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Inflammatory and immune responses are impaired in mice deficient in intercellular adhesion molecule 1

(cell adhesion/immune deficiency/homologous recombination/gene targeting/lymphocyte interactions)

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ABSTRACT **Gene targeting was used to produce mice deficient in intercellular adhesion molecule 1 (ICAM-1) or CD54, an immunoglobulin-like cell adhesion molecule that binds β_2 integrins. Homozygous deficient animals develop normally, are fertile, and have a moderate granulocytosis. The nature of the mutation, RNA analysis, and immunostaining are consistent with complete loss of surface expression of ICAM-1. Deficient mice exhibit prominent abnormalities of inflammatory responses including impaired neutrophil emigration in response to chemical peritonitis and decreased contact hypersensitivity to 2,4-dinitrofluorobenzene. Mutant cells provided negligible stimulation in the mixed lymphocyte reaction, although they proliferated normally as responder cells. These mutant animals will be extremely valuable for examining the role of ICAM-1 and its counterreceptors in inflammatory disease processes and atherosclerosis.**

Intercellular adhesion molecule 1 (ICAM-1) or CD54, a cell-surface protein with five immunoglobulin-like domains, plays an important role in transendothelial migration of leukocytes (1–3) through its expression on vascular endothelium and binding to β_2 leukocyte integrins (4, 5). The β_2 integrins are heterodimers composed of a common β subunit encoded by the *CD18* gene, combined with one of three α chains: CD11a for lymphocyte function-associated antigen 1 (LFA-1), CD11b for Mac-1, and CD11c for p150,95. Immunoglobulin domains 1 and 2 of ICAM-1 are involved in binding to LFA-1 (6), while immunoglobulin domain 3 of ICAM-1 mediates binding to Mac-1 (7). ICAM-1 is upregulated through synthesis of new protein *in vivo* and/or *in vitro* in response to inflammatory cytokines (8, 9), phorbol esters (10), or lipopolysaccharide (11).

Transendothelial migration of leukocytes begins with leukocyte rolling, which is largely dependent on selectins (12), followed by activation of integrins, firm attachment to endothelium, and migration across the endothelial surface (13). Integrin binding to ICAM-1 is particularly important for firm attachment and migration across the endothelium (13); for example, migration of human neutrophils through a monolayer of umbilical vein endothelium was inhibited >85% by anti-ICAM-1 monoclonal antibodies (mAbs) (1). Blocking antibodies to ICAM-1 inhibit migration of neutrophils *in vivo* in response to inflammation in the lung (14) and myocardium (15).

ICAM-1 is also implicated in various immune responses (16). Using allogeneic mouse or human cells *in vitro*, there is profound inhibition of the mixed lymphocyte reaction (MLR) by mAbs to ICAM-1 (11). ICAM-1 as well as other adhesion molecules can provide costimulatory signals for B-cell (17) and T-cell activation *in vitro* (18, 19). mAbs to ICAM-1 resulted in 50% reduction in contact hypersensitivity in mice (20).

No animals with mutations in ICAM-1 are reported. We sought to test the role of ICAM-1 in intact animals by disrupting the gene in murine embryonic stem (ES) cells.

MATERIALS AND METHODS

Targeting Construct and Generation of Mutant Mice. To prepare the targeting construct, a 5.5-kb segment of the *Icam-1* gene (21) containing exons 4–7 was cloned into pBluescript II KS(−) (Stratagene). A neomycin-resistance gene (*neo*) cassette containing a short version of the RNA polymerase II promoter and the bovine growth hormone polyadenylylation signal (22) was inserted at the *Hin*dIII site in exon 5.

The AB1 ES cell line was electroporated as described (23) with the construct after digestion with *Bgl* I for use as a replacement vector. Selection was performed with G418 (300 μg total weight per ml) for 9 days at which time individual G418-resistant colonies were picked. Screening for targeted recombination was performed either by Southern blotting using a microextraction procedure (24) or by PCR analysis of a portion of the colony using a primer contained within the *neo* cassette (oligonucleotide 2, 5′-GGACAGGTCGGTCTTGACAA-3′) paired with an outside primer (oligonucleotide 1, 5′-TGTGGGTAAAGGAAGGGACT-3′) located in the 5′ flanking region. PCR screening was performed by recovery of individual colonies in 20 μl of trypsin solution; half of the cell suspension was added to a final volume of 20 μl containing 50 μg of proteinase K per ml, 1.7 μM SDS, and 10 mM Tris at pH 8.0 and was incubated at 55°C for 1 hr. PCR was carried out for 35 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C for 1.5 min as described (25).

