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Protective Role of Sialophorin (CD43)-Expressing Cells in Experimental *Staphylococcus aureus* Infection

TOMAS BREMELL,[1]* RIKARD HOLMDAHL,[2] AND ANDRZEJ TARKOWSKI[1]

Departments of Clinical Immunology and Rheumatology, University of Göteborg, Göteborg,[1] and Department of Medical Inflammation Research, University of Lund, Lund,[2] Sweden

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Sialophorin (CD43) is a major surface mucin on most hematopoetic cell lineages, including phagocytes. Defects of CD43 expression occur in Wiskott-Aldrich syndrome, a disease characterized by susceptibility to pyogenic infections. In a newly established rat model of septic *Staphylococcus aureus* arthritis, we have investigated the role of CD43-expressing cells in the progression of the disease. A single injection of a monoclonal antibody specific for CD43 induced a highly erosive course of arthritis and increased mortality in animals exposed to a suboptimal dose of bacteria. Our results demonstrate that sialophorin-expressing cells play a protective role in the early stage of staphylococcal infection.

Sialophorin (leukosialin, CD43) is a major surface mucin expressed on many hematopoetic cells. It interferes with lymphocyte function-associated antigen 1–intracellular adhesion molecule 1 interaction (3, 13) and is involved in polymorphonuclear cell adhesion to endothelial cells (7). A deficiency and/or defect of the surface sialophorin expression is a hallmark of Wiskott-Aldrich syndrome, a disease characterized by susceptibility to pyogenic infections (12). However, the precise role of sialophorin-expressing cells in the disease process still remains unclear.

We have used a recently established rat model of intravenously (i.v.) induced *Staphylococcus aureus* infection (5) to study, in vivo, the influence of sialophorin-expressing cells on the outcome of septicemia and septic arthritis. The rationale of this investigation is an impairment of host defense mechanisms in the pathogenesis of septic arthritis (9). We demonstrate that a single pretreatment of *S. aureus*-inoculated rats with the monoclonal antibody specific for CD43 leads to malaise, severe arthritis, and death within a few days. Thus, our results indicate that CD43-expressing cells play a protective role in *S. aureus* infection.

Outbred Sprague-Dawley rats were obtained from ALAB (Stockholm, Sweden) and used throughout the study. They were inoculated i.v. with *S. aureus* AB-1, originally isolated from a swollen talocrural joint of a Sprague-Dawley rat (5). All of the rats were monitored individually. Limbs were inspected at regular intervals. Arthritis was defined as visible joint swelling or erythema of at least one joint (4, 5). The overall condition was evaluated by assessment of weight, general appearance, alertness, and skin abnormalities.

The monoclonal antibody W3/13 (anti-sialophorin antibody, anti-CD43), recognizing surface determinants on rat T lymphocytes, monocytes, polymorphonuclear cells, NK cells, and a subset of B lymphocytes (15), was injected i.v. (0.75 mg per rat) at specified intervals. Analyses of leukocytes, platelets, and differential counts were performed with a Sysmex analyser (K-1000; Toa Medical Electronics, Kobe, Japan). Serum interleukin 6 levels were estimated by using the cell line B13.29, subclone B9, which is dependent on interleukin 6 for its growth, by a method described previously (4).

Bacterial sampling and tests for catalase and coagulase activities were performed as described previously (6).

The histopathological processing included routine fixation, decalcification, paraffin embedding, and staining with hematoxylin and eosin (4). Tissue sections from ankles, tarsal bones, toes, wrists, carpal bones, and fingers were studied.

To analyze T-cell-dependent inflammatory reactivity in vivo, rats were sensitized and given an epicutaneous booster application of 4-ethoxymethylene-2-phenyloxazolone (oxazolone; BDH Chemicals, Poole, United Kingdom) as described previously (8). The thicknesses of both ears were measured before and 24 h after challenge with an Oditest spring caliper (Kröplin, Hessen, Germany). The intensity of the delayed-type hypersensitivity reactions was expressed as (ear thickness at 24 h − ear thickness at 0 h) $\times 10^{-3}$ cm.

