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Activation-Induced Expression of Carcinoembryonic Antigen-Cell Adhesion Molecule 1 Regulates Mouse T Lymphocyte Function

Atsushi Nakajima,* Hideki Iijima,* Markus F. Neurath,* Takashi Nagaishi,* Edward E. S. Nieuwenhuis,* Raktiama Raychowdhury,* Jonathan Glickman,‡ Dianna M. Blau,¶ Sara Russell,* Kathryn V. Holmes,‡ and Richard S. Blumberg2*

Carcinoembryonic Ag cell adhesion molecule 1 (CEACAM1) consists of highly related homologs in humans and rodents that are characterized by significant alternate splicing generating isoforms capable of negative intracellular signaling by virtue of two immunoreceptor tyrosine-based inhibition motifs in its cytoplasmic (cyt) tail. Although human T cells have been recently observed to express CEACAM1, the expression and function of CEACAM1 in mouse T cells have not been defined. Although resting mouse spleen T cells exhibited no evidence of CEACAM1 on the cell surface, CEACAM1 was rapidly up-regulated on CD4+ and CD8+ T cells after activation with either Con A or anti-CD3 without a requirement for either de novo transcription or translation due to the fact that CEACAM1 was present intracellularly before activation. Using a GST-CEACAM1-cyttoplasmic tail fusion protein, it was shown that the cytoplasmic tail of CEACAM1 bound the src homology domain-containing phosphatase 1 and adaptor protein 1 complex in its phosphorylated and nonphosphorylated states, respectively. CEACAM1 ligation with an anti-CEACAM1 mAb resulted in inhibition of an allogeneic MLR and anti-CD3 plus anti-CD28 Ab-induced proliferation of spleen T cells in vitro and inhibition of a delayed-type hypersensitivity response to oxazolone in vivo. Inhibition of the delayed-type hypersensitivity response required that the anti-CEACAM1-specific mAb be present at the time of T cell sensitization. These studies support a role for CEACAM1 as a novel class of immunoreceptor tyrosine-based inhibition motif-bearing regulatory molecules on T cells that are active during early phases of the immune response in mice.
variants of the IgV-like N domain called CEACAM1α and significant homologies that exist between human and mCEACAM1. Most inbred mouse strains, except SJL/J, express the CEACAM1α allele (3).

A major proven function of CEACAM1 is intercellular adhesion. Homophilic adhesion of the CEACAM1 has been linked to development of the intestine, placenta, muscle, tooth, and vascular systems and to the regulation of cell growth (11–14). Heterophilic adhesion of CEACAM1 has also been observed with other CEACAM members (2), E-selectin through expression of the sialyl-Lewisα Ag by CEACAM1 (15), and cell surface structures of bacteria (including Escherichia coli, Salmonella, Hemophilus influenza, and Neisseria sp.) (7, 16, 17) and the murine coronavirus, murine hepatitis virus (5, 6). Such heterophilic interactions may be involved in regulating microbial colonization of the gut, leukocyte trafficking, bacterial phagocytosis, and the pathogenesis of coronavirus infections.

These regulatory functions associated with intercellular adhesion are highly associated with the ability of the long CEACAM1-associated cyt tail to deliver intracellular signals. The long cyt tail of CEACAM1 of mouse and rat, for example, has been shown to bind calmodulin and src-related tyrosine kinases (18–20). The latter leads to tyrosine phosphorylation of the two immunoreceptor tyrosine-based inhibition motifs (ITIM) contained within the long cyt tail of CEACAM1 and the binding to src homology 2 domain-containing protein tyrosine phosphatases (SHPs), such as SHP-1 and SHP-2 in epithelial cells (18, 19). Such attributes of CEACAM1 have been associated with inhibition of tumor cell growth (21–23). Whereas CEACAMS (formerly called CEA) has been shown to be up-regulated in a wide variety of human epithelial tumors, CEACAM1 expression is commonly diminished (22, 24). As a corollary, transfection of CEACAM1 into tumors of the colon, breast, prostrate, endometrium, and lung leads to inhibition of their growth, presumably through homophilic CEACAM1 interactions and recruitment of SHP-1 (23).