Cells confirmed by Southern blotting to carry the replacement mutation were injected into day 3.5 C57BL/6J blastocysts and transferred into foster mothers (26). Chimeric males were mated with C57BL/6J females, and germ-line transmission of the mutation was documented by Southern blotting of tail DNA by using *Bam*HI digestion and the 5′ flanking probe.

Reverse Transcription–PCR (RT–PCR). RNA was isolated from fresh tissues by using guanidinium isothiocyanate (27). Single-stranded cDNA was prepared with the SuperScript Moloney murine leukemia virus reverse transcriptase kit (Bethesda Research Laboratories). The cDNA product was

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Abbreviations: ICAM-1, intercellular adhesion molecule 1; MLR, mixed lymphocyte reaction; LFA-1, lymphocyte function-associated antigen 1; ES cells, embryonic stem cells; DNFB, 2,4-dinitrofluorobenzene; RT–PCR, reverse transcription–PCR; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody.

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amplified by using primers within exon 4 (oligonucleotide 3, bases 668–684: 5′-CTTCCAGCTACCATCCC-3′), exon 7 (oligonucleotide 4, antisense to bases 2244–2228: 5′-AGAACCACTGCTAGTCC-3′), and exon 5 (oligonucleotide 5, bases 1049–1064: 5′-GTTCTTCTGAGCGGCGT-3′); base pair numbering is as reported (28). PCR conditions were as described above with 10 cycles of 94°C for 30 sec, 65°C for 30 sec (decreasing 1°C each cycle), and 72°C for 30 sec followed by 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec.

Peripheral Blood Analysis and Chemical Peritonitis. Peripheral blood cell counts and chemical peritonitis were performed as described (29).

Contact Hypersensitivity. Contact hypersensitivity was elicited in the mice by using the 2,4-dinitrofluorobenzene (DNFB) sensitization protocol exactly as described (30). Ear thickness was measured 24, 48, and 72 hr after DNFB challenge and the change in ear thickness (T) was calculated as ΔT.

Mixed Lymphocyte Reaction (MLR). MLRs were performed by mixing spleen cells from BALB/c animals with spleen cells harvested from wild-type or mutant hybrid (129/Sv × C57BL/6J) animals. Spleen cells were isolated and cultured in supplemented Dulbecco's modified Eagle's medium (sDMEM), and T lymphocytes were prepared as described (31). Stimulator cells were irradiated with 1500 R (1 R = 0.258 mC/kg). Enriched T cells (0.6 to 8 × 10^6 per ml) and irradiated stimulator spleen cells (4 or 8 × 10^6 per ml) were cocultured for 5 days in 96-well flat-bottom microtiter plates in sDMEM and then incubated for the final 18 hr with [^{3}H]thymidine (32).

Histology and Immunohistochemistry. Animals were sacrificed with or without a 6-hr-previous i.p. injection with 50 μg of lipopolysaccharide from *Salmonella typhosa* (Difco) dissolved in 0.5 ml of H_2O. Immunohistochemistry was performed by fixing freshly isolated tissues in 10% formalin in phosphate buffer. Fluorescent staining for ICAM-1 was performed by using the fluorescein isothiocyanate (FITC)-conjugated 3E2 antibody (20).

Flow Cytometry. Indirect immunofluorescent analysis was performed on leukocytes with a EPICS Profile flow cytometer (Coulter) using FITC-conjugated 3E2 antibody to ICAM-1 and with the phycoerythrin-conjugated RA3-6B2 antibody to CD45R (B220) (both from PharMingen). Two-color staining was performed by incubating 5 × 10^5 splenocytes in 100 μl containing 1 μg of each specific antibody or isotype-matched antibodies (PharMingen) on ice for 20 min.

RESULTS

Mutating the *Icam-1* Gene by Homologous Recombination. Homologous recombination was used to introduce a *neo* expression cassette within exon 5 of the *Icam-1* gene as described (Fig. 1*A*). Recombinant colonies were identified among the G418-resistant clones by screening with either PCR or Southern blotting. The mean frequency of homologous recombination was 1 in 70 G418-resistant colonies.

Targeted clones were injected into blastocysts, giving rise to male chimeric mice that transmitted the mutated *Icam-1* gene to the germ line of offspring as documented by Southern blotting. Heterozygous (+/−) mutant animals were intercrossed, and homozygous mutant animals were born in the expected ratio representing 26.5% of 260 progeny tested. The homozygous mutant (−/−) animals gained weight normally, were fertile, and did not demonstrate any obvious phenotype or susceptibility to infection when maintained with sterilized cages and food. Homozygous mutant inbred 129/Sv animals also were obtained and showed viability similar to animals of hybrid (129/Sv × C57BL/6J) background; all data shown below are for hybrid animals.

