Intercellular Adhesion Molecule 1 Is Critical for Activation of CD28-Deficient T Cells

Jason L. Gaglia, Edward A. Greenfield, Aditaya Mattoo, Arlene H. Sharpe, Gordon J. Freeman and Vijay K. Kuchroo

*J Immunol* 2000; 165:6091-6098; doi: 10.4049/jimmunol.165.11.6091

http://www.jimmunol.org/content/165/11/6091

References
This article cites 42 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/165/11/6091.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Interacellular Adhesion Molecule 1 Is Critical for Activation of CD28-Deficient T Cells

Jason L. Gaglia,* Edward A. Greenfield,*† Aditaya Mattoo,* Arlene H. Sharpe,‡ Gordon J. Freeman,§ and Vijay K. Kuchroo²*

Presentation of Ag to T lymphocytes in the absence of the requisite costimulatory signals leads to an Ag-specific unresponsiveness termed anergy, whereas Ag presentation in conjunction with costimulation leads to clonal expansion. B7/CD28 signaling has been shown to provide this critical costimulatory signal and blockade of this pathway may inhibit in vitro and in vivo immune responses. Although T cells from CD28-deficient mice are lacking in a variety of responses, they nonetheless are capable of various primary and secondary responses without the induction of anergy expected in the absence of costimulation. This suggests that there may be alternative costimulatory pathways that can replace CD28 signaling under certain circumstances. In this paper, we show that ICAM-1 becomes a dominant costimulatory molecule for CD28-deficient T cells. ICAM-1 costimulates anti-CD3-mediated T cell proliferation and IL-2 secretion in CD28-deficient murine T cells. Furthermore, splenocytes from ICAM-1-deficient mice could not activate CD28-deficient T cells and splenocytes lacking both ICAM and CD28 fail to proliferate in response to anti-CD3-induced T cell signals. This confirms that not only can ICAM-1 act as a CD28-independent costimulator, but it is the dominant, requisite costimulatory molecule for the activation of T cells in the absence of B7/CD28 costimulation. The Journal of Immunology, 2000, 165: 6091–6098.

Optimal activation of T cells by APCs requires at least two signals. The first signal is provided by the recognition of MHC-peptide complex by the TCR and the second signal is provided by the interaction of costimulatory molecules with their respective receptors on T cells. Engagement of the TCR without costimulation is insufficient to induce sustained T cell proliferation and IL-2 production; instead T cells fail to proliferate and become anergic or unresponsive to further activation (1).

Of the cell surface costimulatory molecules studied thus far, B7-1 (CD80) and B7-2 (CD86) are the only ones that have been shown to consistently activate T cells, leading to T cell proliferation and sufficient IL-2 production with protection from TCR stimulation induced anergy (2). Similarly, blockade of B7 in vitro has been shown to induce anergy (3). The effects of these ligands are mediated through their coreceptors, CD28 and CTLA4 (CD152). Several attempts have been made to interrupt the B7/CD28 pathway in vivo. As one would expect from the in vitro data, CD28-deficient mice are impaired in a number of T cell immune responses, most notably defects in Ig class switching and memory T cell-dependent Ab responses (4–6). Interestingly, their IgG responses to chronic infections including lymphocytic choriomeningitis virus are only marginally affected (6). T cells from CD28-deficient mice have greatly reduced in vitro responses to lectins and unstimulated allogeneic spleen cells (1, 6), but are still capable of raising a number of in vivo immune responses. These include allograft rejection (7), induction of allogeneic graft-versus-host disease (8), generation of cytotoxic T cells (7, 9), and mounting certain delayed-type hypersensitivity responses (6).

Similarly, mCTLA4-Hy1-transgenic mice, which produce CTLA4Ig capable of blocking B7, can generate normal primary T cell responses and show Ag-specific CD4+ T cell expansion after secondary or tertiary immunization (10). Thus, T cells from CD28-deficient mice and the mCTLA4-Hy1-transgenic mice were not anergic after in vivo priming and were capable of clonal expansion and Ag-specific responses in vitro. This suggests the existence of alternate non-B7/CD28 costimulatory pathways that can supplant CD28 signaling under these conditions. A number of cell surface molecules including CD44H, heat-stable Ag, OX40 ligand, CD54, and B7h have been shown to be capable of CD28-independent costimulation (11–15). However, none of these have been shown to be capable of replacing all of the functions of the B7/CD28 pathway (2).