To test T-cell-independent inflammatory reactivity in vivo, rats were exposed to an intradermal injection of 100 μl of olive oil (Apoteksbolaget AB, Göteborg, Sweden) in the left hind footpad as described previously (10). Before and 24 h after the olive oil injection, the thickness of the footpad was measured with an Oditest spring caliper (Kröplin). The inflammatory response was expressed as the increase of footpad thickness in 10^{-3} cm.

In the first experiment, the rats treated with the anti-CD43 antibody 1 h before and 4 days after the i.v. challenge with 5×10^8 CFU of *S. aureus* AB-1 developed severe illness. Two of five animals died, and histopathological evidence of erosive arthritis was noted in the remaining three. In contrast, in the control group, which was treated with mouse immunoglobulin G i.v. and challenged with the same dose of *S. aureus*, there were no deaths and three of five rats displayed erosive arthritis. The anti-CD43-treated rats displayed significant weight loss in comparison with the controls, who gained weight (results not shown).

In the second set of experiments, we used a suboptimal dose of bacteria (1/10 of the dose in the first experiment) and a single pretreatment with anti-CD43 antibodies. The rats inoculated with 5×10^7 CFU of *S. aureus* developed severe illness with clinical arthritis in 90% of the cases as compared with 30% of the control animals. While the control rats exhibited a significant weight gain (+22%), the anti-CD43-pretreated animals lost weight (−19%) during the 5-day experiment. Also,

* Corresponding author. Mailing address: Department of Clinical Immunology, Guldhedsgatan 10, S-413 46 Göteborg, Sweden. Phone: 46 31 60 10 00. Fax: 46 31 82 67 91.

