INFECTION AND IMMUNITY, July 1996, p. 2804–2807 Vol. 64, No. 7
0019-9567/96/$04.00+0

NOTES

Role of Intercellular Adhesion Molecule 1 in Pathogenesis of Staphylococcal Arthritis and in Host Defense against Staphylococcal Bacteremia

MARGARETA VERDRENGH,[1]* TIMOTHY A. SPRINGER,[2] JOSE-CARLOS GUTIERREZ-RAMOS,[2] AND ANDREJ TARKOWSKI[1,3]

Departments of Clinical Immunology[1] and Rheumatology,[3] University of Göteborg, Göteborg, Sweden, and Center for Blood Research, Harvard Medical School, Boston, Massachusetts[2]

Received 25 January 1996/Returned for modification 13 March 1996/Accepted 5 April 1996

Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin superfamily that interacts with two integrins, LFA-1 and Mac-1. These interactions are critical for leukocyte extravasation into inflamed tissue. To assess the role of ICAM-1 expression in the pathogenesis of bacterial infection, homozygously mutant mice lacking the ICAM-1 gene were exposed to *Staphylococcus aureus*. Within 6 days after inoculation 50% of the animals in the ICAM-1$^{-/-}$ group, but none of the controls, had died. Despite the high level of mortality, ICAM-1$^{-/-}$ mice developed less frequent and less severe arthritis than their wild-type littermates. In agreement, normal mice inoculated with staphylococci and administered anti-ICAM-1 antibodies exhibited a higher frequency of mortality but less severe arthritis than the controls. Our results indicate that ICAM-1 on the one hand provides protection against systemic disease but on the other hand aggravates the local disease manifestation.

Intercellular adhesion molecule 1 (ICAM-1) is an integral membrane protein that is a member of the immunoglobulin superfamily (12). ICAM-1 plays an important role in cell adhesion and migration, as well as in immune responses. Under normal conditions, the level of ICAM-1 expression is quite low, but it is upregulated during inflammation by a variety of cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and gamma interferon (IFN-γ) (13). The counterreceptors for ICAM-1 are the β_2 integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18). We have recently established a superantigen-enhanced and T-cell-mediated (1, 2, 5) model of *Staphylococcus aureus* septicemia and arthritis in which a single intravenous inoculation of the toxic shock syndrome toxin 1 (TSST-1)-producing *S. aureus* strain LS-1 within days leads to a rapid deterioration of joints (3, 9). One of the hallmarks of the disease is a rapid extravasation of phagocytic cells and T lymphocytes into the synovial tissue (5, 8), which in *S. aureus*-infected mice contains a plethora of activated and clonally expanded Vβ11$^+$ CD4$^+$ cells. These cells produce large amounts of IFN-γ, an arthritogenic cytokine (22). The aim of this study was to assess the role of ICAM-1 expression in the pathogenesis of *S. aureus* septicemia and arthritis. Homozygous female ICAM-1 mutant (ICAM-1$^{-/-}$) and wild-type (ICAM-1$^{+/+}$) mice were obtained as previously described (20). Swiss mice were used in the monoclonal antibody depletion experiments. Hybridoma cell line YN1/1.7.4, obtained from the American Type Culture Collection (Rockville, Md.), produces a rat immunoglobulin G2b (IgG2b) antibody specific for mouse ICAM-1. A rat IgG2a antibody specific for ovalbumin was used as a control antibody.

S. aureus LS-1 was originally isolated from a swollen joint of a spontaneously arthritic NZB/W mouse (7).

Twelve mice deficient in ICAM-1 and 12 wild-type littermates were injected intravenously with an amount of *S. aureus* corresponding to 4×10^6 CFU per mouse. They were sacrificed on day 6 after inoculation of bacteria.

In another experiment, 45 Swiss mice were given 2×10^7 CFU of an *S. aureus* suspension. Three days after the inoculation, 25 mice were injected intraperitoneally with 0.2 mg of anti-ICAM-1 antibody (YN1/1.7.4.), and 20 mice were injected with 0.2 mg of control rat IgG. The antibodies were administered three times per week until sacrifice 14 days later.