We have previously studied untransfected Chinese hamster ovary (CHO) cells capable of CD28-independent costimulation of T cell proliferation and IL-2 secretion (our unpublished results). By generating blocking anti-CHO Abs, we were able to express clone several candidate molecules that may be involved in Ag presentation and T cell activation. Among the gene products identified in this system was hamster CD54 (ICAM-1). By transiently expressing hamster ICAM-1 on COS cells we could activate CD28-deficient T cells with soluble anti-CD3. In our system, there was significant amounts of IL-2 secretion as well as an increased proliferative response of the CD28-deficient T cells (when compared with wild type) to both CHO cells and ICAM expressing COS transfectants (our unpublished results). This suggests that the...

Received for publication July 3, 2000. Accepted for publication August 29, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by National Institutes of Health Grants R01NS30843, R01NS35685, P01AI39671-01A1, CA40216, AI39671, and AI25082 and by National Multiple Sclerosis Society Grants RG2571 and RG2320. J.L.G. was supported by the Howard Hughes Medical Institute as a Medical Student Research Fellow.

2 Address correspondence and reprint requests to Dr. Vijay K. Kuchroo, Department of Neurology, Center for Neurologic Diseases, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115. E-mail address: kuchroo@ndc.bwh.harvard.edu

Abbreviations used in this paper: CHO, Chinese hamster ovary; CD40L, CD40 ligand.
study of costimulation in the absence of B7/CD28 signaling may be required to study the more subtle interactions that must be responsible for the unexpected phenotypes of mice with disruptions of the B7/CD28 pathway. Our results demonstrate that ICAM-1 plays a dominant role in the activation of T cells in the absence of B7 costimulation.

Materials and Methods

Experimental animals
BALB/c, C57BL/6J, C57BL/6-CD28 tm1Mak >, and C57BL/6J-ICAM tm1Bay > mice were purchased from The Jackson Laboratory (Bar Harbor, ME). We intercrossed the C2D2 tm1 and ICAM tm1 mice in our facility to generate CD28 and ICAM double-deficient mice. All mice were housed at the animal facility of the Harvard Institutes of Medicine (Boston, MA).

Cell lines

COS cells were grown in RPMI 1640 supplemented with 10% heat-inactivated FCS, 25 IU/ml penicillin, 25 µg/ml streptomycin, 2 mM t-glutamate, and 100 µg/ml gentamicin. All T cell proliferation assays were conducted in the following culture medium: DMEM supplemented with 10% heat-inactivated FCS, 2 mM t-glutamate, 1 mM sodium pyruvate, 25 IU/ml penicillin, 25 µg/ml streptomycin, 1× NEAA (BioWhittaker, Walkersville, MD), folic acid, arginine, asparagine, 1× MEM vitamin solution (BioWhittaker), 2-ME, and 100 µg/ml gentamicin. The IL-2-dependent cell line CTLL2 was maintained in an IL-2 supplemented medium consisting of the above medium supplemented with 10% T-Stim (Collaborative Biomedical Products, Bedford, MA) as an IL-2 source. The IL-2 in the medium was allowed to become depleted by consumption before use of the CTLL2 cells in assays.

Antibodies

Anti-CD3 145.2C11 (16) (hamster IgG) culture supernatant was used to cross-link the TCR-CD3 complex. Anti-hFcRIIa (CD32) mAb 2E1 was purchased from Immunotech (Westbrook, ME). PE-conjugated goat anti-mouse F(ab’)2 was purchased from Southern Biotechnology Associates (Birmingham, AL). Antihamster ICAM Abs capable of inhibiting CHO-mediated costimulation were generated in BALB/c mice after repeated immunization with whole CHO cells using the methods of Kohler and Milstein (17) and Kearney et al. (18).

T cell isolation

T cells were enriched using a modification of the technique of nonadherence to nylon wool. Briefly, mouse spleens were harvested and a single-cell suspension was prepared by mincing, followed by passage through a 70-µm nylon mesh. Erythrocytes were lysed by incubation in ACK lysis buffer (150 mM NH4Cl, 1 mM KHCO3, 0.1 mM EDTA, pH 7.4) on ice (19). The remaining cells were resuspended in warm complete medium containing DMEM (Life Technologies, Gaithersburg, MD) supplemented with 5% FCS (5 medium), layered onto a sterile equilibrated nylon wool column, and adhered for 45 min at 37°C incubator. The nonadherent cells were eluted in 5% medium. Residual adherent cells were removed by incubating the eluent over tissue culture-treated plastic for 1 h at 37°C.