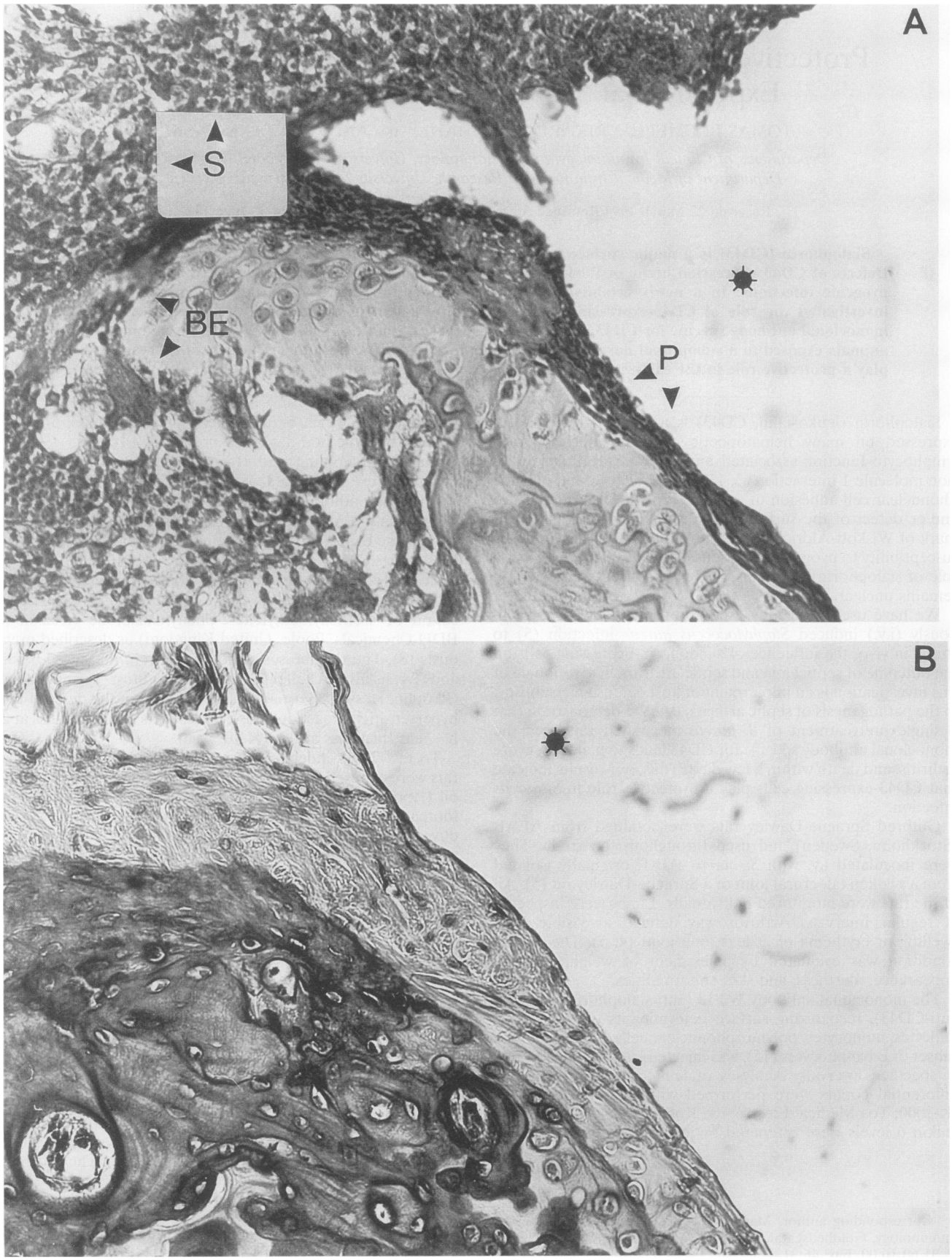
A
S
BE
P
B

FIG. 1. (A) Micrograph showing an arthritic knee joint of a male 6-week-old Sprague-Dawley rat administered anti-CD43 antibodies and inoculated i.v. with *S. aureus* AB-1. There is synovial hypertrophy (S), pannus formation (P), and bone erosion (BE) at the cartilage-synovial tissue junction. (B) For comparison, a micrograph of a morphologically intact knee joint of a control rat that received a matching dose of *S. aureus* is shown. The joint cavity is marked with an asterisk. Magnification, ×64.

3 of 10 anti-CD43-pretreated rats died during the experiment (one after 2 days and two after 5 days), while no rats in the control group died. The histopathological examination revealed that 7 of 8 anti-CD43-pretreated rats had erosive arthritis with pannus formation (synovial tissue overlying joint cartilage) and cartilage and bone erosions (Fig. 1), as compared with 2 of 10 control rats ($P < 0.01$). There was an extensive infiltration of polymorphonuclear cells and mononuclear cells into the synovia, with polymorphonuclear cells being the dominant infiltrating cell type. Extra-articular manifestations were recorded in 6 of 8 anti-CD43-treated rats, as compared with 1 of 10 control rats ($P < 0.01$). *S. aureus* growth in joints was detected in 6 of 8 anti-CD43-pretreated rats compared with 2 of 10 control rats ($P < 0.01$) (Table 1). There were significant increases of circulating leukocytes, mainly neutrophils. In contrast, the numbers of lymphocytes and platelets were not altered by pretreatment with the anti-CD43 antibody. Moreover, there was a significant increase of serum IL-6 among anti-CD43-pretreated rats (477 ± 194 pg/ml), as compared with controls (73 ± 25 pg/ml; $P < 0.001$) (Table 1).

In the third set of experiments, we analyzed the impact of anti-CD43 antibodies on T-cell-dependent and -independent inflammatory responses in noninfected rats. The delayed-type hypersensitivity response to oxazolone, a T-cell-dependent inflammatory reaction, was $(42.0 \pm 3.0) \times 10^{-3}$ cm for the phosphate-buffered saline-challenged rats in comparison with $(18.3 \pm 5.1) \times 10^{-3}$ cm ($P = 0.002$) for the rats receiving anti-CD43 antibodies, indicating a moderate T-cell suppression caused by the anti-CD43 antibody. There were no differences between the groups with regard to footpad swelling in response to olive oil, a T-cell-independent inflammatory stimulus. Finally, administration of anti-CD43 antibodies alone to healthy rats did not cause any clinically visible disease manifestations.

