes were photographed and the diameter of the colored areas was measured with a ruler to the nearest millimeter. The diameter of a positive reaction corresponds to serum RF levels (32).

(v) **Anti-ssDNA antibodies.** Levels in serum of antibodies to denatured single-stranded DNA (ssDNA) were measured by an ELISA with methylated bovine serum albumin (BSA) ($10 \mu\text{g/ml}$) to precoat wells and $50 \mu\text{g}$ of heat-denatured (boiled for 20 min and then cooled rapidly on ice) calf thymus DNA (Sigma) per ml to coat wells. All sera were serially diluted in PBS-BSA (0.5%) and incubated in wells. To measure the level and class specificity of anti-ssDNA anti-

TABLE 1. Histopathological findings in joints of Swiss mice after a single i.v. injection of 10^7 *S. aureus* LS-1 cells

Time after injection	No. of mice tested	No. of mice with synovial hypertrophy	Predominant infiltrating cell type in synovia ^a		No. of mice with:				
			PMNCs	MNCs	Pannus formation	Cartilage destruction	Bone destruction	Extra-articular manifestations	Tail lesions
0 h	16	0	0	0	0	0	0	0	0
6 h	10	0	0	0	0	0	0	0	0
24 h	10	9	+++	0	5	2	1	6	0
48 h	10	6	++++	0	5	4	2	7	0
72 h	10	8	++++	++	6	6	6	5	2
8 days	10	7	++++	+++	7	7	7	7	3
15 days	9	9	++++	+++	9	9	9	9	2
22 days	10	10	+++	++++	10	9	9	6	1
29 days	10	8	+++	++++	8	8	8	8	5

^a 0, no infiltrating cells; +, minimal infiltration; ++, mild infiltration; +++, moderate infiltration; +++++, massive infiltration.

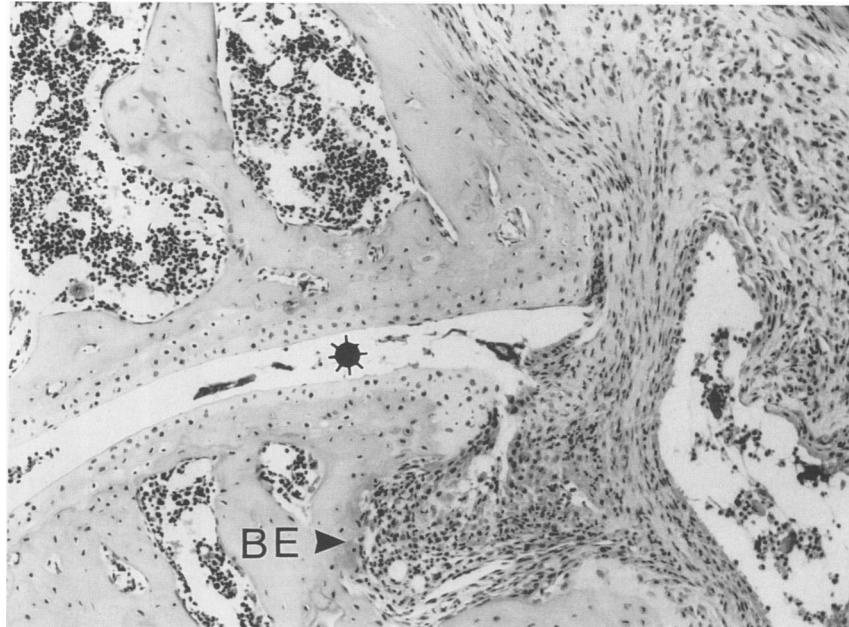


FIG. 4. Micrograph showing a tarsal joint of a 6-week-old male Swiss mouse 8 days after injection of *S. aureus*. Marked synovial hypertrophy is visible, and there is bone erosion (BE) at the cartilage-synovium junction. There are also PMNCs in the joint cavity, which is marked with an asterisk. Magnification, $\times 32$.

bodies bound to the solid phase, we added to wells affinity-purified and biotinylated $F(ab')_2$ fragments of goat anti-mouse IgG and IgM (Jackson Laboratories), diluted 1:3,000 in PBS-Tween 20, and then added stepwise 0.5 μg of avidin-horseradish peroxidase (Sigma) per ml and 2.5 mg of the enzyme substrate 2,2-azino-bis-(3-ethylbenzothiazoline sulfonic acid) (Sigma) per ml in citrate buffer (pH 4.2), containing 0.0075% H_2O_2 . The A_{414} was measured in a Titertek Multiscan photometer (Flow Laboratories, McLean, Va.). All optical density values were converted to antigen-specific arbitrary units with calibration curves based on the optical density values obtained from serial dilutions of a reference pool of sera. The calibration curves were constructed with a computer program based on weighted logit-log models (23, 29).

