ORIGINAL ARTICLE

Experimental endocarditis in the rat secondary to septic arthritis induced by *Staphylococcus aureus*

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Objective: To develop a modified model for experimental infective endocarditis (IE) in the rat. The goal was to induce a primary infectious focus in the temporomandibular joint (TMJ) of a rat. Hematogenous translocation of the bacteria to the traumatized aortic valve was desired.

Methods: Catheterization of the right carotid artery through the aortic valve was performed 7 days after induction of arthritis, which was done by intra-articular injection of glucocorticosteroid (triamcinolone acetonide, 1 mg) and intravenous challenge with 10^7 CFU *Staphylococcus aureus*.

Results: TMJ arthritis could be induced by intra-articular triamcinolone acetonide followed by intravenous bacterial challenge. Joints not given glucocorticosteroid were not affected. Only rats with arthritis developed IE subsequent to catheterization as a result of bacteremia generated from the arthritis.

Conclusions: The present model may serve as a complement to the conventional method for induction of IE, in which a high intravenous challenge has to be given. In the present model, IE was instead the result of a continuous low level of bacteremia from an infectious focus in the TMJ. This model mimics the natural development of IE in patients, and may assist as a setting for prophylactic and therapeutic trials.

Key words: *Staphylococcus aureus*, septic arthritis, endocarditis, corticosteroids, experimental rat model

Staphylococci are responsible for a large proportion of cases of septicemia, endocarditis and arthritis. Despite progress in medicine, microbiology and the development of new antimicrobial agents, these diseases may still be life-threatening conditions with high morbidity and even mortality. The situation is becoming more complex with the emergence of multiresistant strains of staphylococci in hospitals throughout the world.

Hematogenously acquired bacterial arthritis is a serious medical problem. This rapidly progressive and highly destructive disease is difficult to treat, with less than 50% of the infected patients recovering without serious joint damage. Long-term oral or intra-articular corticosteroids or other immunosuppressive agents are probably important factors in the promotion of bacterial arthritis. Intra-articular corticosteroids implement their anti-inflammatory effect by genetic downregulation of several pro-inflammatory proteins. In general, the effect of corticosteroids on leukocyte circulation is more profound than that on cellular immunity, with humoral immunity being least affected [1]. *Staphylococcus aureus* is the most common etiologic agent of bacterial arthritis, causing up to 80% of such cases [2,3]. The synovium is highly vascularized and contains no limiting basement membrane, thus promoting easy access of blood contents to the synovial space. Bacteria may also directly infect a joint, e.g. from a deeply penetrating wound, contiguous osteomyelitis rupturing into the joint, but may also be introduced by arthroscopy or open surgery [4–7].

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An infection of a joint may function as a primary infectious focus and may be responsible for the spread of the infection by the slow release of bacteria into the bloodstream, causing septicemia and endocarditis in patients at risk.

An animal model of this type of secondary spread of infections mimics the clinical situation when a contagious focus leads to a systemic infection. This model could be useful when studying the initiating events of the infection, such as bacterial adherence and other virulence factors, in an experimental system. Studies of the natural history of intravascular infections [8–14] have mainly been conducted in rats and rabbits. All these studies have been performed according to conventional techniques, which require an intravenous challenge of microorganisms for direct establishment of the infection.

In this report, we describe a modified rat model of catheter-induced endocarditis which is secondary to temporomandibular joint (TMJ) arthritis induced by intra-articular injection of triamcinolone acetonide followed by intravenous challenge with *S. aureus*. We have induced arthritis and investigated the hematogenous translocation of the primary infection to the aortic valve.

This model of endocarditis mimics the natural pathway of endocarditis better than the conventional method, where a high intravenous challenge dose of microorganisms for direct valvular infection has to be given.

MATERIALS AND METHODS

Rats

Female pathogen-free Wistar rats weighing approximately 185 g were used in this study. They were fed laboratory chow and water ad libitum under standard conditions of temperature and light, and were housed five per cage.