In the present study, we addressed the question of whether in vivo interaction with cells expressing CD43 modulates the course of infection. We demonstrated that a single administration of a monoclonal antibody specific for CD43 significantly alters the course of disease by increasing both morbidity and mortality. This outcome is not unexpected since patients with defects of CD43 expression (Wiskott-Aldrich syndrome) display increased susceptibility to pyogenic infections (12). In vivo interaction with CD43-expressing cells giving rise to dramatically increased susceptibility to infections is, to our knowledge, the first experimental model of human Wiskott-Aldrich syndrome.

The CD43 molecule is expressed on lymphocytes as well as on phagocytic cells of the immune system. Thus, interaction with the CD43 molecule may potentially affect a multitude of immunologic effector functions during an infection. Large numbers of phagocytic cells of both monocyte and granulocyte lineages can be seen already within 24 h in the joints of rats and mice inoculated i.v. with *S. aureus* (4, 5). These CD43-expressing cells may be assumed to exert the first-line defense during early infection. Thus, the early accelerated course of septic arthritis in the anti-CD43-pretreated rats speaks in favor of disturbance within the phagocytic cell population. Hypothetically, homing and extravasation of phagocytes, known to require intracellular adhesion molecule 1–lymphocyte function-associated antigen 1 interaction, might have been altered by the interaction with the sialophorin antibody (13). Alternatively, bacterial phagocytosis, another major function of phagocytes, could have been affected by the interaction with the CD43 molecule. We have both circumstantial and experimental evidence indicating that the migratory and extravasating capacity of phagocytic cells remains unaltered despite infusion of the anti-CD43 antibody. First, in rats administered the anti-CD43 antibody, large numbers of synovial tissue-infiltrating granulocytes were noted (Fig. 1). Second, the migratory capacity of phagocytic cells to the peritoneal cavity in response to glycogen challenge (14) in rats exposed to anti-CD43 antibodies is intact, as compared with that of control animals (unpublished results). Third, the granulocyte-dependent inflammatory response to olive oil is not affected by in vivo interactions with the CD43 molecule. Consequently, we believe that monoclonal antibodies to the CD43 molecule interact with the process of phagocytosis. A significantly increased bacterial count in the target organ of infection (Table 1) supports this notion. Other cells besides the phagocytes, such as T lymphocytes (11), might also have been influenced by the anti-CD43 treatment. The role of T lymphocytes in murine septic arthritis has been investigated extensively by us (1, 2, 5).

TABLE 1. Increased frequency of erosive arthritis in male, 6-week-old, Sprague-Dawley rats pretreated with a single i.v. injection of sialophorin (CD43)-specific monoclonal antibody W3/13 and then challenged with a single i.v. injection of 5×10^7 CFU of *S. aureus* AB-1 per rat

Rats[a]	Clinical features		Histopathology[b]		Growth of *S. aureus* in joints[b]	Hematology		
	Body wt (g [mean ± SD])[c]	No. of rats dead/total no.	Arthritis[d]	Extra-articular manifestations		No. of neutrophils $(10^3)/mm^3$[e]	No. of lymphocytes $(10^3)/mm^3$[e]	IL-6 (pg/ml)[e,f]
Anti-CD43 injected	121 ± 16	3/10	7/8	6/8	6/8	6.0 ± 3	5.6 ± 2.0	477 ± 194
Control, IgG injected	186 ± 12	0/10	2/10	1/10	2/10	1.4 ± 0.6	7.6 ± 2.1	73 ± 25
P	<0.001	NS	<0.01	<0.01	<0.01	<0.001	NS	<0.001

[a] $n = 7$ to 10 (in each group).
[b] Number of rats exhibiting characteristic/total number.
[c] Body weight of rats at day zero: anti-CD43-injected rats, 149 ± 6 g; mouse immunoglobulin G-injected controls, 153 ± 6 g.
[d] All of the arthritic mice displayed erosive changes.
[e] Mean ± standard deviation.
[f] IL-6, interleukin 6.

These studies demonstrate that suppression of T-cell activation clearly alleviates the morbidity and mortality in this condition. Thus, interaction with sialophorin molecules on T lymphocytes, leading to T-cell depression, should be beneficial rather than harmful to the host during staphylococcal infection.

To summarize, our results demonstrate that sialophorin-expressing cells play a protective role in the early stage of staphylococcal infection.

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