CEACAM1 has recently been reported on human B and T lymphocytes and NK cells (25–27). Although CEACAM1 expression on B cells was observed to be constitutive (25), the expression on T cells was inducible and required prior activation (26, 27). Moller and colleagues (26) first reported that CEACAM1, but not other CEACAM members, is expressed on a subset of activated peripheral blood NK cells that are CD16+ CD56− and on conventional T cells using a panel of mAbs. Subsequently, the same group reported confirmatory data (27) and showed that CEACAM1 was expressed on activated CD4+ and CD8+ T cells expressing either αβ or γδ TCR. Additionally, it was shown that immobilized and soluble anti-CEACAM1 mAbs enhanced and inhibited the proliferation of human T cells induced by anti-CD3 mAbs, respectively (27). Although the expression of CEACAM1 by activated human T cells was recently confirmed by two additional groups in mucosal and peripheral tissues (28, 29), both a negative (28) and a positive (29) costimulatory role was ascribed to the cell surface expression of this molecule in human T cells as defined in vitro, respectively. Such inconsistencies may relate to the complex splicing patterns attributable to CEACAM1 (3) and the methodologic approaches used, among other possibilities.

The in vivo function of CEACAM1 in T cell-dependent models has not, however, yet been examined in rodents that express highly related homologs (3). In fact, previous studies have concluded that mouse T cells are CEACAM1α negative (30, 31). Given the significant homologies that exist between human and mCEACAM1, we have re-examined the expression and function of mCEACAM1α on T lymphocytes to determine the immunoregulatory function of this molecule in this species, if any. In this report we show that cell surface expression of mCEACAM1α can be detected after T cell activation, including ligation of the TCR/CD3 complex. Moreover, we provide evidence that mCEACAM1α is present in resting T cells and is rapidly mobilized to the cell surface after T cell activation. In association with this, we observed that the nonphosphorylated and phosphorylated long cyt tails of mCEACAM1α differentially associate with adaptor protein 1 and SHP-1, respectively. Finally, mCEACAM1α ligation with an anti-CEACAM1α-specific mAb was associated with inhibition of T cell activation in vitro and in vivo as defined in an oxazolone-mediated, delayed-type hypersensitivity (DTH) model.

Materials and Methods

Antibodies

The mouse mAb specific for mCEACAM1α (CC1; mouse IgG1) and polyclonal rabbit anti-mCEACAM1α Ab have been previously described (5, 6, 32, 33). Normal rabbit and mouse control Abs, anti-mouse IgG1-FITC, anti-CD3-PE, anti-CD3-PerCP, anti-CD3, anti-CD28, anti-CD4-PE, anti-CD8-PE, anti-CD19-FITC, anti-CD16/32, and anti-CD69-biotin were purchased from BD PharMingen (San Diego, CA). Anti-SHP-1 Ab was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell preparations

Spleen mononuclear cells were prepared from 6- to 8-week-old male BALB/c or C57BL/6 mice (Charles River Breeding Laboratories, Wilmington, MA) and activated with either Con A (2.5 μg/ml) or plate-bound anti-CD3 mAb by standard methods. To purify T cells from spleen, negative selection was performed using Dynabeads (Dynal Biotech, Lake Success, NY) containing anti-CD19 and CD11b mAbs, resulting in preparations that contained <2% B cell contamination based upon FACS analysis of anti-CD19-stained samples. A mouse T cell lymphoma line, 4A2 (ATCC TIB-154), and a mouse B cell line, SP20 (ATCC CRL-1581), were purchased from American Type Culture Collection (Manassas, VA).

FACS analysis

FACS was performed using a FACSsort (BD Biosciences, San Diego, CA) after staining with standard methods (34). Briefly, the cell suspensions were incubated with an anti-FcγR Ab (anti-CD16/32, 2.4G2) to prevent non-specific binding by the secondary Ab for 1 h and were washed twice in suspension medium (SM: PBS without calcium, but with 1% FBS). The cell suspension was incubated with the primary Ab for 1 h at a concentration of 1–10 μg/ml, washed twice with SM, and labeled with secondary Ab, followed by two washes with SM before suspension in PBS containing 0.5% paraformaldehyde. FACS was then performed. Control staining with an irrelevant isotype–matched Ab was performed for every FACS analysis. To identify mCEACAM1 on splenic T cells by two- and three-color staining, the cell suspension was stained with the mCEACAM1α mAb, CC1, as the primary Ab, followed by an anti-mouse IgG1-FITC conjugate as the secondary Ab and staining with an anti-CD3-PE conjugate and/or a biotin-labeled anti-CD69, -CD4, or -CD8 mAb, with development of the latter with tricolor-conjugated avidin (BD Pharmingen).