(vi) **Antibodies to cell walls of *S. aureus* LS-1.** Levels in serum of IgG and IgM antibodies to *S. aureus* cell wall constituents were estimated by the same ELISA as that described above, with the exception that the wells were coated with 100 μl of whole, formalin-treated (4%, 20 min) *S. aureus* LS-1 cells ($10^8/\text{ml}$) instead of denatured ssDNA. This concentration of bacteria was chosen after checkerboard titration (results not shown).

(vii) **Anti-TSST-1 antibodies.** Levels in serum of IgG antibodies to TSST-1 were estimated by an ELISA with 0.5 μg of highly purified TSST-1 (Toxin Technology, Sarasota, Fla.) per ml as a solid-phase coating.

In all the serological analyses described above, sera from noninfected, saline-injected mice were used as controls.

Statistics. Comparisons regarding weights were made by Student's two-tailed t test. All values are reported as the mean \pm the standard error of the mean.

RESULTS

Clinical course of infectious arthritis. Within 48 h after injection, approximately 40% of the mice already displayed

clinical signs of joint swelling. Hindpaws and forepaws were equally affected. There was often concomitant joint erythema. The incidence of swollen joints was greatest during the first week of the experiment. Only in one mouse did arthritis start later than 8 days after injection of *S. aureus*. The proportion of mice with clinical evidence of arthritis peaked to 67% 15 days after injection and levelled off thereafter (Fig. 1). Concomitant changes in the intensity of arthritis occurred with increased severity of joint swelling and a greater number of affected joints. Beginning 3 weeks after the bacterial injection, joint swelling and erythema decreased and joint contractures became more evident. Nine mice displayed signs of transient arthritis. Nodose tails, appearing later than arthritis, were observed clinically for four mice. Saline-injected control mice did not exhibit arthritis, osteitis, or any extra-articular manifestations.

The mice injected with *S. aureus* as well as saline-injected control mice continuously put on weight during the experiments. However, the controls had a larger weight gain at the beginning. The difference in weight between the two groups was significant at days 16 ($P < 0.05$) and 22 ($P < 0.05$). One mouse died within 3 days after inoculation of *S. aureus*.

Histopathological course of arthritis and osteitis. Within 24 h after injection of *S. aureus*, 90% of the mice exhibited arthritis with hypertrophic synovia (Fig. 2). In some joints, dilated venules with polymorphonuclear cells (PMNCs) adhering to the blood vessel wall were visible (Fig. 2). In addition, 50% of the mice displayed pannus formation (Fig. 3) and 20% displayed signs of cartilage or bone destruction (Table 1). By the third day, six of eight arthritic mice displayed cartilage and/or bone erosions, and by day 8, all arthritic animals exhibited severe erosions of cartilage and bone (Fig. 4). The maximal frequency of arthritis was noted on days 15 and 22, when all the mice were affected. Also, the intensity of arthritis, manifested as the mean number of arthritic joints per mouse, was most pronounced on days 15

