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Preferential Induction of Septic Arthritis and Mortality by Superantigen-Producing Staphylococci

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Staphylococcal enterotoxins A through D (SEA through SED) and toxic shock syndrome toxin-1 display superantigen properties, i.e., they stimulate a great fraction of T cells expressing certain T-cell receptor Vβ sequences. Using a newly established rat model of septic *Staphylococcus aureus* arthritis, we have recently shown that an *S. aureus* strain producing SEA showed marked arthritogenic properties. In the present study we decided to employ another five *S. aureus* strains, each one producing a distinct exotoxin. Almost all rats injected with superantigen-producing strains developed arthritis. In contrast, only 20% of rats injected with an *S. aureus* strain lacking superantigen production displayed mild and transient arthritis. Mortality was preferentially induced among the rats inoculated with the *S. aureus* strains producing SEB and SED. This study emphasizes that superantigen production is an important virulence factor in the development of septic *S. aureus* arthritis. Differences concerning mortality between staphylococcal strains producing different exotoxins may be dependent on the degree of activation of the immune system.

Staphylococcal enterotoxins A through D (SEA through SED) and toxic shock syndrome toxin-1 (TSST-1) display superantigen properties, i.e., they stimulate a great fraction of T cells expressing certain T-cell receptor Vβ sequences (15). We have recently reported that a *Staphylococcus aureus* strain producing SEA induced severe arthritis in rats (6). The disease was characterized by T-lymphocyte infiltration in the joints and could be ameliorated, in vivo, by T-cell depletion (6). Also in mice, TSST-1 production by *S. aureus* has proved to be an arthritogenic factor (3), leading to a clonal expansion of T cells in affected joints (1). The aim of the present study was to evaluate if other exotoxins display similar virulence with respect to the development of arthritis.

We have used a recently established rat model of intravenously induced *S. aureus* infection (6) to study the influence of different superantigen-producing staphylococcal strains on the development of inflammation, arthritis, and mortality.

Outbred, healthy 3-month-old female Sprague-Dawley rats, obtained from ALAB (Stockholm, Sweden), were used throughout the study. They were housed in the animal facility at the Department of Clinical Immunology, University of Göteborg, Sweden, under standard conditions of light and temperature and fed standard laboratory chow and water ad libitum. The rats were housed five in each cage and kept in the animal house for 1 to 2 weeks before use.

Six different *S. aureus* strains, including five different superantigen-producing strains and one non-superantigen-producing control strain, were used in the study. *S. aureus* AB-1, producing SEA, was originally isolated from a swollen talocrural joint of a Sprague-Dawley rat (6). *S. aureus* NCTC 10654 (ATCC 14458) (10), producing SEB; NCTC 10655 (ATCC 19095) (4), producing SEC; and NCTC 10656 (ATCC 23235) (9), producing SED, were obtained from the Culture Collection of the University of Göteborg. *S. aureus* LS-1, producing TSST-1, was originally isolated from a swollen mouse joint (7). As a control strain lacking superantigen production, *S. aureus* 8325-4, a kind gift from T. J. Foster, University of Dublin, Dublin, Ireland, was used. This strain is a derivative of NCTC 8325 cured of prophages (13). *S. aureus* 8325-4 produces significant amounts of alpha-, beta-, gamma-, and delta-hemolysin; lipase; hyaluronate lyase; staphylokinase; metallo- and serine-proteinase; and acid phosphatase but does not produce detectable amounts of enterotoxins or TSST-1.

Bacteria were kept frozen at −20°C, in phosphate-buffered saline (PBS; 0.13 M NaCl, 10 mM sodium phosphate [pH 7.4]) containing 5% bovine serum albumin and 10% dimethyl sulfoxide (C_2H_6OS), until use. Before use, the bacterial solution was thawed and washed in PBS. Viable counts were used to check the number of bacteria in each bacterial solution.