Inhibitor treatment

Before activating the spleen cells and assessment by FACS, the cell suspension was treated with actinomycin D, cycloheximide, or brefeldin A for 30 min at maximum concentrations of 200 μM, 10 ng/ml, and 20 μM, respectively.

GST long cyt tail fusion protein and GST pull-down

mRNA were prepared by poly(A) selection, and cDNA were prepared from murine spleen cells by standard methods (35); the long cyt tail of mCEACAM1α (aa 420–492) was cloned by RT-PCR using forward (5′-GGCGATCTCTATTTCTCCTCAGGAAG-3′) and reverse (5′-CGG GAGATTATCTCTTTTTACTCTGCA-3′) primers incorporating BamHI and EcoRI restriction sites, respectively, and subcloned into the pGEX-4T vector (Amersham Pharmacia Biotech, Piscataway, NJ). The
GST-mCEACAM1 1-3 fusion protein was synthesized and purified using the GST fusion protein system (Amersham Pharmacia Biotech) and modified to contain phosphorylated tyrosine residues using the TK-Phos System (Stratagene, La Jolla, CA). Lysates were prepared by solubilizing cells in immunoprecipitation buffer containing 100 mM NaCl, 10 mM Tris (pH 7.8), 1 mM EDTA, protease inhibitors, and 1% Nonidet P-40 as previously described (36, 37), and GST pull-downs were performed with the fusion proteins followed by Western blotting with an Ab specific for SHP-1 by standard methods (36, 37). mRNA and cDNA were also used to clone the cDNA encoding AP47 (µl chain of adaptor protein 1) using forward (5'-CCAAAGCTTTACCTCCAGCGGCCTGCATC-3') and reverse (5'-TACCTGGATCCACTGGTGCCGGAGCTGTAATC-3') primers incorporating HindIII and XhoI sites, respectively, and subcloned into the pCDNA3.1 expression vector (Invitrogen, Carlsbad, CA). In vitro transcription and translation were performed using the TNT kit (Promega, Madison, WI) in the presence of [ 35S]methionine (Amersham Pharmacia Biotech) (36). In vitro synthesized AP47 was pulled down with either the tyrosine-phosphorylated or the nonphosphorylated GST-CEACAM1 1-3 fusion protein, the immunoprecipitates were resolved by SDS-PAGE under reducing conditions, and autoradiography was performed.

**MLR and cell proliferation assay**

Spleen cells from C57BL/6 were used as the responder cells, and spleen cells from BALB/c, which were treated with mitomycin C (100 µg/ml; Sigma-Aldrich, St. Louis, MO) for 90 min, followed by extensive washing with complete medium, were used as the stimulator cells. The responder cells and stimulator cells were mixed in equal ratios in a total volume of 200 µl at a cell concentration of 1 x 10^5 cells/ml and cultivated in 96-well, round-bottom microtiter plates for 3 days at 37°C in 5% CO₂. Subsequently, [ 3H]Thymidine (Amersham Pharmacia Biotech) was added 5 h before harvesting (38). Incorporation of [ 3H]thymidine was assessed by scintillation using a 96-well plate reader (Packard Instrument, Meriden, CT). For cell proliferation assays, plate-bound anti-CD3- and anti-CD28-specific Abs were prepared according to a standard protocol, described previously (38), in either the presence or the absence of varying concentrations of the anti-mCEACAM1 1-3 mAb CC1. Freshly prepared mouse T cells from spleen were incubated in 96-well, flat-bottom microtiter plates for 3 days at 37°C in 5% CO₂. Incorporation of [ 3H]thymidine was assessed by scintillation as described above.

**Immunohistochemical staining**

Cytoplasts of purified mouse T cells were prepared with a cytocentrifuge (Cytospin 2; Shandon, Norwood, MA) onto silane-coated glass slides and air-dried, followed by fixation and permeabilization in acetone for 30 s. After dehydration with alcohol, staining was performed with a polyclonal rabbit anti-mCEACAM1 1-3 Ab or control rabbit serum using the avidin biotin complex kit (Vector Laboratories, Burlingame, CA) for visualization of the primary Ab.