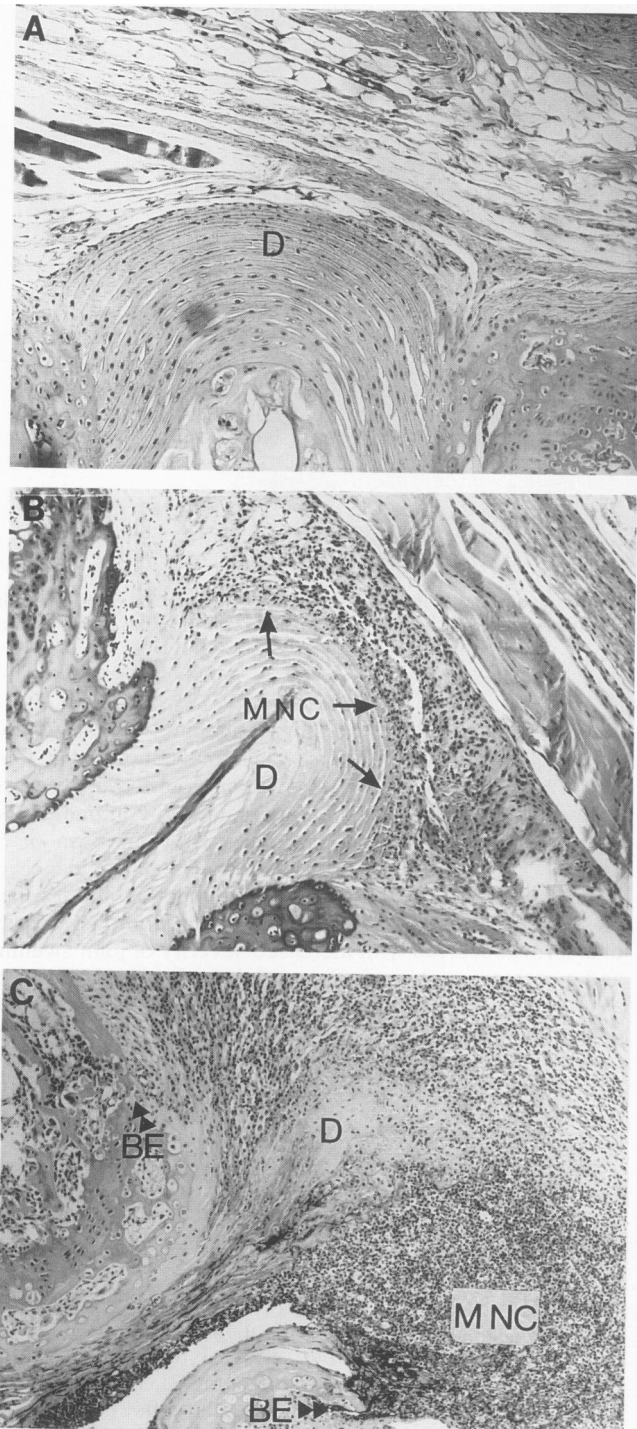


FIG. 5. Micrographs showing the evolution of tail lesions in Swiss mice after injection of *S. aureus*. (A) Tail from a saline-injected mouse with a normal disk (D). (B) MNCs infiltrating the disk (D) 72 h after bacterial inoculation. The disk structure is still well preserved. (C) Massive infiltration of MNCs (MNC) into the disk (D) and bone erosion (BE) 8 days after bacterial inoculation. The disk has undergone partial atrophy because of an intense inflammatory reaction. Magnification of all panels, ca. $\times 24$.

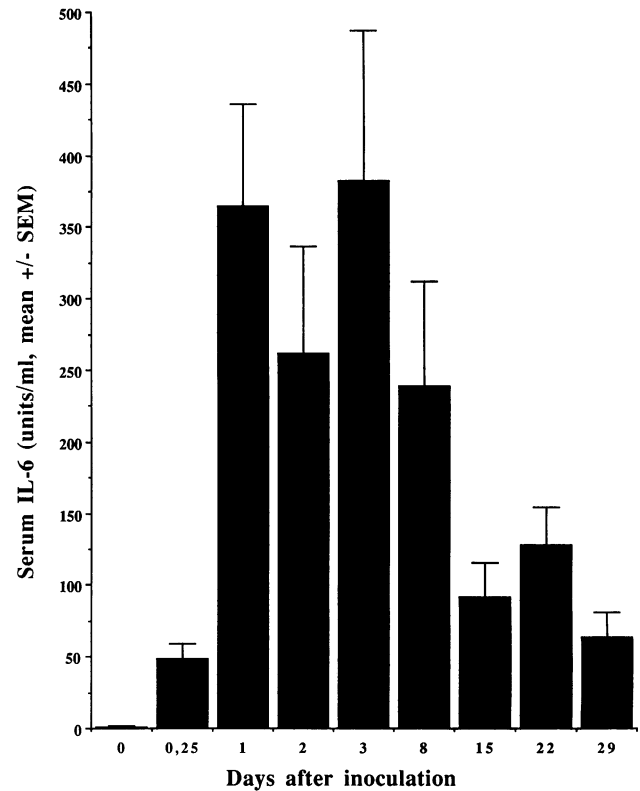


FIG. 6. Serum IL-6 levels in Swiss mice ($n = 9$ or 10) after a single i.v. injection with 10^7 *S. aureus* cells.

and 22, with 4.2 ± 0.5 and 4.0 ± 0.7 arthritic joints per mouse, respectively.

The initial focus of joint destruction was often found in the cartilage-synovium junction (Fig. 4). In most cases, there was a severe and prompt destruction of the joint. On the other hand, mild synovial hypertrophy and sparse pannus formation were seen in some joints even after 3 to 4 weeks. In the early arthritic lesions, infiltrates of PMNCs dominated, but starting 3 days after bacterial inoculation, increasing numbers of mononuclear cells (MNCs) became more evident. The majority of synovial cells in the cartilage-synovium junction participating in the destructive process had the appearance of macrophages.