With this method of purification, >95% of the resulting cells were CD3 positive by flow cytometry, and the maximal proliferative response of 5 × 105 purified cells to anti-CD3 (culture supernatant diluted 1:50) was under 1000 cpm.

Costimulation and proliferation assays

To prevent proliferation of accessory cells, splenocytes were γ-irradiated (5000 rad), whereas all other stimulator cells were incubated in medium containing mitomycin C (50 µg/ml for 15–18 h). Purified T cells were incubated with stimulators incapable of proliferation and soluble anti-CD3 (145.2C11 hybridoma culture supernatants at 1:50–1:100 dilutions) for 66 h. The incorporation of [3H]TdR was used as an index of mitogenic activity and proliferation. During the last 18 h of culture, the cells were incubated with 1 µCi/well [3H]TdR (NEN, Boston, MA). The incorporated radiolabeled thymidine was measured by harvesting the cells onto filters and measuring the radioactivity of the dried filters using the Beta Plate scintillation counter (Wallac, Gaithersburg, MD).

Measurement of IL-2 secretion

The IL-2-dependent cell line CTLL2 (ATCC TIB 214; American Type Culture Collection, Manassas, VA) was used to measure IL-2 secretion. Culture supernatants were harvested from wells 16–20 h after activation of T cells with anti-CD3 plus APCs. Contaminating cells were removed by centrifugation. To 100 µl of culture supernatant, 106 CTL2 cells were added in an equal volume. After 16 h of incubation at 37°C, 1 µCi/well [3H]TdR was added. The plates were harvested at 22 h. Data shown are the means of triplicate wells and fall within the linear range of a standard IL-2 curve run concurrently.

Immunoprecipitation

CHO cells were surface labeled in an aqueous solution of 1 mM sulfo-NHS-LC-biotin (Pierce, Rockford, IL) in PBS with calcium (0.1 mM CaCl2) and magnesium (1 mM MgCl2). Unreacted biotin was quenched with DMEM and removed by subsequent washings. The labeled cells were lysed in immunoprecipitation buffer (150 mM NaCl, 50 mM Tris (pH 8.0), 1% Nonidet P-40, 1 mM PMSF, 1 µg/ml leupeptin, 1 µg/ml aprotonin, and 1 µg/ml pepstatin) for 20 min at 4°C. The lysate was centrifuged at 15,000 × g for 10 min at 4°C and then precleared with goat anti-mouse Sepharose 4B beads (Cappel, West Chester, PA) preloaded with normal mouse serum. Immune complexes were collected onto preblocked goat anti-mouse Sepharose 4B beads armed with anti-ICAM-1 mAb. The Sepharose bead pellets were rinsed and the immunoprecipitated material was eluted from the beads by boiling in Laemmli sample buffer and resolved by SDS-PAGE under reducing conditions (20). Proteins were electrophoreted to nitrocellulose membranes and visualized with NeutrAvidin-HRP (Pierce) using ECL (Amersham, Arlington Heights, IL) detection reagents.

Transient expression system for costimulation

CHO cells were cotransfected with hFcRIIa (CD32) cDNA and hamster ICAM-1 cDNA or mB7 cDNA using the DEAE-dextran method described by Seed and Araujo (21). Briefly, 100-mm dishes of 50% confluent COS cells were transfected with 4 µg of CScl preparation of plasmid DNA for each plasmid. Vector DNA was added to transfections to keep the total DNA concentration constant. The cells were trypsinized and replated after 24 h. At 72 h, the cells were detached by incubation in PBS/0.5 mM EDTA (pH 7.4) for 30 min. The detached cells were resuspended in mitomycin C (50 µg/ml) and incubated at 37°C for 3 h to inhibit proliferation, with subsequent washing to remove residual mitomycin C. Expression of hFcRIIa and candidate molecules was verified by flow cytometric analysis. The mitomycin C-treated transfected COS cells were also tested in T cell proliferation assays as described.