Arthritis was defined as visible joint swelling and/or erythema of at least one joint. A clinical evaluation of arthritis was carried out with a system in which macroscopic evaluation of arthritis yields a score of 0 to 3 for each paw. An arthritic index was constructed by the addition of scores for all four paws in each mouse (1).

Histopathological sections of the joints were evaluated with regard to synovial hypertrophy, pannus formation, and cartilage and subchondral bone destruction. The phagocytic activities of circulating monocytes and granulocytes in healthy ICAM-1-deficient and wild-type congenic mice were determined by incubation of fluorescein isothiocyanate-labeled bacteria (Orpegen Pharma, Heidelberg, Germany), and the fluorescence was recorded with a FACScan (Becton-Dickinson, Mountain View, Calif.). Leukocyte counts were determined in a hemacytometer (Toa Medical Electronics, Kobe, Japan). Splenic mononuclear cells from healthy mutant and wild-type mice were isolated and suspended at a concentration of 10^6/ml in 24-well culture dishes in Iscove's medium (Gibco, Paisley, United Kingdom) supplemented with 10% heat-inactivated fe-

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

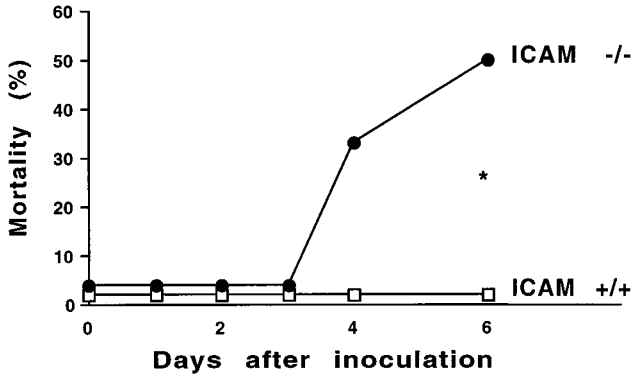

FIG. 1. Mortality in 12 ICAM-1-deficient and 12 wild-type mice after inoculation with *S. aureus*. Six days after intravenous bacterial injection, 50% of the mutants but none of the normal congenic mice had died. *, $P < 0.05$.

tal calf serum, L-glutamine, mercaptoethanol, and gentamicin. The cell suspensions were incubated with 2.5 μg of concanavalin A (ConA) per ml, 10 μg of TSST-1 (Toxin Technology, Madison, Wis.) per ml, or 10^7 CFU of formalin-killed *S. aureus* LS-1 per ml. The proliferation assay was performed in 96-well microtiter plates, with culture for 72 h. During the last 12 h, 1 μCi of [^{3}H]thymidine per well was added. The analysis of levels of IFN-γ, TNF-α and IL-6 in sera and supernatants was performed by enzyme-linked immunosorbent assay or bioassay as previously described (21, 23). Levels of total immunoglobulin, IgG1, IgG2a, IgG2b, IgG3, and IgM in serum were measured by the single radial immunodiffusion technique (16).

Statistical analysis of the differences between means was carried out with Student's two-tailed *t* test. The chi-square test was used to analyze categorical data. *P* values of below 0.05 are regarded as significant.

Intravenous inoculation of ICAM-1-deficient mice with *S. aureus* resulted in a high level of mortality. At the termination of the experiment, 6 days after the bacterial inoculation, 6 of 12 ICAM-1-deficient mice were dead. With a single exception, the remaining animals did not develop arthritis. In the wild-type group, all 12 animals stayed alive (Fig. 1). However, the animals in the wild-type group developed a more prevalent and more severe arthritis than the mutants (Fig. 2). Two days after bacterial injection, 6 of 12 wild-type mice but only 1 of 12 ICAM-1 mutant mice displayed clinical signs of arthritis. Four days later, at the time of sacrifice, 5 of 12 in the wild-type group and 1 of 6 in the ICAM-1-deficient group showed clinical arthritis (Fig. 2A). When the joints were analyzed for histopathological signs of arthritis, the mice in the ICAM-1 mutant group exhibited less destruction of joint cartilage and bone (33 versus 45%) and less hypertrophy of synovial tissue (50 versus 82%) than the wild-type littermates. In addition, three mice from the wild-type group but none from the ICAM-$1^{-/-}$ group displayed osteitis and extra-articular granulomas. The histopathological findings are thus in agreement with the clinical findings, where the wild-type mice were found to have signs of a more severe arthritis.