At 24 h after *S. aureus* injection, 60% of the mice displayed extra-articular manifestations. The most common finding was granulomas in bones such as the femur, tibia, humerus, and radius. However, muscular abscesses could also be noted. The first sign of commencing tail involvement already was seen within 72 h, with infiltrations of inflammatory cells around one or two disks in two mice (Fig. 5B). Within 1 week, three mice displayed severe destruction of disks and adjacent bone tissue (Fig. 5C). After 4 weeks, 50% of the mice exhibited frank tail osteitis, with obvious destruction of bone tissue. The osteitic lesions in the tail showed a mixture of infiltrating cells comprising both MNCs and PMNCs, whereas in the other extra-articular manifestations, PMNCs were predominant and were often located in granuloma formations. PMNCs were also frequently found in the synovial cavity (Fig. 2 and 4).

In saline-injected controls, no pathological changes were found in joints, the tail, or other parts of the locomotor system.

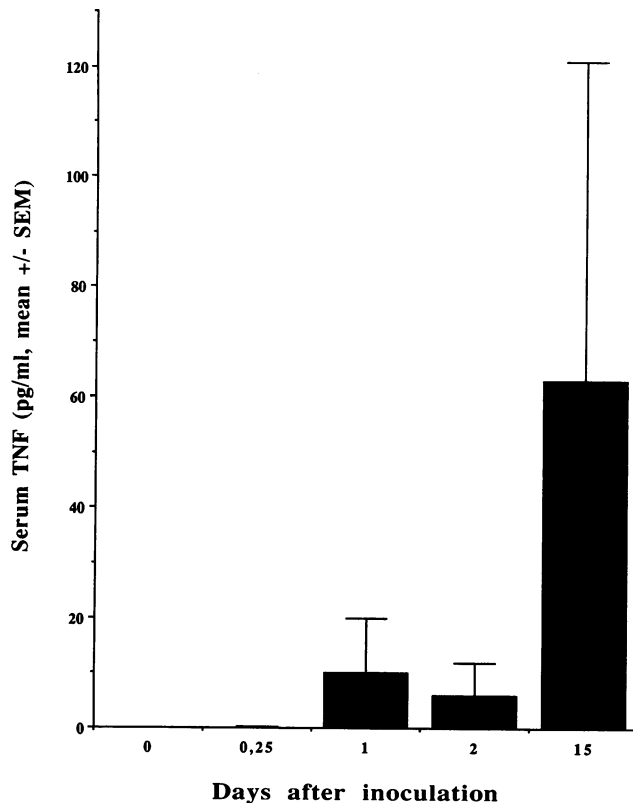


FIG. 7. Serum TNF levels in Swiss mice ($n = 5$ to 9) after a single i.v. injection with 10^7 *S. aureus* cells.

Serological manifestations during *S. aureus* arthritis. (i) **IL-6.** Within 6 h after i.v. inoculation of *S. aureus*, the levels in serum of IL-6 were already significantly increased (Fig. 6). During the first week of infection, IL-6 levels peaked to levels often exceeding 400 U/ml. However, even 4 weeks after bacterial inoculation, there was significant production of IL-6. Saline-injected control mice did not exhibit any IL-6 production. Also, sera from mice injected with dead bacteria (formalin treated, 4%, 20 min) and sacrificed on days 1, 3, 4, 7, 14, and 21 after inoculation did not display increased IL-6 levels.

(ii) **TNF.** Levels in serum of TNF were increased within 24 h after inoculation of live *S. aureus*, the highest value being reached on day 15 (Fig. 7). No measurable serum TNF levels were recorded in control mice.

(iii) **Immunoglobulins.** During the course of *S. aureus* infection, a marked increase in immunoglobulin production was noted (Fig. 8). Thus, within 4 weeks after inoculation of *S. aureus*, levels in serum of immunoglobulins were increased 20-fold (IgG1), 2-fold (IgG2a), 4-fold (IgG3), and 2-fold (IgM), in comparison with those in saline-injected control mice. Injection of dead *S. aureus* did not affect serum immunoglobulin levels.

(iv) **RF.** The first signs of the production of RF of the IgM class were seen, in some mice, within 2 days after injection of *S. aureus*. The production of IgM RF peaked 15 days after inoculation (mean, 5 ± 1 mm). In contrast, serum IgG RF levels reached their highest value later and were of a considerably greater magnitude (11 ± 1 mm after 29 days) (Fig. 9). Saline-injected control mice displayed background serum RF levels.

(v) **Antibodies to denatured ssDNA.** The levels of serum IgG antibodies to denatured ssDNA showed an increase within 2 weeks, reaching the highest value on day 29, with a fivefold increase in comparison with the levels in noninfected control mice (Fig. 10). Changes in IgM antibodies to ssDNA were not prominent (data not shown).