**In vivo assessment of DTH response**

C57BL/6 mice were injected i.p. with either the CC1 mAb (1 mg in PBS) or the control IgG1 mAb. Twenty-four hours after mAb treatment mice were presensitized by painting 150 µl of the haptenating agent, 3% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; Sigma-Aldrich), a classic low m.w. chemical hapten, dissolved in 100% ethanol onto a shaved abdomen (39, 40). Five days after presensitization, 1% oxazolone in 10 µl of 10% HCl in 0.9% NaCl control or ethanol alone as a control was painted on the right and left ears, respectively, in either the presence or the absence of a second i.p. injection of the CC1 or control IgG1 mAbs administered 24 h before ear challenge. Ear swelling was measured before and 24 h after the ear challenge with a dial thickness gauge (Mitutoyo, Kanagawa, Japan). DTH responses were expressed as the increase in ear swelling after oxazolone painting on the ear following subtraction of the thickness before the challenge for the control (IgG1 mAb) and anti-CEACAM1 1-3 (CC1 mAb)-treated groups. A fragment (5 mm²) of the center portion of the ear from six mice in each group was assessed by paraffin embedding by standard H&E staining, and three sections from each tissue block were examined.

**Statistics**

Data were expressed as the mean ± SEM and were analyzed using a two-tailed Student’s t test for independent samples.

**Results**

mCEACAM1 1-3 is expressed on the cell surface at early stages of T cell activation

Confirming previous observations, mCEACAM1 1-3 could not be detected on resting splenic T cells using the CC1 mAb (30). However, when mouse splenocytes from C57BL/6 animals were activated with Con A, mCEACAM1 1-3 was rapidly up-regulated on the cell surface of CD8⁺ cells, as defined by two-color FACS. mCEACAM1 1-3 expression was detected by 0.5 h after T cell activation, with expression peaking at 12–24 h, by which time all T cells exhibited mCEACAM1 1-3 expression on the cell surface (Fig. 1A) with persistence for up to 72 h (data not shown). This was in contrast to CTLA-4, which was up-regulated after 72 h as previously described by others (41). mCEACAM1 1-3 could be detected on large (blast-like) CD3⁺ cells as assessed by forward scattering analysis and overlapped nearly completely with the expression of CD69, which was observed early after T cell activation (data not shown). Identical observations were made after anti-CD3 stimulation of purified T cells from spleen and were confirmed with a second polyclonal rabbit anti-mCEACAM1 1-3 Ab (data not shown). mCEACAM1 1-3 could be detected on the cell surface of both CD4⁺ and CD8⁺ T cells as defined by three-color FACS with anti-mCEACAM1 1-3, anti-CD3, and either anti-CD4 or anti-CD8 mAbs (Fig. 1, B and C). By 2 h of activation with Con A, 48–53% of T cells, as defined by anti-CD3 staining, expressed CEACAM1 1-3 (Fig. 1B). Of these, 36% of the CD4⁺ T cells (Fig. 1B) and 37% of the CD8⁺ T cells (Fig. 1B) expressed mCEACAM1 1-3, suggesting that mCEACAM1 1-3 is regulated similarly in both T cell subsets. These results were confirmed by examining mCEACAM1 1-3 expression on the CD4⁺ and CD8⁺ subsets after gating on the CD3⁺ cells which showed mCEACAM1 1-3 expression on blastic CD4⁺ and CD8⁺ cells (Fig 1C and data not shown). These studies indicate that murine CEACAM1, like human CEACAM1 (26–29), is expressed on the cell surface of activated, but not resting, CD4⁺ and CD8⁺ T cells.