No significant differences were found between the wild-type and the mutant groups with respect to bacterial growth in the spleen, kidneys, blood, or joints (data not shown). Table 1 shows levels of cytokines and immunoglobulins in serum during *S. aureus* infection in ICAM-1-deficient mice and wild-type littermates. There were no significant differences with respect to levels of circulating IFN-γ or IL-6 between the two groups. In contrast, ICAM-1-deficient mice displayed significantly higher levels of IgG2a ($P < 0.05$) and IgM ($P < 0.05$).

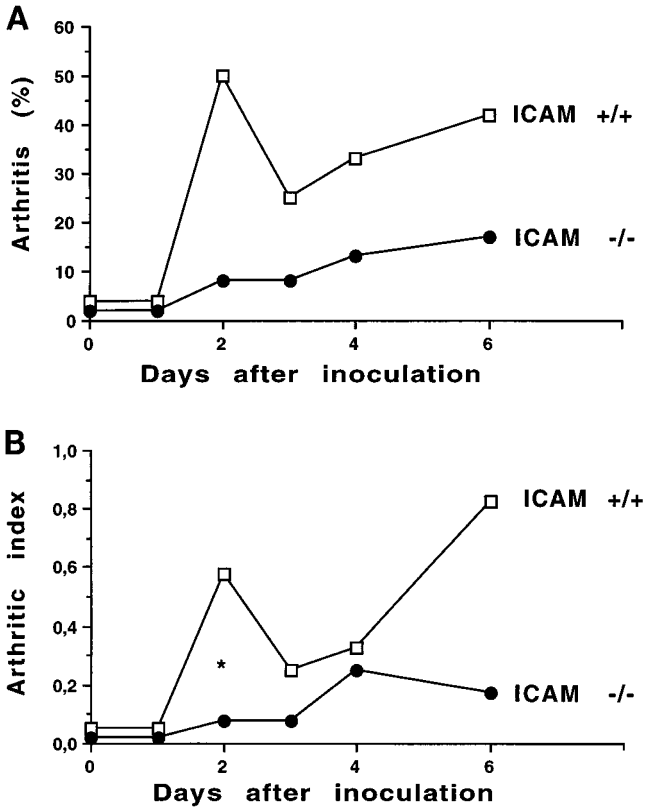

FIG. 2. Prevalence (A) and severity (B) of arthritis in 12 ICAM-1-deficient and 12 wild-type mice after a single intravenous injection of *S. aureus* LS-1. The details of the clinical evaluation are presented in the text. The number of animals in the ICAM-$1^{-/-}$ group decreases with time because of mortality.

Spleen cells from ICAM-1-deficient and wild-type congenic animals proliferated similarly in response to the T-cell mitogen ConA and the superantigen TSST-1. In contrast, formalin-killed staphylococci induced significantly higher proliferative responses in ICAM-$1^{+/+}$ mice than in ICAM-$1^{-/-}$ mice (14,774 ± 3,353 versus 5,790 ± 825 cpm; $P < 0.05$). As expected from the in vivo results, IFN-γ production in vitro in response to *S. aureus* was higher in spleen cells from ICAM-$1^{+/+}$ mice than in those from ICAM-$1^{-/-}$ mice (1,029 ± 292 versus 409 ± 63 U/ml; $P < 0.05$). IFN-γ production in response to ConA and TSST-1 was not significantly different in the two

TABLE 1. Levels of cytokines and immunoglobulins in serum during *S. aureus* infection in ICAM-1-deficient mice ($n = 5$) and wild-type littermates ($n = 11$)