(vi) **Antibodies to cell walls of *S. aureus* LS-1.** The levels of serum IgG anti-*S. aureus* antibodies showed a pattern similar to that of total IgG1 levels. There was a sharp increase in production within 2 weeks, with increasing levels until 4 weeks that exceeded by more than 20-fold the levels in control mice (Fig. 11). The levels of IgM anti-*S. aureus* antibodies showed a slight increase within a few days and then leveled off (data not shown).

(vii) **Antibodies to TSST-1.** The levels of serum IgG antibodies to TSST-1 showed a pattern similar to that of total IgG1 levels. Within 2 weeks after inoculation, there was a sharp increase in antibody production, with the highest levels, on day 29 after inoculation, exceeding the levels in noninfected control mice 1,000-fold (Fig. 12).

DISCUSSION

In this report, we describe the histopathological, serological, and clinical features of a new mouse model of *S. aureus* septic arthritis. Our model is characterized by i.v. administration of the bacteria. This experimental design has several advantages over earlier models of infectious arthritis. First, the use of hematogenic spread of bacteria makes it possible to study important virulence mechanisms of *S. aureus* arthritis, for example, factors influencing bacterial survival in the blood before the bacteria reach the joint as well as factors influencing bacterial ability to penetrate bone and synovia and interact with these tissues. Second, human bacterial arthritis usually spreads in a hematogenic manner (14), a fact that further strengthens the argument to use this route of administration in experimental models. Third, mice are immunologically very well characterized, compared with the commonly used outbred rabbits.

There are several striking similarities between our mouse model and *S. aureus* arthritis in humans. Thus, in both conditions, the speed of joint destruction is very high, giving rise to severe sequelae (14). The rate of progression of the arthropathy and the severity of sequelae are closely related to the causative microbial agent. *S. aureus* is, in this respect, highly virulent. In contrast, Tissi et al. (34) showed that mice with group B streptococcus arthritis displayed destructive changes later, with fibrous ankylosis not being observed until day 60. The more favorable outcome of group B streptococcus arthritis than of *S. aureus* arthritis has also been reported in humans (10). A possible reason for the less favorable outcome of *S. aureus* arthritis could be related to the production of toxins.

Another similarity between our model and human septic arthritis is the cellular composition of the inflamed synovium. Thus, in both circumstances, there is an early dominance of PMNCs, a later appearance of MNCs, and occasionally the formation of granulomas (13). In murine *S. aureus* arthritis, infiltrating PMNCs were seen within 24 h, whereas MNCs started to appear 2 days later. Tail lesions, characterized predominantly by an infiltration of MNCs, consequently developed later than arthritis. The high proportion of cases with granulomas in bone and bone marrow was due to the direct penetration of bacteria via the joint (especially at the cartilage-synovium junction) but may also have been due to hematogenic spread. The latter route was