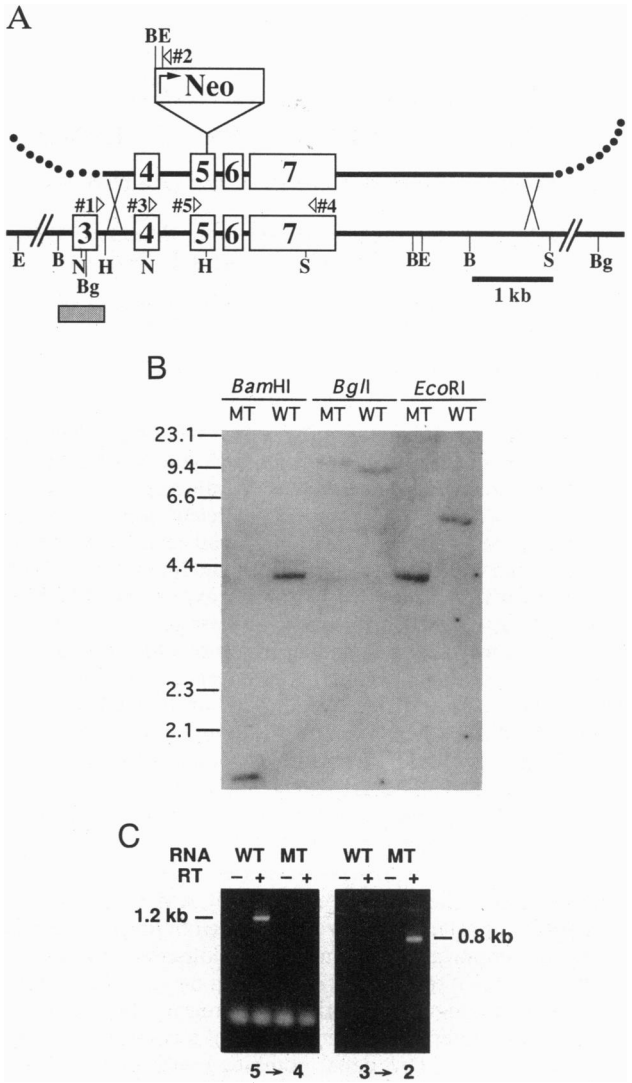

FIG. 1. Preparation and analysis of targeted mutation. (*A*) The targeting vector (upper diagram) is drawn to scale with exons numbered within boxes, the location of the *neo* cassette indicated, a solid line for mouse genomic DNA, and a dotted line for plasmid sequence. The lower diagram is of the mouse genomic DNA to be targeted. The location of oligonucleotide primers for PCR and RT–PCR are designated 1 to 5. The location of a flanking probe is indicated by the shaded box below. B, *Bam*HI; Bg, *Bgl* I; E, *Eco*RI; H, *Hin*dIII; N, *Nhe* I; and S, *Sal* I. (*B*) Southern blot analysis was performed with the flanking probe and genomic DNA isolated from tails of wild-type (lanes WT) and homozygous mutant (lanes MT) mice. (*C*) RT–PCR was performed by using RNA isolated from lung. RNA was prepared from wild-type (lanes WT) or mutant (lanes MT) tissue, and RT was omitted or added as indicated. Oligonucleotide primer pairs were 4 and 5 (*Left*) and 2 and 3 (*Right*).

Southern blotting analysis comparing DNA from wild-type and homozygous mutant animals was consistent with the expected mutation (Fig. 1*B*). Use of an upstream flanking DNA probe and digestion with *Bam*HI or *Eco*RI, both of which cut within the *neo* cassette, revealed the smaller 1.8- or 4.3-kb DNA fragments, respectively, consistent with the predicted replacement mutation. Digestion with Bgl I, which cuts outside the sequences contained in the vector, demonstrated a larger fragment that is increased in size by the 1.3-kb length of the inserted *neo* cassette.

***Icam-1* Mutation Eliminates Cell-Surface Expression.** To analyze expression of mutant transcripts, RNA was isolated from lung for RT–PCR. Using primers 5 and 4, which flank the mutation site from upstream of the *neo* cassette in exon

5 to exon 7, the expected 1.2-kb product is obtained with RNA from wild-type animals but not from mutant animals (Fig. 1*C*). Similar results were obtained with the primer in exon 5 and a primer in exon 6 (not shown), confirming the absence of normal mRNA in homozygous mutant animals. A sense-oriented primer in exon 4 (primer 3) and an antisense primer in the *neo* coding region (primer 2) were used to detect a transcript that might arise if exon 4 were spliced to the mutated exon 5, and the 0.8-kb product predicted for such a transcript was detected with RNA from mutant but not from wild-type animals (Fig. 1*C*). It is possible that a truncated form of ICAM-1 could be produced ending with aberrant sequence at the *Hin*dIII site in exon 5, but such a product would not have a transmembrane domain.