Parameter (unit)	Value (mean ± SEM) for:		*P*
	ICAM-$1^{+/+}$ mice	ICAM-$1^{-/-}$ mice	
IFN-γ (U/ml)	1,636 ± 373	900 ± 437	NS[a]
TNF-α (pg/ml)	<1.4	<1.4	
IL-6 (pg/ml)	1,557 ± 196	1,545 ± 166	NS
IgG1 (μg/ml)	848 ± 212	1,296 ± 150	NS
IgG2a (μg/ml)	1,902 ± 215	3,376 ± 733	<0.05
IgG2b (μg/ml)	1,256 ± 188	1,576 ± 16	NS
IgG3 (μg/ml)	1,324 ± 96	1,348 ± 91	NS
IgM (μg/ml)	545 ± 108	1,136 ± 189	<0.05

[a] NS, not significant.

groups of mice (data not shown). In contrast, ICAM-$1^{+/+}$ mice displayed lower in vitro production of TNF-α in response to ConA (53 ± 16 versus 130 ± 57 pg/ml), *S. aureus* (63 ± 13 versus 80 ± 16 pg/ml), and TSST-1 (66 ± 15 versus 126 ± 23 pg/ml) ($P < 0.05$) than did ICAM-$1^{-/-}$ mice.

There were no significant differences with respect to the secretion of IL-6 when spleen cells from the mutant and wild-type groups were stimulated with ConA, TSST-1, or *S. aureus* cell walls. The number of circulating neutrophils was significantly higher in ICAM-1 mutants than in the wild-type congenic mice, as previously reported ($1{,}130 \times 10^9 \pm 182 \times 10^9$/liter versus $404 \times 10^9 \pm 98 \times 10^9$/liter; $P < 0.01$) (20). However, the ICAM-1-deficient mice proved to have a significantly lower proportion of phagocytizing cells than their wild-type littermates (17.7% ± 1.2% versus 25.3% ± 2.0%; $P < 0.01$).

Consistent with the results from the experiment with ICAM-1-deficient mice, there was a tendency for a higher level of mortality in the group of animals treated with anti-ICAM-1 antibody. Four days after bacterial inoculation, 5 of 25 mice died, but only 1 of 20 mice in the control group died. By the time the experiment was terminated on day 14 after inoculation of bacteria, 72% in the anti-ICAM-1-treated group and 40% in the control group had died. The frequency of septic arthritis in mice treated with anti-ICAM-1 antibody was lower than that in rat IgG-treated controls during the first week of *S. aureus* infection. Thus, on day 4 after bacterial administration, the anti-ICAM-1-treated animals displayed a significantly lower frequency of arthritis than the controls (15 versus 68%; $P < 0.01$). The severity of arthritis parallelled this observation; the arthritic index decreased in the anti-ICAM-1-treated animals from 0.6 ± 0.1 on day 2 to 0.35 ± 0.2 on day 4 after the bacterial inoculation. In contrast, control mice displayed an increased arthritic index, from 0.5 ± 0.1 to 1.1 ± 0.2, during the corresponding period ($P < 0.01$ with regard to arthritic index at day 4 between anti-ICAM-1-treated mice and controls).