mCEACAM1 1-3 is preformed in resting T cells

We subsequently observed that actinomycin D, an inhibitor of DNA-dependent RNA synthesis, was unable to inhibit the cell surface expression of mCEACAM1 1-3 on T cells activated by Con A (Fig. 2A, ↔), although it could inhibit the expression of CD69 (Fig. 2A, ↔ in a dose-dependent manner. Identical findings were made with cycloheximide, an inhibitor of protein synthesis, and after direct anti-CD3 stimulation (data not shown). These results indicate that the expression of mCEACAM1 1-3 on T cells requires neither de novo transcription nor translation, suggesting that mCEACAM1 1-3 is presynthesized in resting T cells. Consistent with this, mCEACAM1 1-3 could be detected in lysates of splenic T cells by Western blot analysis. Fig. 2B shows that when protein lysates of total splenocytes and purified T cells were subjected to Western blot analysis with a rabbit anti-mCEACAM1 1-3 polyclonal Ab, two bands with molecular masses of ~120 and 65 kDa, consistent with the four-extracellular domain (Fig. 2B, ▲) and two-extracellular domain (Fig. 2B, ◀) isoforms of mCEACAM1 1-3, respectively, could be detected. As a confirmation of this result, cytoplasts prepared from purified spleen T cells were permeabilized with acetone and stained with a rabbit anti-mCEACAM1 1-3-specific Ab. As shown in Fig. 2C, a punctate, reddish-brown staining pattern was detectable with the specific polyclonal Ab (Fig. 2B, left panel), but not the isotype-matched control Ab (Fig. 2B, right panel), in virtually all cells examined. Although the cellular location of mCEACAM1 1-3 remains to be defined, interestingly, we observed that the activation-induced expression of mCEACAM1 1-3 on the cell
surface was not affected by brefeldin A treatment, which blocks protein trafficking in the biosynthetic pathway (data not shown). These results strongly support the hypothesis that mCEACAM1 α is presynthesized in resting T cells, which has not previously been appreciated in human T cells (26–29), and either rapidly exported to and/or stabilized upon the cell surface after activation.

mCEACAM1 α associates with SHP-1 and adaptor protein 1

The factors that regulate the function and cellular sorting behavior of mCEACAM1 α in T cells are unknown. Previous studies in non-T cells have shown that the long cytoplasmic tail of mCEACAM1 α can associate with SHP-1 and SHP-2 through binding to the two ITIMs present in the cytoplasmic tail when phosphorylated, an event that has been linked to the inhibitory functions of this molecule (2, 18, 19, 23, 42). Moreover, our studies described above, which show that mCEACAM1 α is present in resting T cells but is only detected on the cell surface after activation, suggest that the sorting of mCEACAM1 α is an actively regulated process. These observations caused us to examine whether the long cytoplasmic tail of mCEACAM1 α...
could associate with SHP-1 in T cells and bind the adaptor protein 1 complex that regulates the cellular sorting of transmembrane proteins between the trans-Golgi network (TGN) and the endolysosomal system (43). Therefore, we used a GST-CEACAM1α-cyt tail fusion protein to show that the phosphorylated, but not the nonphosphorylated, cyt tail could pull down SHP-1 from protein lysates derived from splenic T cells, a mouse T cell lymphoma line (4A2), and a mouse B cell line (SP20; Fig. 3A, arrowhead); these results were consistent with previous studies in mouse epithelial cells (18, 19).

We also examined the association of the cyt tail of mCEACAM1α with adaptor protein 1. Adaptor protein 1 is a heterotetramer that consists of γ, β1, μ1 (AP47), and σ1 chains that link transmembrane proteins to clathrin. This is effected through association between tyrosine-based motifs within the cyt tail of transmembrane proteins and the μ1 (AP47) chain of adaptor protein 1 and between clathrin and the β1 subunit of adaptor protein 1 (43). Binding of the μ1 chain of adaptor protein 1 to this tyrosine-based motif, which consists of YXXΦ (tyrosine-lysine-amino acid-lysine acid containing a bulky hydrophobic side chain), regulates protein transport between the TGN and the endolysosomal system. The YXXΦ motif only binds adaptor protein 1 in the nonphosphorylated state (43) and is contained in the long cyt tail of mCEACAM1α. We thus examined the association between the GST-mCEACAM1α-cyt tail fusion protein and the μ1 (AP47) chain generated by in vitro translation in the presence of [35S]cysteine-methionine from the respective gene that we cloned into plasmids were in vitro translated in the presence of [35S]cysteine-methionine from the respective gene that we cloned using specific primers and RT-PCR amplification. As shown in Fig. 3B, we observed that the nonphosphorylated, but not the phosphorylated, GST-mCEACAM1α-cyt tail fusion protein was capable of associating with AP47. This association between the nonphosphorylated cyt tail of mCEACAM1α and adaptor protein 1 has not been previously reported. Taken together, these results suggest that phosphorylation and dephosphorylation of the mCEACAM1α cyt tail in T cells may regulate its reciprocal association with SHP-1 and adaptor protein 1 and its consequent cellular localization and function.