Flow cytometry

For flow cytometric analysis, cells were rinsed in PBS. Approximately 106 cells were rinsed and then resuspended in FACS buffer (1% FCS and 2 mM Na3, in PBS). The cells were incubated with a saturating concentration of primary Ab for 45 min at 4°C. The cells were washed three times to remove excess unbound Ab, and then incubated with the appropriate secondary Ab for 45 min at 4°C. Excess Ab was again removed by washing three times, and the cells were resuspended in 1% Formalin in PBS and incubated for at least 30 min at 4°C before analysis on a FACScan (Becton Dickinson, Mountain View, CA). Alignment of the flow cytometer was verified with Immunocheck beads (Becton Dickinson) and Autoomp software (Becton Dickinson).

FIGURE 1. Immunoprecipitation of cell surface-labeled CHO cells. Cell surface-labeled CHO cells were immunoprecipitated as described. mAbs J5.3F9 and J5.6D12 identified an ~120-kDa protein.
Results

We found that a subline of untransfected CHO cells in our laboratory was able to costimulate T cell proliferation and IL-2 secretion for CD28-deficient T cells. To characterize the costimulatory molecule(s) capable of costimulating CD28<sup>-/-</sup> T cells, we generated mAbs that were capable of blocking this activity. Two mouse anti-hamster Abs, J5.3F9 and J5.6D12 (both IgG1 isotype), that bind the same 120-kDa protein blocked the costimulatory activity (Fig. 1), which we identified as the hamster homologue of CD54 (ICAM-1) by expression cloning using the immunoselection method of Seed and Aruffo (21). This hamster gene was highly conserved throughout its length, having 72% amino acid identity with the murine ICAM-1 precursor MALA-2 (Fig. 2). Furthermore, the hamster cDNAs encode all five Ig domains of ICAM-1 and therefore did not represent a splice variant. The two mAbs thus generated not only blocked CHO-mediated costimulation of CD28<sup>-/-</sup> cell but also of CD28<sup>-/-</sup> T cells (data not shown).

We have produced COS cell transfectants expressing only FC<sub>R</sub> (CD32), which cross-links anti-CD3 mAb on the T cell surface, or COS cells expressing FC<sub>R</sub> plus the hamster ICAM-1 to determine whether hamster ICAM-1 could costimulate CD28-deficient T cells. Along with anti-CD3 mAb, COS cells cotransfected with

---

**FIGURE 2.** Sequence homology between hamster and murine ICAM-1. The consensus sequence presented obtained by double-stranded sequencing multiple clones identified by Abs J5.3F9 and J5.6D12. Amino acid identities (●), highly conserved substitutions (●●), and moderately conserved substitutions (●) are indicated. Predicted N-linked glycosylation sites are marked with an asterisk. The signal peptide (signal), transmembrane (TM), and cytoplasmic domains are indicated.
ICAM-1 and FcR induced significantly higher T cell proliferation (Fig. 3A) and IL-2 secretion (Fig. 3B), as compared with COS cells transfected with FcR alone. Thus, hamster ICAM-1 expressed on COS cells is capable of costimulating both T cell proliferation and IL-2 secretion by wild-type T lymphocytes; COS cells cotransfected with FcR and mB7-1 were used as a positive control in these experiments and induced strong responses (Fig. 3). Cotransfection with mB7-1 and ICAM-1 in addition to FcR proved additive (Fig. 3). When the response of wild-type T cells to costimulation by FcR/ICAM-1 transfectants is compared with that of CD28-deficient T cells, the CD28-deficient T cells showed an increased proliferative response as compared with wild-type T cells at higher concentrations (Fig. 4A). Similarly, the IL-2 secretion by the CD28-deficient T cells was induced by the ICAM transfectants, although the amounts produced by CD28<sup>+/−</sup> T cells was greater (Fig. 4B). Such differences may represent an increased sensitivity of CD28-deficient T cells for alternate costimulatory pathways.