Histopathologic examination performed on three male and three female mice ranging in age from 8 weeks to 8 months did not reveal any abnormalities in tissue architecture. Thymus, spleen, liver, brain, eye, heart, skeletal muscle, bone, testis, ovary, skin, pancreas, stomach, small and large intestine, mesenteric and superior cervical lymph node, submandibular gland, adrenal gland, kidney, seminal vesicle, uterus, and lung were examined. Immunofluorescent staining of lung was performed by using the 3E2 antibody directed against ICAM-1. ICAM-1 is known to be expressed abundantly on alveolar capillary endothelium and on the luminal surface of type 1 alveolar epithelial cells in the mouse (C. Doerschuk, personal communication). Sections of lung taken from animals 6 hr after i.p. injection of lipopolysaccharide demonstrated abundant expression of ICAM-1 in cells surrounding airspaces in wild-type animals, but no immunostaining was visible with homozygous mutant animals (Fig. 2).

As a quantitative assessment of ICAM-1 expression, flow cytometric analysis of B lymphocytes was performed by using double-color analysis of cells stained with the 3E2 mAb directed against murine ICAM-1 and anti-B220 directed against the B-cell form of CD45R (Fig. 3). Flow cytometric analysis showed substantial expression of ICAM-1 on activated B lymphocytes from wild-type animals (mean fluorescence 4.2), but there was no significant expression of ICAM-1 on B lymphocytes from homozygous mutant animals (mean fluorescence 0.22 compared with 0.14 for the isotype-matched control antibody). The RT–PCR data, the immunohistochemistry, and the flow cytometry are all consistent with the complete loss of surface expression of ICAM-1.

Mutant Animals Show Granulocytosis but Normal Lymphocyte Populations. Although animals appeared phenotypically normal, subtle abnormalities could be identified in the resting state. Since ICAM-1 is strongly implicated in neutrophil emigration, peripheral blood neutrophil counts were performed. The neutrophil count ± SD was 1.0 ± 0.5 × 10^3 per μl for wild-type animals (n = 12) and was increased to 4.1 ± 1.6 × 10^3 per μl for homozygous mutant animals (n = 14) at 2–4 months of age ($P = 5 \times 10^{-6}$). As an additional evaluation of the cellular phenotype of the mutants, analyses of cell populations in spleen and thymus were performed by flow cytometry. No differences were found for wild-type and mutant animals for populations of $CD11a^+$, $ICAM\text{-}2^+$, $CD3^+$, $CD45R^+$ (B220), $CD4^+$, and $CD8^+$ cells in the spleen. Similarly no differences were found for thymic T-cell subsets of $CD4^+$ $CD8^-$, $CD4^-$ $CD8^+$, or $CD4^+$ $CD8^+$ cells.

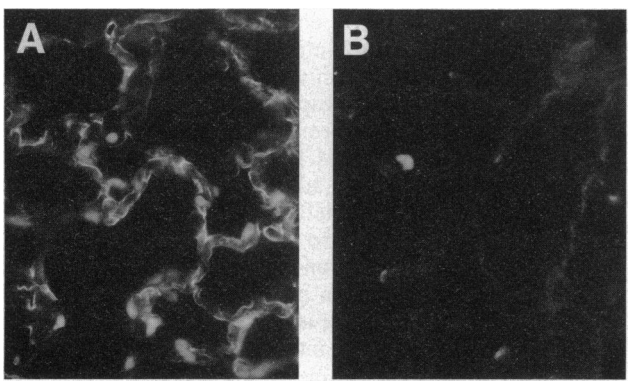

FIG. 2. Immunohistofluorescent staining of lung from wild-type and homozygous mutant mice. Wild-type (*A*) and mutant (*B*) animals were sacrificed 6 hr after i.p. injection of 50 μg of lipopolysaccharide. Lungs were stained with the 3E2 monoclonal antibody to mouse ICAM-1. Only weak autofluorescence is seen in mutant tissue.