**Ligation of mCEACAM1α regulates T cell function in vitro and in vivo**

The physiologic role of mCEACAM1α in the immune response has yet to be elucidated. The association between mCEACAM1α and SHP-1 in T cells established above and the presence of two ITIMs within the cyt tail suggested a potential immunoregulatory role. Therefore, we examined the effects of the murine CEACAM1α-specific mAb, CC1, in the context of T cell activation. mAb CC1, but not control IgG1, significantly suppressed the allo-MLR between C57BL/6 and BALB/c spleen cells (Fig. 4A). These results were not due to a toxic effect of the CC1 mAb based upon both trypan blue exclusion and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assays (data not shown). We further examined the response of mouse spleen T cells to plate-bound anti-CD3 and anti-CD28 mAbs in the presence or the absence of soluble anti-mCEACAM1α mAb, CC1. As shown in Fig. 4B, left panel, cross-linking of mCEACAM1α with the CC1 mAb significantly inhibited the proliferation of splenic T cells induced by the anti-CD3 and anti-CD28 mAbs in a concentration-dependent manner compared to the isotype-matched Ab control (Fig. 4B, right panel). Either increased mAb CC1 or decreased anti-CD3 and CD28 mAbs resulted in increased inhibition.

The studies described above support the hypothesis that mCEACAM1α regulates mouse T cells as has previously been
shown with human in vitro model systems (26–29). To further extend these observations, we investigated whether ligation of mCEACAM1 by the CC1 mAb can affect a T cell response in vivo. To do so, we examined the effects of the CC1 mAb on the T cell-mediated DTH response associated with exposure to the haptenating agent, oxazolone (39, 40). As shown in Fig. 5A, the CC1 mAb-treated mice exhibited a 64.3% decrease in ear swelling compared to that in mice treated with the control mAb (p = 0.017) when the anti-CEACAM1a-specific mAb was administered both before initial oxazolone sensitization and at the time of the secondary ear challenge exposure. However, blockade of the DTH response required that the anti-CEACAM1a mAb be present at the time of oxazolone-specific T cell priming, because administration of the anti-CEACAM1a mAb at this time resulted in a 65.3% decrease in ear swelling (p = 0.0079; Fig. 5B). However, administration of the CC1 mAb at the time of the secondary ear challenge exposure alone was unable to significantly inhibit the DTH response (Fig. 5B). Although administration of the CC1 mAb at this latter time point resulted in a 30.6% inhibition of ear swelling, this did not reach statistical significance (p = 0.1829), indicating that the effect was marginal. The inhibition of skin swelling observed in association with the DTH response was shown to be due to a dramatic diminution in the lymphoid infiltrates observed within the dermis and thickness of the epithelial layer imposed by the CC1 (Fig. 5C, right panel), but not control (Fig. 5C, left panel), mAb. Anti-CD3 immunostaining of the tissue sections revealed that the lymphoid subset affected by the CC1 mAb was indeed derived from the T cell compartment (data not shown). Therefore, our studies support an important role for mCEACAM1a as an activation-induced, cell surface molecule involved in regulating Ag-driven T cell responses.

Discussion

It has recently become appreciated that CEA-related molecules may provide unique positive and negative signals to human lymphocytes. Mayer and colleagues (44) have shown that CEA, which is normally expressed by human intestinal epithelial cells, can bind to CD8 on PBLs and possibly function as a positive costimulatory molecule. We and others have recently observed that CEACAM1 is expressed on the cell surface of activated, but not on resting, human NK and T lymphocytes (26–29). Moreover, we have made the novel observation that ligation of CEACAM1 by specific mAbs on human intraepithelial lymphocytes, which are predominately CD8⁺, inhibits the cytolysis associated with this subset of cells that is initiated by cross-linking the TCR/CD3 complex in a redirected lysis assay (28). This has suggested that CEACAM1, by virtue of an ITIM-bearing, long cyt tail, may inhibit positive intracellular signals delivered by immunoreceptor tyrosine-based activation motif-containing CD3 molecules. Other studies have concluded that expression of CEACAM1 on activated human T cells is associated with amplification of the T cell response, as defined by proliferation and cytokine responses (27, 29). Whether these observations are due to differences in the functional role of CEACAM1 on CD8- and CD4-bearing T cells and/or different T lymphocyte functions remains to be defined.