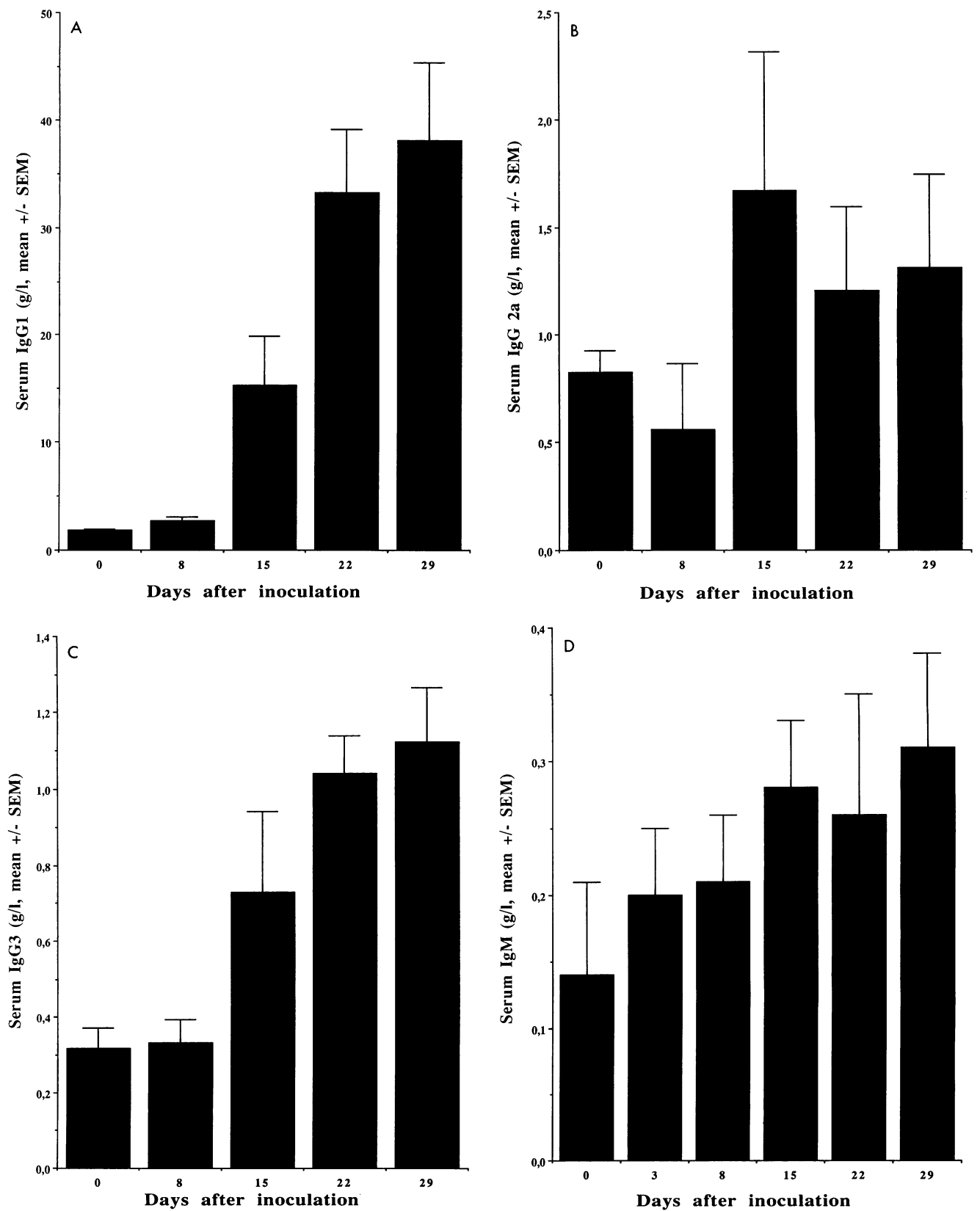


FIG. 8. Levels in serum of IgG1 (A), IgG2a (B), IgG3 (C), and IgM (D) in Swiss mice ($n = 9$ or 10) after a single i.v. injection with 10^7 *S. aureus* cells.

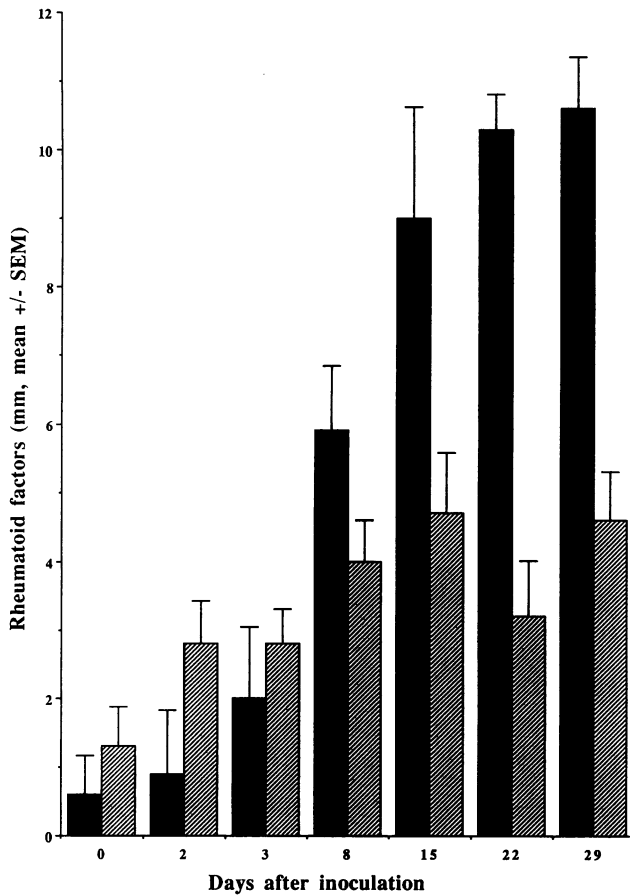


FIG. 9. Levels in serum of IgG (■) and IgM (▨) RF in Swiss mice ($n = 9$ or 10) after a single i.v. injection with 10^7 *S. aureus* cells.

noted in some early cases of bone marrow granulomas, when the adjacent joint was intact.

Notably, macrophagelike cells were seen at the front edge of cartilage and bone erosions in the joints, indicating that the immune system plays an important part in the process of joint destruction. In this respect, the production of macrophage-derived cytokines, such as IL-1 and TNF- α , leads to increased leucocyte chemotaxis, increased leucocyte-endothelial cell interaction, and enhancement of bone and cartilage degradation (5).