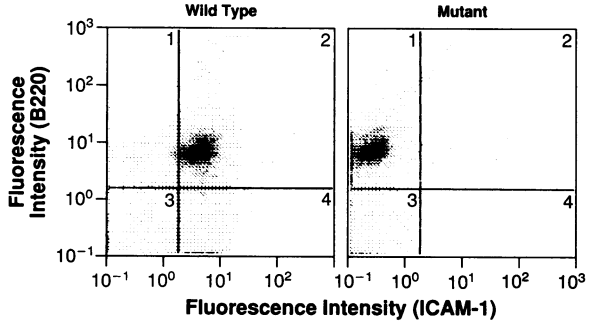

FIG. 3. Expression of ICAM-1 on B lymphocytes from wild-type (*Left*) and mutant (*Right*) mice. Cells were isolated from spleen and stimulated in culture for 15 hr with 200 ng of ionomycin per ml and 1 ng of phorbol 12-myristate 13-acetate per ml. Cells were stained with phycoerythrin-labeled anti-B220 (RA3-6B2) directed against the B-cell form of CD45R and FITC-labeled anti-ICAM-1 (3E2).

Neutrophil Migration Is Impaired in Mutant Mice. To assess the role of ICAM-1 in transendothelial migration, a peritonitis study was initiated. The total number of neutrophils in the peritoneal cavity and the percentage of neutrophils relative to all leukocytes in the exudate were reduced in mutant animals, whereas the neutrophil count in the blood 3 hr after thioglycollate injection was even more elevated than in the resting state (Fig. 4). Analysis of peritonitis at 8 hr revealed 76% neutrophils in +/+ animals (n = 4) and 43% neutrophils in −/− animals (n = 6), indicating the alteration is not simply a delay in emigration.

Contact Hypersensitivity Is Reduced in Mutant Mice. Since ICAM-1 is thought to be important in lymphocyte interac-

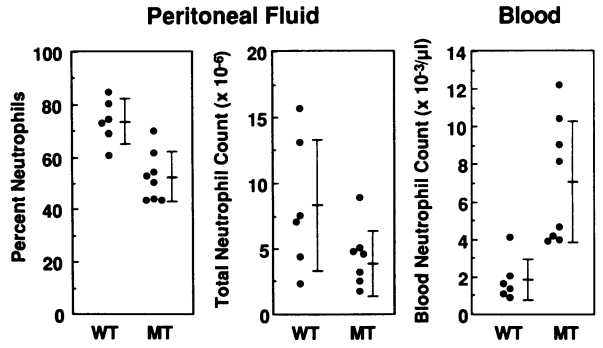

FIG. 4. Altered response to chemical peritonitis in mutant mice. Wild-type (+/+) or homozygous mutant (−/−) mice were injected i.p. with 1 ml of fluid thioglycollate medium and sacrificed after 3 hr. The mean ± SD are shown as bars and from left to right are as follows: 73.8 ± 8.4 for +/+ and 52.6 ± 9.5 for −/− for the percentage of neutrophils in peritoneal fluid (P = 0.001), 8.3 ± 5.0 × 10^6 for +/+ and 3.9 ± 2.5 × 10^6 for −/− for the total neutrophils in peritoneal fluid (P = 0.09), and 1.85 ± 1.1 × 10^3 per μl for +/+ and 7.07 ± 3.2 × 10^3 per μl for blood neutrophil count (P = 0.002). Analysis of peritoneal fluid from four +/+ and four −/− animals without instillation of thioglycolate revealed 0.6–3.0 × 10^6 cells per animal with a mean of 4.2% neutrophils for +/+ animals and 1.1% neutrophils for −/− animals.

tions, we examined the ability of the ICAM-1-deficient animals to generate a contact hypersensitivity response. Mice were challenged at 7–19 weeks of age with application of DNFB to one ear 5 days after sensitization by two applications of DNFB to the abdomen. Naive animals received the challenge to the ear but do not undergo abdominal sensitization with DNFB. Maximal ear swelling in all test groups occurred 24 hr after challenge as reported (33) and was reduced by 74% in homozygous mutant animals as shown in Table 1 ($P < 0.0001$, unpaired T test). Histologic study of punch biopsies of the ears confirmed the difference in thickness, and sections from sensitized wild-type animals revealed prominent edema separating normal tissue structures and a moderately dense infiltrate of lymphoid cells and neutrophils (not shown). Both of these changes were essentially absent in mutant animals. These studies indicate that ICAM-1 plays a prominent role in mediation of contact hypersensitivity and demonstrate a significant inflammatory abnormality in the mutant animals.