Mice genetically and functionally deficient in ICAM-1 exhibit greater mortality but less-destructive arthritis than their wild-type littermates during *S. aureus* infection. At the time of sacrifice, 50% of the animals in the ICAM-1 mutant group were dead and the remaining were in poor condition, while all of the mice in the wild-type group were alive and in good condition. By contrast, in a previous study we found that ICAM-$1^{-/-}$ mice are more resistant to *S. aureus* enterotoxin B-induced septic shock than ICAM-$1^{+/+}$ controls (20). The present study highlights the difference between infection with a superantigen-producing bacterium and exposure of healthy animals to superantigen alone. Despite septicemia-induced death, ICAM-1 mutants showed only sparse clinical and histological signs of arthritis. Similar findings regarding the clinical development of septic death and arthritis were obtained in an independent experiment using in vivo administration of anti-ICAM-1. What are the reasons for the highly discrepant outcomes regarding septic mortality and septic arthritis in ICAM-1 deficiency? One of the key events in all localized inflammatory conditions (e.g., septic arthritis) is extravasation of inflammatory cells. In this respect, the interactions between ICAM-1 and LFA-1/Mac-1 are critical for leukocyte migration and adhesion. Our results showing that ICAM-1 deficiency decreases the severity of arthritis are supported by recent studies on rheumatoid arthritis (11, 18) and on animal models of autoimmune arthritis (14, 15, 17). Apart from its importance for extravasation, the ICAM-1 molecule is also involved in the interaction between T lymphocytes and antigen-presenting cells (10, 13). Given that *S. aureus* arthritis is a T-cell-mediated disease (2, 5), deficient expression of ICAM-1 should reduce the antigen-presenting capacity and hence contribute to a less destructive course of the disease. In striking contrast to the case for arthritis, the same *S. aureus*-inoculated animals with either genomic or phenotypic ICAM-1 deficiency developed severe systemic manifestations such as sepsis, which frequently led to death. The main participants in the host defense system against staphylococcal infections are phagocytic, CD43-expressing cells (6), whereas T and B lymphocytes aggravate the systemic manifestations of the disease (4, 21). In this respect it should be noted that despite the presence of high numbers of circulating polymorphonuclear cells, the percentage of cells able to phagocytize bacteria was significantly lower in ICAM-1-deficient mice than in wild-type congenic mice. The nonphagocytized, circulating bacteria and, more importantly, their exotoxins, such as TSST-1, may be potent inducers of TNF-α, a cytokine mediating lethality in septic shock. Indeed, whereas we could not detect circulating TNF-α, the in vitro results clearly show that TNF-α production in response to TSST-1 is significantly increased in ICAM-1-deficient animals in comparison with wild-type congenic animals. IFN-γ is produced by antigen-, superantigen-, or mitogen-activated T lymphocytes. It enhances phagocytic functions (19) and increases major histocompatibility complex expression. In our study ICAM-1-deficient mice displayed lower levels of circulating IFN-γ than the controls. Further in vitro analysis demonstrated that lymphocytes from ICAM-$1^{-/-}$ mice have a decreased capacity to produce IFN-γ in response to staphylococcal antigens. It is thus plausible that an insufficient IFN-γ response to staphylococcal infection might have resulted in decreased phagocytic capacity leading to deficient bacterial elimination in ICAM-$1^{-/-}$ mice.

Together our results point to a dual role of ICAM-1 expression during *S. aureus* infection. Whereas ICAM-1 is a critical participant in the host defense against systemic, extracellular bacterial infections, its absence or downregulation may be protective in septic arthritis.

We thank Ing-Marie Nilsson for excellent technical assistance.

This work was supported by grants from the Göteborg Medical Society, the Swedish Association against Rheumatism, the King Gustaf V:s 80 Years Foundation, the Swedish Medical Research Council, the Nanna Svartz' Foundation, the A-G Crafoord Foundation, the Förenade Liv Foundation, the University of Göteborg, and the Tornspiran Foundation.

REFERENCES

1. **Abdelnour, A., T. Bremell, R. Holmdahl, and A. Tarkowski.** 1994. Clonal expansion of T lymphocytes causes arthritis and mortality in mice infected with toxic shock syndrome toxin-1-producing staphylococci. Eur. J. Immunonol. **24:**1161–1166.
2. **Abdelnour, A., T. Bremell, R. Holmdahl, and A. Tarkowski.** 1994. Role of T lymphocytes in experimental *Staphylococcus aureus* arthritis. Scand. J. Immunol. **39:**403–408.
3. **Abdelnour, A., T. Bremell, and A. Tarkowski.** 1994. Toxic shock syndrome toxin 1 contributes to the arthritogenecity of *Staphylococcus aureus*. J. Infect. Dis. **170:**94–99.
4. **Abdelnour, A., and A. Tarkowski.** 1993. Polyclonal B-cell activation by an arthritogenic *Staphylococcus aureus* strain: contribution of T-cells and monokines. Cell. Immunol. **147:**279–293.
5. **Bremell, T., R. Holmdahl, and A. Tarkowski.** 1994. Immunopathological features of rat *Staphylococcus aureus* arthritis. Infect. Immun. **62:**2334–2344.
6. **Bremell, T., R. Holmdahl, and A. Tarkowski.** 1994. Protective role of sialophorin (CD43)-expressing cells in experimental *Staphylococcus aureus* infection. Infect. Immun. **62:**4637–4640.
7. **Bremell, T., S. Lange, L. Svensson, E. Jennische, K. Gröndahl, H. Carlsten, and A. Tarkowski.** 1990. Outbreak of spontaneous staphylococcal arthritis and osteitis in mice. Arthritis Rheum. **33:**1739–1744.
8. **Bremell, T., S. Lange, A. Yacoub, C. Rydén, and A. Tarkowski.** 1991. Experimental *Staphylococcus aureus* arthritis in mice. Infect. Immun. **59:**2615–2623.
9. **Bremell, T., and A. Tarkowski.** 1995. Preferential induction of septic arthritis and mortality by superantigen-producing staphylococci. Infect. Immun. **63:** 4185–4187.