These data confirm that ICAM-1 can effectively costimulate T cells and induce IL-2 production in the absence of CD28. Since earlier studies indicated that ICAM-1 may directly affect T cell-APC interactions, we next tested whether ICAM-1 is required for costimulation in the absence of CD28. Ab blockade of ICAM-1 has proven ineffective in determining ICAM-1’s relative contribution to costimulation since Abs against ICAM-1 cause disruption of cell-cell adhesion and therefore block all costimulation (22). Instead, we measured costimulation in the absence of ICAM-1 by using splenocytes from mice with a targeted disruption of ICAM-1, rendering them deficient in the common isoform of ICAM-1 (23). Interestingly APCs from these mice are able to maintain adequate adhesion interactions for wild-type T cell activation. Fig. 5 shows that LPS-activated splenocytes from ICAM-1-deficient mice can act as competent costimulators of T cells purified from wild-type but not CD28-deficient mice. LPS-activated wild-type splenocytes were used as positive controls and were able to effectively stimulate the CD28-deficient T cells.

Furthermore, when splenocytes derived from CD28-ICAM double-deficient mice were tested for their ability to respond to anti-CD3 T cell activation, they could not proliferate or secrete IL-2 after anti-CD3 stimulation (Fig. 6). The splenocytes from wild-type and ICAM<sup>−/−</sup> mice responded equally well to the anti-CD3 whereas the splenocytes from CD28<sup>−/−</sup> mice responded only at high concentrations of anti-CD3 (Fig. 6A) and produced relatively little IL-2 (Fig. 6B). These data indicate that although CD28 is normally important for costimulation, ICAM-1 is crucial to activate T cells in its absence.
Discussion

Increasing evidence from studies of CD28-deficient mice and CTLA4-Ig-transgenic mice suggests that alternative costimulatory pathways are used for immune function in the absence of B7/CD28 costimulation. Although CD28-deficient mice have many immunologic defects, the T cells from these mice are not anergic. CD28-deficient mice are capable of in vivo priming of T cells and can show in vitro proliferative responses to anti-CD3 or specific Ags (1). While investigating CD28-independent costimulation mediated by CHO cells, we noted the prominent role played by ICAM-1 under these conditions.

For quite some time, the role of ICAM-1 in providing costimulatory signals has remained controversial, as it has been difficult to separate the role of ICAM-1 in costimulation from its role in adhesion. Some published reports suggest that ICAM-1 is not sufficient for costimulation (24), whereas others suggest that ICAM-1 is sufficient for T cell activation but not IL-2 secretion (25) and still others report that ICAM-1 can costimulate both T cell proliferation and IL-2 secretion (26). Damle et al. (27) proposed a model suggesting that ICAM-1 may have a differential costimulatory activity in that ICAM-1 can costimulate naive T cells but may provide a significantly weaker signal to preactivated T cells. This model is supported by a recent study which shows that costimulation by ICAM-1 leads to a transient expression of IL-2 mRNA which results in detectable IL-2 secretion by naive CD4+ T cells but not T cell clones (28). Furthermore, Zuckerman et al. (28) have reported that the level of IL-2 expression associated with ICAM-1-mediated costimulation is not capable of protecting against the induction of anergy associated with TCR-mediated signal alone. Although ICAM-1 can provide the signals necessary to up-regulate IL-2 gene transcription, it apparently does not stabilize IL-2 mRNA in CD4+ T cells (28). The ability of ICAM-1 to induce but not stabilize IL-2 mRNA may account for the synergistic effect reported between ICAM-1 and B7 (26) (Fig. 3), as CD28 signaling is known to stabilize IL-2 mRNA (29, 30).

Although our studies only used unfractionated CD4+ T lymphocytes, other groups have examined the differential effects of ICAM-1-mediated costimulation on the activation of CD4+ and CD8+ T cells. Recent reports suggest that ICAM-1 can costimulate the proliferation of both CD4+ and CD8+ T cells but preferentially costimulates IL-2 production by CD8+ and not CD4+ T cells (14, 31). Deeths and Mescher (14) have reported that