ICAM-1-Deficient Cells Are Defective as Stimulators in the MLR. In the MLR, the activating stimulus is the foreign histocompatibility antigen expressed on allogeneic stimulator cells, and a proliferative T-cell response is induced. Previous studies demonstrated that antibodies to ICAM-1 inhibit the MLR but did not distinguish the role of its expression on stimulator cells in comparison with the role of induced ICAM-1 expression on responder T cells. Unfractionated spleen cells were irradiated and used as stimulators while T lymphocytes were isolated from spleen for use as responder cells. Cells from wild-type and homozygous mutant mice were of hybrid (C57BL/6J × 129/Sv) background (both $H\text{-}2^b$). Allogeneic cells expressing $H\text{-}2^d$ were isolated from BALB/c mice. The normal and homozygous mutant T lymphocytes responded equally well to allogeneic stimulation with irradiated BALB/c cells and a wide range of concentration of responder cells; for example, mean incorporation was 79,900 cpm for wild-type cells and 68,100 cpm for mutant cells with 5×10^6 responder cells and 4×10^6 BALB/c stimulator cells. However, cells isolated from the spleen of homozygous mutant animals demonstrated a marked reduction in the ability to function as stimulator cells with a wide range of concentrations of BALB/c responder cells (Fig. 5). These data show that the defect involves the function of ICAM-1 primarily or exclusively on the stimulator cells as opposed to the responder cells.

DISCUSSION

The *Icam-1* gene was disrupted by homologous recombination, and homozygous mutant mice are viable but show an absence of surface expression of ICAM-1. The phenotype in the mice might be expected to resemble that seen in CD18 deficiency in humans and animals (34, 35), but the phenotype might be milder, since β_2 integrins are the only proven counterreceptors for ICAM-1, although ICAM-2 and ICAM-3 are suggested to serve as counterreceptors for LFA-1 (36–38), and Mac-1 is the receptor for the iC3b component of complement. The ICAM-1-deficient mice display some phenotypic features similar to partial deficiency of CD18 in human and mice (29), including a mild increase in neutrophil count and impaired neutrophil emigration.

Table 1. Impaired contact hypersensitivity in ICAM-1-deficient mice

Genotype	$\Delta T^* \times 10^2$, mm ± SD	
	Naive	Sensitized
Wild type	0.9 ± 1.5 (n=18)	16.1 ± 7.5 (n=15)
Homozygous mutant	1.0 ± 2.3 (n=14)	5.0 ± 4.7 (n=26)

*ΔT = (ear thickness 24 hr after elicitation) − (ear thickness before DNFB challenge). The difference between sensitized mutant and wild-type ΔT is significant; $P < 0.0001$, unpaired t test; 74% reduction.

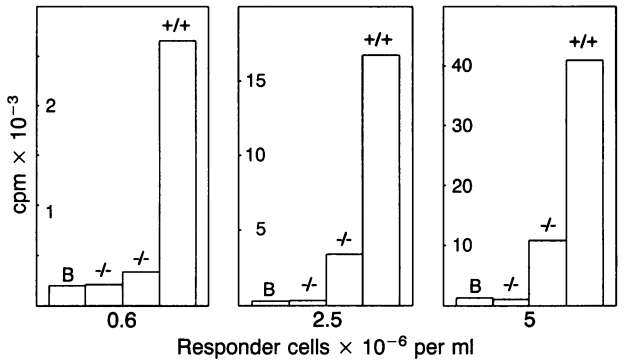

FIG. 5. Stimulator cell capacity of mutant cells in MLR. Spleen cells were isolated from wild type (+/+) or homozygous mutant (−/−) hybrid (129/Sv × C57BL/6J) mice and from BALB/c (B) mice and were irradiated for use as stimulator cells. Responder cells were from BALB/c mice.

In the chemical peritonitis studies of ICAM-1-deficient mice, the reduction in neutrophils in the peritoneal exudate and the accumulation of granulocytes in the blood is indicative of decreased transendothelial migration of neutrophils in the mutant mice. Since the migration defect in the mutant mice is not complete, there is evidence for an ICAM-1-independent mechanism for transendothelial migration. The results are consistent with *in vitro* studies in which mAb to ICAM-1 inhibited transendothelial migration by only 55%, while mAb to CD18 inhibited by 90% (39).

The ICAM-1-deficient animals exhibited a 74% suppression of contact hypersensitivity. Based on the MLR results with ICAM-1-deficient cells, it seems probable that the defect in contact hypersensitivity will involve the afferent or sensitization phase of the response. The contact hypersensitivity data are compatible with the hypothesis that ICAM-1 is a critical accessory molecule for T-cell function. It is also possible that the defect in the contact hypersensitivity response may be caused by abnormalities of migration involving the antigen-presenting cell or the T cell.