Bacterial strain and culture conditions

S. aureus strain Phillips was used for infection [15]. Bacteria were cultured in Luria–Bertani (LB) medium overnight at 37°C, and then washed in phosphate-buffered saline (PBS), adjusted to the desired concentration and kept in aliquots at −70°C. The viable count was determined before freezing at −70°C and prior to each experiment. The same batch of strain Phillips was used for all experiments.

Induction of septic arthritis of the TMJ

The rats were anesthetized by intramuscular injection of 0.5 mL/kg fentanyl citrate (0.315 mg/mL) and 0.5 mL/kg of fluanisone (10 mg/mL) (Hypnorm, Janssen, Animal Health, Division of Janssen-Cilag LTD, Saunderton, High Wycombe, UK) per kilogram. Under aseptic conditions, 0.1 mL of triamcinolone acetonide was administered by intra-articular injection into one of the TMJs. The contralateral joint was always used as control and received 0.1 mL of physiologic saline. After insertion of triamcinolone acetonide and saline, the rats were intravenously challenged with 1.0 mL of *S. aureus* (Phillips). All procedures were carried out under aseptic conditions. The rats were weighed and clinically examined daily for 1 week.

The overall clinical condition was evaluated, with assessment of weight, general appearance, alertness, and skin abnormalities of each rat. Arthritis was defined as visible joint swelling or erythema of the infected joint or in conjunction with a subsequent positive microbiological analysis from TMJ specimens.

Optimization of variables for induction of arthritis

The optimal sampling day was determined by using 14 rats, divided into four groups, receiving 0.01 mg of triamcinolone acetonide (0.1 mL) and 10^7 colony-forming units (CFU) of Phillips (1.0 mL). The animals were sacrificed on days 4 ($n=4$), 8 ($n=5$), 12 ($n=5$) and 15 ($n=5$). The TMJs and the spleen were aseptically removed for microbiological analysis.

The optimal dose of triamcinolone needed to produce the necessary conditions to facilitate hematogenous arthritis without causing severe clinical effects in non-infected animals was estimated in a pilot experiment. Five groups of 15 animals each were given an intra-articular injection into the TMJ with 0.1 mL containing 1, 0.1, 0.01, 0.001 or 0 mg triamcinolone acetonide, respectively. The contralateral joint was used as control, receiving 0.1 mL of PBS intra-articularly. All animals were then intravenously challenged with 10^7 CFU of strain Phillips (1.0 mL).

Another group of five animals received 1 mg of triamcinolone acetonide (0.1 mL) intra-articularly without any bacterial challenge to allow the study the clinical systemic effect of the highest dose used in the experiment.

The optimal challenge dose of *S. aureus* was determined by giving 42 rats 1 mg of triamcinolone acetonide intra-articularly (0.1 mL) (which was found to be the optimal dose in the pilot test described). Thirteen rats were given 10^5, 14 rats were given 10^6 and 15 rats were given 10^7 CFU of strain Phillips. The contralateral joint was used as control receiving 0.1 mL of PBS intra-articularly. The rats were observed clinically for 2 weeks and were then killed for microbiological sampling.

The percentage weight change subsequent to arthritis was determined, using 18 animals divided into two

groups. Animals in both groups were infected with 10^7 CFU of Phillips (1.0 mL). Animals in one group were also injected with 1.0 mg triamcinolone acetonide intra-articularly, whereas the control group was not given corticosteroid.

Estimation of bacteremia induced by arthritis was done by collecting blood samples from the carotid artery 6 days (three rats), 7 days (four rats) and 8 days (three rats) after induction of arthritis. The samples were serially diluted and cultured on blood agar plates for 24 h at 37°C.

Endocarditis, secondary to arthritis

Seven days post-challenge, sterile aortic vegetations were induced in the rats by introducing a polyethylene catheter through the right carotid artery as described previously [8,16]. After 24 h with the catheter left in place, the animals were killed. The right and left TMJs, aortic valvular vegetations and the spleen were aseptically removed for microbiological analysis.