The initial focus of joint destruction in *S. aureus* arthritis, localized to the cartilage-synovium junction, with pannus formation and subsequent cartilage and bone destruction as well as the presence of various types of infiltrating cells, shows similarities to those in adjuvant arthritis, collagen type II arthritis, streptococcal cell wall arthritis, and human rheumatoid arthritis (18). It is notable that autoimmune MRL *lpr/lpr* mice, which spontaneously develop arthritis, mice with collagen type II arthritis, and patients with rheumatoid arthritis all display significant production of RF, as do our mice with *S. aureus* arthritis. These similarities speak in favor of common pathogenic mechanisms leading to joint destruction, even if initiated by quite different stimuli.

One of the most striking features of our *S. aureus* arthritis model is the occurrence of polyclonal B-cell activation. Immunoglobulin production, especially of the IgG class, as well as autoantibody production, including that of IgG RF and IgG anti-ssDNA antibodies, was increased up to 20-fold.

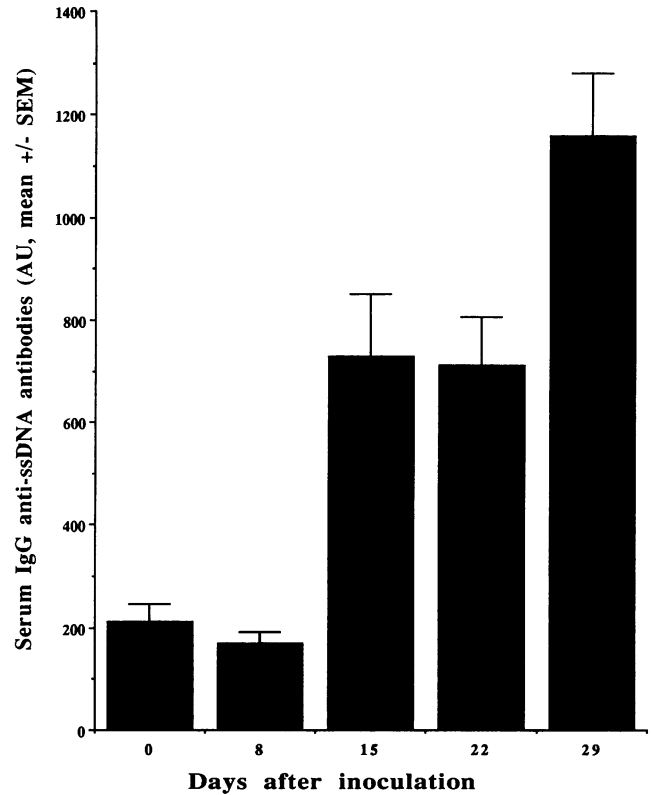


FIG. 10. Levels in serum of IgG anti-ssDNA antibodies in Swiss mice ($n = 9$ or 10) after a single i.v. injection with 10^7 *S. aureus* cells. AU, arbitrary units.

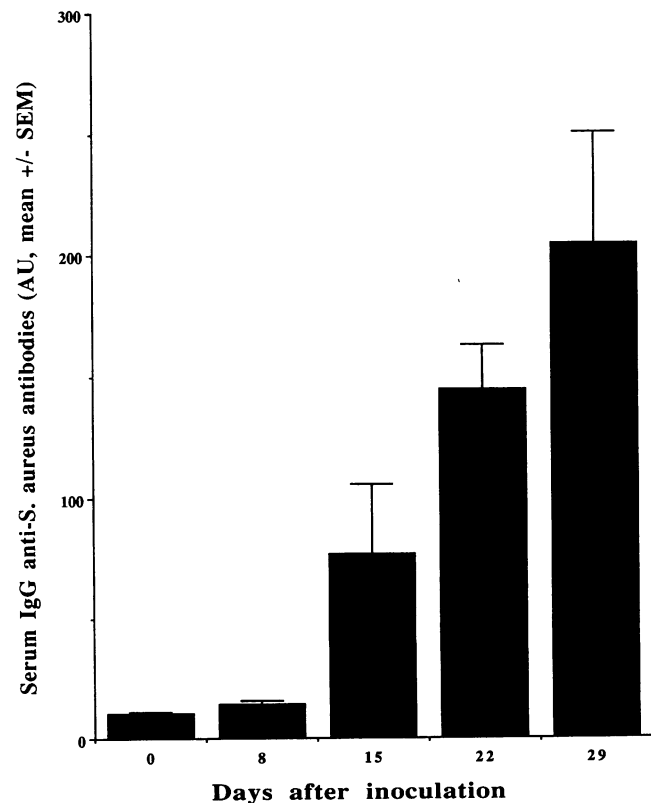


FIG. 11. Levels in serum of IgG anti-*S. aureus* antibodies in Swiss mice ($n = 9$ or 10) after a single i.v. injection with 10^7 *S. aureus* cells. AU, arbitrary units.