The rats were inoculated intravenously with 10^9 CFU of the above-mentioned *S. aureus* strains per rat and monitored individually. Limbs were inspected visually at regular intervals. Arthritis was defined as visible joint swelling and/or erythema of at least one joint (6). The overall condition was evaluated by assessment of weight, general appearance, alertness, and skin abnormalities. The animals were sacrificed 12 days after bacterial inoculation.

Serum interleukin-6 (IL-6) levels were estimated by using the cell line B13.29, subclone B9, which is dependent on IL-6 for its growth, by a method previously described (5). Levels of total immunoglobulin G (IgG) and IgM in serum were measured by an enzyme-linked immunosorbent assay (ELISA), using rabbit anti-rat IgG and IgM as the antisera and appropriate immunoglobulin standards (Zymed Laboratories, San Francisco, Calif.). Levels of IgG and IgM rheumatoid factors in serum were measured by diffusion-in-gel ELISA (11) as previously described (16).

The differences between mean values were tested for significance by the use of the two-tailed Student's *t* test. All values reported are means ± standard errors of the mean, unless stated otherwise.

The rats injected with superantigen-producing *S. aureus* displayed arthritis in almost 100% of the cases (Table 1). By contrast, *S. aureus* 8325-4, without superantigen production, induced mild and transient arthritis in only 20% of the rats. Notably, *S. aureus* strains producing SEB and SED gave rise to

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TABLE 1. Superantigen-producing staphylococci preferentially induce arthritis and mortality

S. aureus strain	No. of rats	Superantigen produced	Frequency of arthritis (%)	Cumulative mortality (%)
8325-4	5	None	20	0
AB-1	10	SEA	90	0
NCTC 10654	5	SEB	100	60
NCTC 10655	5	SEC	100	0
NCTC 10656	5	SED	80	40
LS-1	5	TSST-1	100	0

60 and 40% mortality, respectively, whereas infection with the other staphylococcal strains did not induce mortality at all.

Bacterial inoculation with superantigen-producing staphylococci induced a significant increase in the level of IL-6 in serum in contrast to inoculation with the control strain (Fig. 1). Serum IgG and IgM levels were also elevated (data not shown). Marked increases in the levels of IgM and IgG rheumatoid factors in serum (Fig. 2), interpreted as a polyclonal B-cell activation, were noted 12 days after bacterial inoculation. Notably, superantigen-producing bacterial strains and the control strain displayed no differences with regard to B-cell activation, despite different clinical courses of the disease.

The arthritogenicity of superantigen-producing staphylococci is well documented in this study. The reason for the arthritogenicity should be sought in the physiological function of superantigens, i.e., the stimulation of a great fraction of T cells expressing certain T-cell receptor Vβ sequences. The arthritogenicities for all the different superantigen-producing staphylococci were similar. Since different superantigens use different Vβ families, when interacting with the T-cell receptor, the results would indicate that no specific Vβ family is more arthritogenic than another. Instead, one could speculate that the number of activated T cells with subsequent stimulation of other cells, e.g., monocytes, granulocytes, or endothelial