The data from the MLR suggest that the deficiency of ICAM-1 on the stimulator cells and not the responder cells is responsible for the diminished response. The critical step for T-cell activation is the recognition of antigen peptides in association with MHC molecules by the T-cell receptor (TCR-CD3). However, cell adhesion molecules are thought to play an important role in providing costimulatory signals between lymphocytes or in enhancing lymphocyte interactions (40). Although the interaction of CD2 and CD58 (LFA-3) is thought to be an important costimulatory event for generation of an immune response, mice with a disrupted *CD2* gene demonstrated normal immune responses (41). It was suggested that the ICAM-1/LFA-1 or other interactions might provide a redundant adhesive function in the CD2-deficient mice. The severe defect (up to 100%) in the ability of ICAM-1-deficient T cells to function as stimulator cells in the MLR is consistent with an important costimulatory role for ICAM-1. The expression of ICAM-1 in antigen presentation can be a decisive factor in determining whether a T-cell response will occur. This interpretation is supported by transfection studies expressing ICAM-1 and HLA-DR in L cells (42). L cells expressing HLA-DR alone failed to activate T cells, while cells expressing HLA-DR and ICAM-1 were effective. Transfection with ICAM-1 was also effective in correcting the defect in mutagenized clones of antigen presenting cells (43).

The ICAM-1-deficient mice and other mice with gene-targeted mutations in cell adhesion molecules should be valuable resources for the study of inflammatory responses *in vivo*. There is considerable interest in the hypothesis that decreased expression or function of cell adhesion molecules might result in reduced susceptibility to common, multifactorial diseases that have inflammation as a component including arthritis, diabetes mellitus, inflammatory bowel disease, asthma, atherosclerosis, and various other autoimmune and inflammatory diseases. Monoclonal antibodies that block function of ICAM-1 have been shown to reduce inflammatory or immune responses in a variety of disease models (44–47), but these antibodies may induce biological responses apart from their role in blocking adhesion. The mutant mice offer an important alternative strategy to assess the role of ICAM-1, and the mice are more suitable for study of chronic inflammatory disease processes.