Vegetations and spleens were homogenized with a teflon homogenizer in sterile saline. The homogenized tissues were then serially diluted in saline and plated onto blood agar plates and cultured for 24 h at 37°C. The TMJs were homogenized by vigorous vortexing with glass beads, diluted serially and plated onto blood agar plates. Numbers of CFU per piece of extirpated tissue were expressed as $\log_{10}$. Detection level was 2 CFU per piece of tissue. Endocarditis was defined as aortic vegetations with positive microbiological growth.

Arthritis was induced in 11 rats by intra-articular injection of 1 mg of triamcinolone acetonide and intravenous challenge by 10^7 CFU of *S. aureus*. On day 7, seven rats with established TMJ arthritis were catheterized via the carotid artery in order to induce vegetations on the aortic valve. Another four rats with arthritis were used as controls without catheterization. Samples from all the animals were taken 24 h later.

Statistical methods

Statistical comparisons of proportions were made by the chi-square test. All values of log CFU are reported as the mean ± standard deviation (SD).

RESULTS

Optimization of variables for induction of arthritis

The optimal timing for catheterization was determined (data not shown) to be 7 days after application of triamcinolone acetonide and bacterial challenge, signs of arthritis being at a maximum by day 7. It was also observed that after a period of 2 weeks, the affected animals began to recover from the infection and started to regain weight (data not shown). TMJs affected with arthritis displayed abscess formation involving bone tissue and a typical pathologic appearance.

To determine the optimal dose of triamcinolone acetonide, 60 rats were studied and, based on the rate of arthritis, 1 mg of triamcinolone acetonide was the dose chosen for further studies. The contralateral control joints remained intact (Table 1).

Eight animals, which were not given triamcinolone acetonide, did not develop TMJ arthritis. No signs of TMJ arthritis were found in animals (n=3)

Table 1 Infection rates with various doses of triamcinolone acetonide

Dose (mg) (no. of rats)	Steroid-treated TMJ[a] (log CFU/tissue)	Control TMJ (log CFU/tissue)	Spleen (log CFU/tissue)
1.0 (n=15)	14/15 (5.6±1.8)	3/15 (2.7±1.0)	12/15 (3.5±1.6)
0.1 (n=15)	3/15 (3.7±2.4)	0/15 (0±0)	2/15 (2.5±1.44)
0.01 (n=15)	2/15 (3.6±1.8)	0/15 (0±0)	4/15 (1.3±0.8)
0.001 (n=15)	0/15 (0±0)	0/15 (0±0)	2/15 (2.5±1.4)

[a]Number of animals infected per total.
Mean values and SEs of log CFU/tissue are shown in parentheses.

Table 2 Infection rates with various challenge doses

Challenge dose (log CFU) (no. of rats)	Steroid-treated TMJ[a] (log CFU/tissue)	Control TMJ (log CFU/tissue)	Spleen (log CFU/tissue)
10^5 (n=13)	7/13 (6.3±0.9)	2/13 (2.1±0.2)	5/13 (4.6±1.5)
10^6 (n=14)	9/14 (6.6±0.8)	3/14 (3.1±0.7)	12/14 (4.4±2.1)
10^7 (n=15)	14/15 (5.6±1.8)	3/15 (2.7±1.0)	12/15 (3.5±1.6)

[a]Number of animals infected per total.
Mean values and SEs of log CFU/tissue are shown in parentheses.

receiving only triamcinolone acetonide (1 mg) but no bacterial challenge (data not shown).

Forty-two rats were used in order to establish the optimal challenge dose. Based on the rate of infection (infection dose$_{90-95}$), 10^7 CFU was the dose chosen for further studies (Table 2). The contralateral control joints had significantly lower rates of infection ($p<0.05$) and fewer bacteria were recovered compared to the infected rats.

All the animals lost weight postoperatively due to anesthesia. A comparison of percentage weight change showed that animals with active arthritis kept losing weight until days 12–13, whereas control animals that were only anesthetized regained weight from the second day (data not shown). For example, at day 7, animals with septic arthritis ($n=8$) lost 23% weight as compared with control animals ($n=10$) receiving only bacteria but no corticosteroid ($p<0.0001$).