10. **Dang, H. L., M. T. Michalek, F. Takei, B. Benaceraff, and K. L. Rock.** 1990. Role of ICAM-1 in antigen presentation demonstrated by ICAM-1 defective mutants. J. Immunol. **144:**4082–4091.
11. **Davis, L. S., A. F. Kavanaugh, L. A. Nichols, and P. E. Lipsky.** 1995. Induction of persistent T cell hyporesponsiveness *in vivo* by monoclonal antibody to ICAM-1 in patients with rheumatoid arthritis. J. Immunol. **154:** 3525–3537.
12. **Dustin, M. L., R. Rothlein, A. K. Bhan, C. A. Dinarello, and T. A. Springer.** 1986. Induction by IL-1 and interferon-γ: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J. Immunol. **137:** 245–254.
13. **Hogg, N., P. A. Bates, and J. Harvey.** 1991. Structure and function of intercellular adhesion molecule-1. Chem. Immunol. **50:**98–115.
14. **Iigo, Y., T. Takashi, T. Tamatani, M. Miyasaka, T. Higashida, H. Yagita, K. Okumura, and W. Tsukada.** 1991. ICAM-1 dependent pathway is critically involved in the pathogenesis of adjuvant arthritis in rats. J. Immunol. **147:** 4167–4171.
15. **Kakimoto, K., T. Nakamura, K. Ishi, T. Takashi, H. Iigou, H. Yagita, K. Okumura, and K. Onoue.** 1992. The effect of anti-adhesion molecule antibody on the development of collagen-induced arthritis. Cell. Immunol. **142:** 326–337.
16. **Mancini, G., A. O. Carbonara, and J. F. Heremans.** 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry **2:**235–254.
17. **Mikecz, K., F. R. Brennan, J. H. Kim, and T. T. Glant.** 1995. The role of adhesion molecules in the development of autoimmune arthritis. Scand. J. Rheumatol. **24:**99–106.
18. **Oppenheimer-Marks, N., and P. Lipsky.** 1994. Transendothelial migration of T cells in chronic inflammation. Immunologist **2**(2)**:**58–64.
19. **Trinchieri, G., and B. Perussia.** 1985. Immune interferon: a pleiotropic lymphokine with multiple effects. Immunol. Today **6:**131–136.
20. **Xu, H., J. A. Gonzalo, Y. St.Pierre, I. R. Williams, T. S. Kupper, R. S. Cotran, T. A. Springer, and J.-C. Gutierrez-Ramos.** 1994. Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. J. Exp. Med. **180:**95–109.
21. **Zhao, Y., A. Abdelnour, R. Holmdahl, and A. Tarkowski.** 1995. Mice with the xid B cell defect are less susceptible to developing Staphylococcus aureus-induced arthritis. J. Immunol. **155:**2067–2076.
22. **Zhao, Y., A. Abdelnour, Å. Ljungdahl, T. Olsson, and A. Tarkowski.** 1995. Patterns of interferon-γ mRNA expression in toxic shock syndrome toxin-1 expanded $V\beta11^+$ T lymphocytes. Cell. Immunol. **161:**28–33.
23. **Zhao, Y., and A. Tarkowski.** 1995 Impact of interferon-γ receptor deficiency on experimental *Staphylococcus aureus* septicemia and arthritis. J. Immunol. **155:**5736–5742.

Editor: V. A. Fischetti