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1. Smith, C. W., Rothlein, R., Hughes, B. J., Mariscalco, M. M., Rudloff, H. E., Schmalstieg, F. C. & Anderson, D. C. (1988) *J. Clin. Invest.* **82,** 1746–1756.
2. Ebisawa, M., Bochner, B. S., Georas, S. N. & Schleimer, R. P. (1992) *J. Immunol.* **149,** 4021–4028.
3. Dustin, M. L. & Springer, T. A. (1988) *J. Cell Biol.* **107,** 321–331.
4. Marlin, S. D. & Springer, T. A. (1987) *Cell* **51,** 813–819.
5. Smith, C. W., Martin, S. D., Rothlein, R., Toman, C. & Anderson, D. C. (1989) *J. Clin. Invest.* **83,** 2008–2017.
6. Staunton, D. E., Dustin, M. L., Erickson, H. P. & Springer, T. A. (1990) *Cell* **61,** 243–254.
7. Diamond, M. S., Staunton, D. E., Marlin, S. D. & Springer, T. A. (1991) *Cell* **65,** 961–971.
8. Dustin, M. L., Rothlein, R., Bhan, A. K., Dinarello, C. A. & Springer, T. A. (1986) *J. Immunol.* **137,** 245–254.
9. Pober, J. S., Gimbrone, M. A., Lapierre, L. A., Mendrick, D. L., Fiers, W., Rothlein, R. & Springer, T. A. (1986) *J. Immunol.* **137,** 1893–1896.
10. Rothlein, R., Dustin, M. L., Marlin, S. D. & Springer, T. A. (1986) *J. Immunol.* **137,** 1270–1274.
11. Takei, F. (1985) *J. Immunol.* **134,** 1403–1407.
12. Lasky, L. A. (1992) *Science* **258,** 964–969.
13. Smith, C. W. (1992) in *Adhesion: Its Role in Inflammatory Disease*, eds. Harlan, J. M. & Liu, D. Y. (Freeman, New York), pp. 83–115.
14. Barton, R. W., Rothlein, R., Ksiazek, J. & Kennedy, C. (1989) *J. Immunol.* **143,** 1278–1282.
15. Ma, X.-I., Lefer, D. J., Lefer, A. M. & Rothlein, R. (1992) *Circulation* **86,** 937–946.
16. Springer, T. A. (1990) *Nature (London)* **346,** 425–433.
17. Tohma, S., Hirohata, S. & Lipsky, P. E. (1991) *J. Immunol.* **146,** 492–499.
18. Kuhlman, P., Moy, V. T., Lollo, B. A. & Brian, A. A. (1991) *J. Immunol.* **146,** 1773–1782.
19. Van Seventer, G. A., Shimizu, Y., Horgan, K. J. & Shaw, S. (1990) *J. Immunol.* **144,** 4579–4586.
20. Scheynius, A., Camp, R. L. & Pure, E. (1993) *J. Immunol.* **150,** 655–663.
21. Ballantyne, C. M., Sligh, J. E., Dai, X. Y. & Beaudet, A. L. (1992) *Genomics* **14,** 1076–1080.
22. Soriano, P., Montgomery, C., Geske, R. & Bradley, A. (1991) *Cell* **64,** 693–702.
23. McMahon, A. P. & Bradley, A. (1990) *Cell* **62,** 1073–1085.
24. Ramirez-Solis, R., Rivera-Perez, J., Wallace, J. D., Wims, M., Zheng, H. & Bradley, A. (1992) *Anal. Biochem.* **201,** 331–335.
25. Sligh, J. E., Jr., Hurwitz, M., Zhu, C., Anderson, D. C. & Beaudet, A. L. (1992) *J. Biol. Chem.* **267,** 714–718.
26. Bradley, A. (1987) in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, ed. Robertson, E. J. (IRL, Oxford), pp. 113–151.
27. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
28. Ballantyne, C. M., O'Brien, W. E. & Beaudet, A. L. (1989) *Nucleic Acids Res.* **17,** 5853.
29. Wilson, R. W., Ballantyne, C. M., Smith, C. W., Montgomery, C., Bradley, A., O'Brien, W. E. & Beaudet, A. L. (1993) *J. Immunol.* **151,** 1571–1578.
30. Gaspari, A. A. & Katz, S. I. (1991) in *Current Protocols in Immunology*, eds. Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M. & Strober, W. (Greene & Wiley-Interscience, New York), pp. 4.2.1–4.2.5.
31. Lee, H.-M. & Rich, S. (1991) *J. Immunol.* **147,** 1127–1133.
32. Kruisbeek, A. D. & Shevach, E. (1991) in *Current Protocols in Immunology*, eds. Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M. & Strober, W. (Greene & Wiley-Interscience, New York), pp. 3.12.1–3.12.14.
33. Goebeler, M., Gutwald, J., Roth, J., Meinardus-Hager, G. & Sorg, C. (1990) *Int. Arch. Allergy Appl. Immunol.* **93,** 294–299.
34. Anderson, D. C., Smith, C. W. & Springer, T. A. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), pp. 2751–2777.
35. Kehrli, M. E., Jr., Schmalstieg, F. C., Anderson, D. C., Vander-Maten, M. J., Hughes, B. J., Ackermann, M. R., Wilhelmsen, C. L., Brown, G. B., Stevens, M. G. & Whetstone, C. A. (1990) *Am. J. Vet. Res.* **51,** 1826–1836.
36. De Fougerolles, A. R. & Springer, T. A. (1992) *J. Exp. Med.* **175,** 185–190.
37. Fawcett, J., Holness, C. L. L., Needham, L. A., Turley, H., Gatter, K. C., Mason, D. Y. & Simmons, D. L. (1992) *Nature (London)* **360,** 481–484.
38. Vazeux, R., Hoffman, P. A., Tomita, J. K., Dickinson, E. S., Jasman, R. L., St. John, T. & Gallatin, W. M. (1992) *Nature (London)* **360,** 485–488.
39. Furie, M. B., Tancinco, M. C. A. & Smith, C. W. (1991) *Blood* **78,** 2089–2097.
40. Damle, N. K., Klussman, K., Linsley, P. S. & Aruffo, A. (1992) *J. Immunol.* **148,** 1985–1992.
41. Killeen, N., Stuart, S. G. & Littman, D. R. (1992) *EMBO J.* **11,** 4329–4336.
42. Altmann, D. M., Hogg, N., Trowsdale, J. & Wilkinson, D. (1989) *Nature (London)* **338,** 512–514.
43. Dang, L. H., Michalek, M. T., Takei, F., Benaceraff, B. & Rock, K. L. (1990) *J. Immunol.* **144,** 4082–4091.
44. Wegner, C. D., Gundel, R. H., Reilly, P., Haynes, N., Letts, L. G. & Rothlein, R. (1990) *Science* **247,** 456–459.
45. Isobe, M., Yagita, H., Okumura, K. & Ihara, A. (1992) *Science* **255,** 1125–1127.
46. Flavin, T., Ivens, K., Rothlein, R., Faanes, R., Clayberger, C., Billingham, M. & Starnes, V. A. (1991) *Transplant. Proc.* **23,** 533–534.
47. Cosimi, A. B., Conti, D., Delmonico, F. L., Preffer, F. I., Wee, S.-L., Rothlein, R., Faanes, R. & Colvin, R. B. (1990) *J. Immunol.* **144,** 4604–4612.