Blood cultures

In a separate experiment, blood samples were taken on days 6, 7 and 8 after induction of septic arthritis from 10 animals. Eight of these rats were blood culture positive (Figure 1). The amount of bacteria in the 10 animals varied from 0 to $10^{3.5}$ bacteria per mL of blood. Nine of these animals developed arthritis in the corticosteroid-treated TMJs. No correlations between the quantity of bacteria in the affected TMJ, or the severity of the arthritis, and the amount of bacteria in the blood were found.

Nine of the spleens yielded positive cultures. One rat did not show any signs of either septicemia or arthritis.

Endocarditis

Arthritis was induced in 11 rats by 1 mg of steroid and a challenge dose of 10^7 CFU. Seven of these were catheterized on day 7 and all of these developed endocarditis as defined by positive bacterial growth on aortic valvular vegetations. In only two of the rats were the contralateral joints infected. Four rats with arthritis which were not catheterized did not develop endocarditis. Figure 2 shows the log number of total CFU recovered from joints, aortic valves and spleens.

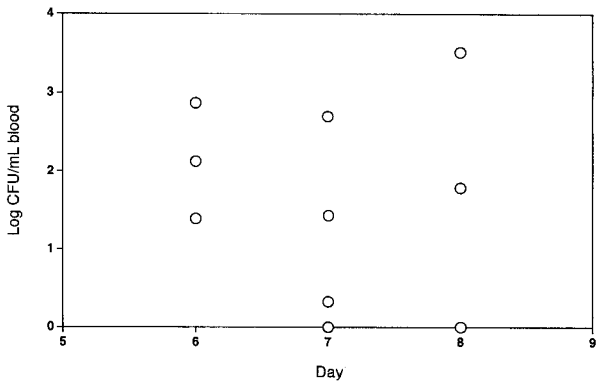

Figure 1 Log number of bacteria/mL blood for each animal, 6 ($n=3$), 7 ($n=4$), and 8 ($n=3$) days after induction of septic arthritis.

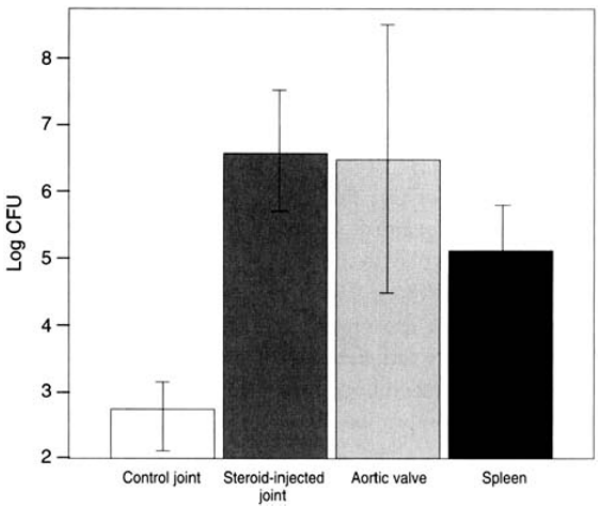

Figure 2 Log number of total bacteria recovered from both TMJs, spleen and aortic valve 24 h post-catheterization and 8 days post-challenge with 10^7 CFU of Phillips and 1 mg intra-articular injection of triamcinolone acetonide. Bars indicate mean values ± SD.

DISCUSSION

Intra- and periarticular corticosteroids for treatment of a variety of rheumatic diseases have been used for nearly 50 years, yet publications that have carefully examined the mechanisms of action, the pharmacokinetics and the comparative safety and efficacy of the available agents are sparse. Highly branched esters of triamcinolone or methylprednisolone are the preferred agents. Pharmacokinetic studies reveal that triamcinolone hexacetonide, the least soluble of all the corticosteroid esters, is retained in the joint for 2–3 weeks [17].

Corticosteroids have been shown to inhibit the synthesis of prostaglandins [18–20]. Glucocorticosteroids are therefore potent anti-inflammatory agents. Glucocorticoids also inhibit leukocyte accumulation and plasma exudation at sites of inflammation. Neutrophil adhesion to endothelial cells is blocked by these drugs, resulting in decreased trapping of neutrophils at inflammatory sites and peripheral blood neutrophilia.