---

**FIGURE 4.** ICAM-1 transfectants costimulate both wild-type and CD28-deficient T cells. A, Microcultures were set up as described with 5 × 10^4 T cells purified from C57BL/6 wild-type or CD28-deficient mice as indicated. Mitomycin C-treated COS cell transfectants were used at the concentrations indicated on the x-axis. cDNA used for transfections was as indicated in the key; vector DNA was used to make the final concentration of DNA for all transfections equal. FcR was concurrently expressed to cross-link the anti-CD3-bound TCR. Expression of transfected molecules was verified by flow cytometry (data not shown). Anti-CD3 mAb 145-2C11 supernatant was added to each of the cultures at 1:100 final dilution. B, After 16–20 h, culture supernatants prepared in parallel with those in A were harvested and analyzed for the presence of IL-2 by the CTLL2 assay as described. CTLL2 cells were incubated for 22 h and pulsed with 1 μCi of [3H]Tdr for the last 6 h of the incubation period. Data are presented as mean cpm ± SD of the [3H]Tdr incorporated into DNA.
ICAM-1 can costimulate both naive and memory CD8 T cells to produce IL-2 levels similar to those seen with B7-1 costimulation. This IL-2 production is comparable to that observed in our experimental systems. However, the in vivo responses to ICAM-1-mediated costimulation may be more complicated. Kim et al. (32) have found that the in vivo expression of ICAM-1, with cDNA expression cassettes, causes enhancement of both immunogen-specific CD4 Th responses and CD8-restricted cytotoxic T lymphocyte responses. This may reflect other costimulators acting in concert with the transfected ICAM-1. The possibility of a CD28-independent costimulator acting preferentially on CD8+ T cells is particularly interesting given the findings of Newell et al. (33), who have reported that B7/CD28 blockade with hCTLA4Ig inhibits intestinal allograft rejection by CD8 knockout mice but not CD4 knockout recipients. These results may reflect the data presented herein that ICAM-1 is required in the absence of CD28 and the previous findings that ICAM-1 can preferentially affect CD8 T cells.

It should be noted that alternative isoforms of ICAM-1 can be generated by RNA splicing (34). These different isoforms have distinct patterns of expression and may play different immunologic functions, including differential adhesive and costimulatory roles.
maintained by the remaining isoforms or alternative adhesion molecules. In the ICAM-1-deficient mouse, this lack of ICAM-1 costimulation may be compensated for by CD7 costimulation. However, in the absence of the CD7/CD28 pathway, the relative importance of ICAM-1 increases and our data would suggest that the common isoform (but not the splice variants expressed) of ICAM-1 then plays a critical role in costimulation. This form of ICAM-1 then becomes necessary for costimulation in the absence of CD28.

In our knockout system, common form ICAM-1 was deficient without grossly affecting the cellular adhesion necessary for functional activation of wild-type T cells. However, in the absence of both CD28 and ICAM-1, there was a lack of response to TCR stimulation. It is unclear whether this finding is secondary to the role of ICAM-1 as a direct costimulator or due to a loss of cell-cell interaction in the absence of both ICAM-1 and CD28. Life et al. (35) have previously noted that CD28 can indeed play a role in cellular adhesion as anti-CD28 treatment of combined B and T cell cultures leads to a decrease in visible cell aggregates and marked impairment of conjugate formation as measured by dual intracellular staining and flow cytometry. It follows that impaired expression of ICAM may increase the importance of the adhesive role of CD28. Similarly, Wülfing and Davis (36) described active T lymphocyte cortical actin cytoskeletal movement triggered by costimulation with either ICAM or B7. They suggested that this was necessary for the active accumulation of receptor pairs and other cytoskeleton-linked molecules at the T cell–APC interface required for T cell activation (36). Although this does not preclude other CD28-independent costimulatory pathways such as the proposed positive signaling through CTLA4 (37), heat-stable Ags., or B7h (via ICOS)-induced costimulation (12, 38), it does necessitate the expression of ICAM-1 under these conditions.

It has been previously shown by Shinde et al. (39) that CD40 ligand (CD40L) is not essential for T cell responses to TCR engagement by costimulatory competent APCs. However, blockade of CD40-CD40L and B7-CD28 is more efficacious at inhibiting engagement by costimulatory competent APCs. However, blockade of CD40-CD40L in-}

References

tional energy is induced by antigen presentation in the absence of B7 costimulation. Proc. Natl. Acad. Sci. USA 90:6586.
8. Deeths, M. J., and M. F. Mescher. 1999. ICAM-1 and B7-1 provide similar but distinct costimulatory activities for CD4+ T cells, while CD4+ T cells are poorly costimu
12. Dohrs, M. J., and M. F. Mescher. 1999. ICAM-1 and B7-1 provide similar but distinct costimulation for CD8+ T cells, while CD4+ T cells are poorly costimu
cellular adhesion molecule-1 or vascular cell adhesion molecule-1 induces func