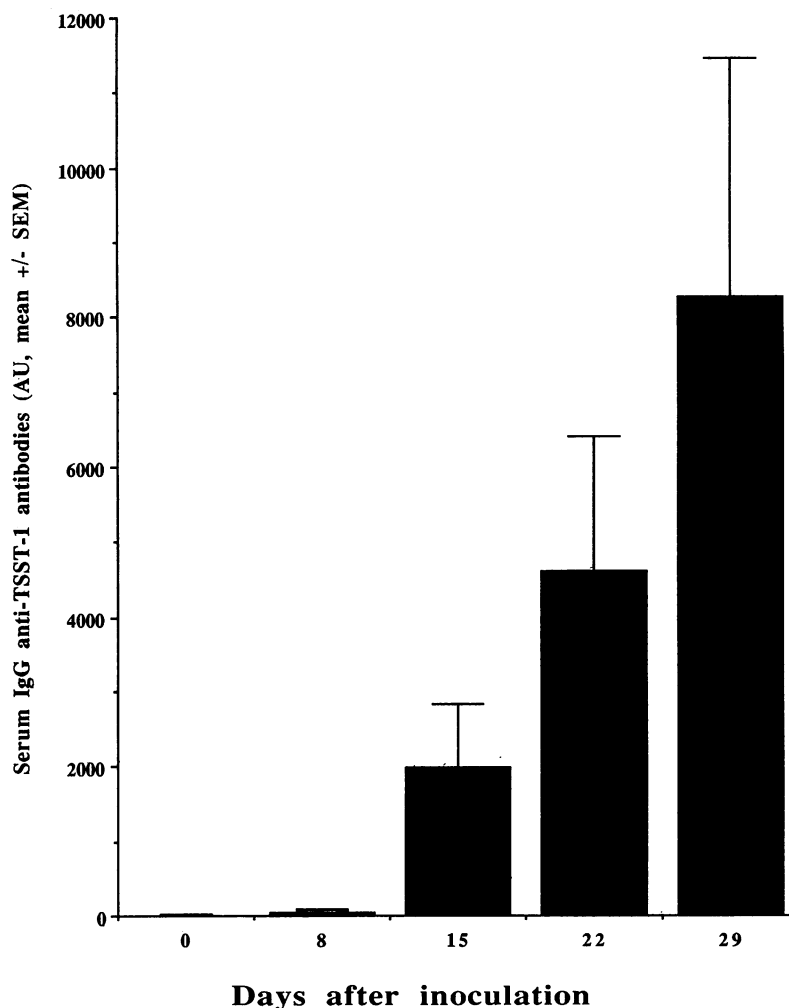


FIG. 12. Levels in serum of IgG anti-TSST-1 antibodies in Swiss mice ($n = 9$ or 10) after a single i.v. injection with 10^7 *S. aureus* cells. AU, arbitrary units.

Several trigger mechanisms might have contributed to this phenomenon. It is known that cell wall constituents of *S. aureus*, such as protein A (22) and peptidoglycans (6, 20, 27), are potent B-cell activators. However, these compounds induce predominantly IgM production (6, 20, 22, 27). In our model, they might have been responsible for the weak early increase in serum IgM levels. However, injection of the mice with dead *S. aureus* did not induce a measurable increase in immunoglobulin production. On the other hand, in vitro studies revealed a marked increase in IgM- as well as IgG-producing cells when mouse spleen cells were incubated with formalin-fixed *S. aureus* (1b). This discrepancy in immunoglobulin responses between in vivo and in vitro studies has also been observed by Dziarski (6). The late and profound IgG response might have been triggered by the release of staphylococcal exotoxins. Several studies have demonstrated that picomolar concentrations of enterotoxins can induce T-cell proliferation and the release of large amounts of cytokines (9), prerequisites for the IgM-IgG switch. One of these cytokines, IL-6, known to promote B-cell differentiation, was shown to be triggered early and produced in high amounts throughout the course of arthritis in our study. Further experimental support of the role of

exotoxins in inducing polyclonal B-cell activation in *S. aureus* arthritis was provided by a pilot study that indicated that mice injected with a mutant *S. aureus* strain that lacked the exotoxin-secreting capacity displayed significantly lower serum IgG1 and IgG3 levels than mice inoculated with the wild-type *S. aureus* strain. The serum IgM responses did not differ between the groups and were of a low magnitude (1a).

This is the first study of the histopathological and serological progression of *S. aureus* septic arthritis (25). The *S. aureus* arthritis model will enable the study of host-bacterium relationships and pathogenetic mechanisms for the development of septic arthritis and osteomyelitis as well as therapeutic and prophylactic approaches.

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