A recent study performed by Verdrengh and Tarkowski emphasized the crucial protective role of neutrophils in the early phase of *S. aureus* infection [21]. Glucocorticoids have a variety of effects on other inflammatory cells, including macrophages, mast cells, and eosinophils [1].

The aim of this study was to develop a modified model of endocarditis secondary to septic arthritis in the rat. A glucocorticoid was given intra-articularly in the TMJ, followed by intravenous challenge with bacteria. Once the septic arthritis was established, on day 7, rats were catheterized for 24 h to induce valvular vegetations, and, in combination with the bacteremia caused by the septic condition, endocarditis resulted in all seven rats studied. In a separate experiment it was shown that eight of nine rats with septic arthritis indeed become bacteremic. In the conventional model of endocarditis, catheterization is done on day 0, challenge on day 1 and sampling at 12 h or later after challenge [16,22,23]. We have in a previous publication [22] reported that no difference in recovered CFU was found with the catheter taken out or left in place at the time of challenge, i.e. on day 1. Non-catheterized rats did not get endocarditis in this study. As in the conventional model of endocarditis, valvular vegetations thus represent a prerequisite for endocarditis to develop.

In the conventional model of rat endocarditis, we have previously found an ID_{50} at a challenge dose of 10^4–10^5 CFU per animal. This is in agreement with observations by others [24,25]. In the study presented here, in eight of 10 animals with septic arthritis, bacteremia was detected at days 6, 7 and 8 and the level of bacteremia was limited to $10^{0.5}$–$10^{3.5}$/mL blood. This level of bacteremia led to 100% endocarditis in a group of seven. We therefore conclude that the continuity, despite the low level, of bacteremia leads to endocarditis.

We have also noted previously [22] that when 10^6 –10^7 CFU were given, between $10^{1.5}$ and $10^{2.5}$ CFU could be recovered from the aortic valves 1 h after bacteria were given, and that no bacteria were left in circulation (unpublished finding). This means that only $1/10^{4.5}$ CFU, adheres during the first hour post-challenge. Consequently, at the ID_{50} dose of 10^4–10^5, very few bacteria attached to the valves are required for endocarditis. The limited numbers of bacteria in the bloodstream in the present model adhere to the vegetations either due to a higher adherence propensity or due to the longer time involved with constant generation of bacteremia, as compared with the situation in the conventional model.

Two of the contralateral joints (2/7) in the endocarditis experiment were shown to be infected but without clinical symptoms. The medical status of these animals indicated a general bacteremia involving most tissues and organs, including the control joints.

It should also be emphasized that the bacterial growth conditions in this model are different from the conditions in the conventional method of infective endocarditis. The bacteria giving rise to endocarditis in this model have multiplied under in vivo conditions. The establishment of the primary infection, septic arthritis, allows in vivo passage of the bacteria, in contrast to the case in the original model, where the bacteria are grown in vitro prior to challenge. The interaction between a host and a microbe during pathogenesis is a dynamic process. The infecting organism encounters diverse environmental conditions during its transition from an external reservoir to a host, as well as during the infectious process within the host. To be able to survive at various locations, the bacteria must be able to control the expression of essential features. The set of virulence factors that are required for survival at one location may confer severe disadvantages in another environment [26].

S. aureus is the most common organism causing septic arthritis in humans and one of the most important pathogens for the development of endocarditis. For experimental models of infectious arthritis, small animals have been selected because of convenience and cost.

In conclusion, we have developed a new model of septic arthritis and modified the model of endocarditis in the rat. The combination of the two models enables the investigator to explore unanswered questions concerning the initiating events of the infection, such as bacterial adherence and the role of other virulence factors required for the translocation of infection from one site to another. The important aspects of the new model of endocarditis can be summarized as follows: (1) endocarditis is caused by bacteria generated by a primary infection, not by the challenge itself; (2) bacteria causing endocarditis are cultivated in vivo, not in vitro; (3) a lower bacteremic level is sufficient; and (4) bacteremia and development of catheter-induced vegetations are simultaneous, whereas in the conventional model, vegetations are induced first, followed by bacterial challenge.

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