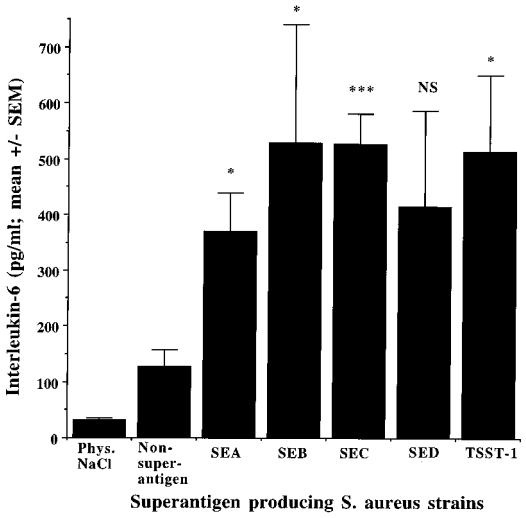

FIG. 1. Serum IL-6 levels in Sprague-Dawley rats 12 days after a single intravenous inoculation of 10^9 CFU of *S. aureus* 8325-4 (control; non-superantigen producer), AB-1 (SEA), NCTC 10654 (SEB), NCTC 10655 (SEC), NCTC 10656 (SED), or LS-1 (TSST-1), respectively. All comparisons are performed with regard to the *S. aureus* 8325-4 inoculated group (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant). Due to mortality, groups inoculated with NCTC 10654 and NCTC 10656 comprise two and three animals respectively. The other groups comprise 5 to 10 rats. Phys. NaCl, physiological saline.

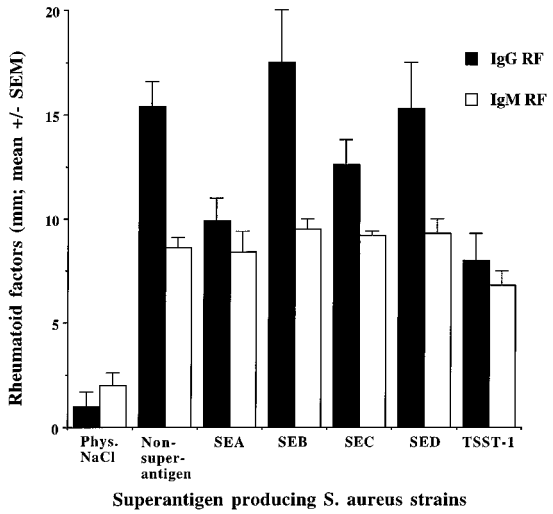

FIG. 2. Levels of IgM and IgG rheumatoid factors (RF) in sera of Sprague-Dawley rats 12 days after a single intravenous inoculation of 10^9 CFU of *S. aureus* 8325-4 (control; non-superantigen producer), AB-1 (SEA), NCTC 10654 (SEB), NCTC 10655 (SEC), NCTC 10656 (SED), or LS-1 (TSST-1), respectively. Due to mortality, groups inoculated with NCTC 10654 and NCTC 10656 comprise two and three animals respectively. The other groups comprise 5 to 10 rats. Phys. NaCl, physiological saline.

cells, is of importance for the induction and persistence of *S. aureus* arthritis. Indeed, in previous studies we have demonstrated the active participation of T cells in joint inflammation (1, 2, 6). The control, non-superantigen-producing *S. aureus* strain also gave rise to a mild and transient arthritis in 20% of the animals. Thus, bacterial factors other than superantigens might be of importance in the pathogenesis of septic arthritis. In this respect we have recently shown that staphylococcal collagen adhesin expression is of significance for the induction and progression of arthritis (14).

Interestingly, rats exposed to superantigen-producing staphylococci displayed a significantly increased production of IL-6. This finding could simply mirror an intense inflammatory reaction as a consequence of severe arthritis. Indeed, intraarticular administration of recombinant IL-6 does not induce arthritis in naive animals (results not shown). However, IL-6 may trigger bone destruction by recruitment of osteoclasts, thereby stimulating bone resorption (12). In addition, we have recently demonstrated that IL-6-deficient mice will not develop a severe destructive arthritis upon inoculation with superantigen-producing staphylococci (12a). Thus, it may be hypothesized that IL-6 does not initiate the arthritic process but accelerates and aggravates the already ongoing joint inflammation.

The role of polyclonal B-cell activation and autoantibody production in septic arthritis remains unclear, since all the staphylococcal strains, irrespective of the capacity to produce superantigens and hence of arthritogenic properties, gave rise to a similar degree of B-cell activation.

We conclude that bacterial superantigen production is a virulence determinant in septic *S. aureus* arthritis. Differences concerning mortality between bacterial strains producing different exotoxins may be dependent on the intensity of activation of the immune system. The correlation between serum IL-6 levels and arthritis may speak in favor of IL-6 as an arthritogenic factor.

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