In the current report we now extend these observations to mouse T cells and provide information on the novel behavior of mCEACAM1a in this cellular population. In contrast to epithelial cells and other cell types, which constitutively express mCEACAM1a on the cell surface (1, 2, 7, 8, 9, 10), mCEACAM1a expression on the cell surface of mouse T cells is revealed only upon activation, as has been shown with human T cells (26–29). Furthermore, our studies suggest that although mCEACAM1a cannot be detected on the cell surface of resting T cells, it is already present in this cell type before activation. Upon activation, we have observed that mCEACAM1a is rapidly detected on the cell surface, and its expression on the cell surface requires neither de novo transcription nor translation. These studies suggest three possible scenarios. First, it cannot be excluded that the epitope recognized on mCEACAM1a by the CC1 mAb is exposed only after T cell activation, although the mCEACAM1a molecule is present on the cell surface of resting T cells. Against this possibility is the fact that we observed activation-induced expression of mCEACAM1a with two different Abs. Second, mCEACAM1a is preformed and retained in an as yet to be defined intracellular compartment and is released from this compartment for transport to the cell surface upon activation. Alternatively, mCEACAM1a is normally directed to the cell surface but is rapidly internalized due to recycling from this cellular location and is only stabilized upon the cell surface as a consequence of activation. Our observation that mCEACAM1a can associate with adaptor protein 1, as shown in this study, is consistent with either scenario. CTLA-4, for example, which is retained intracellularly before T cell activation, associates with adaptor protein 1 and adaptor protein 2 and is inhibited from this association by tyrosine phosphorylation (45, 46).

![FIGURE 5](http://www.jimmunol.org/DownloadedFrom/April162017.png)

**FIGURE 5.** In vivo regulation of T cell function by mCEACAM1. A, Mean ear swelling of oxazolone-sensitized mice treated with either an IgG1 control mAb (n = 16) or the CC1 mAb (n = 16). *, p = 0.017; CC1 mAb vs IgG1 control. A representative experiment of six is shown. B, Comparison of mean ear swelling of oxazolone-sensitized mice with CC1 mAb treatment either pre-skin painting (n = 6; sensitization phase) or pre-ear challenge (n = 6; effector phase) compared with isotype-matched control mAb treatment. *, p = 0.0079, control vs pre-skin painting; p = 0.1829, control vs pre-ear challenge. C, Histologic sections of ears from oxazolone-presensitized mice treated with control (left panel) or CC1 (right panel) mAbs at the time of both sensitization and effector phases. Lymphoid cell infiltrate is indicated by the arrowhead. H&E stain; magnification, ×400.
Therefore, it is possible that interactions between the nonphosphorylated cyt tail of mCEACAM1a and the µ1 (AP47) chain of adaptor protein 1 directs mCEACAM1a to an as yet to be defined intracellular location where it is retained. Based upon our observations that brefeldin A does not inhibit T cell activation-induced cell surface expression of mCEACAM1a, this putative compartment would be predicted to be beyond the TGN, consistent with the known function of adaptor protein 1, which is to direct transmembrane proteins from the TGN to the endolysosomal system (43). T cell activation may recruit src-related tyrosine kinases that are known to associate with and phosphorylate the long cyt tail of CEACAM1 (47). In this case CEACAM1a would be forced to release from adaptor protein 1 binding and potentially be transported to the cell surface. Phosphorylation of the mCEACAM1a cyt tail would, on the other hand, allow for an association with SHP-1 and/or SHP-2, as we have shown in this study and has been previously shown in epithelial cells (18, 19). Such an association is likely to occur on the cell surface (48) wherein the inhibitory functions of mCEACAM1a isoforms containing the long cyt tail could be asserted. As noted above, an alternative and related scenario is the possibility that mCEACAM1a is rapidly recycling between the cell surface and endosomes independent of TCR/CD3 complex signaling through reciprocal interactions between adaptor protein 2 and cellular phosphatases such as SHP-1 and the nonphosphorylated and phosphorylated cyt tails, respectively. In this case T cell activation would be predicted to serve in the stabilization of mCEACAM1a on the cell surface. These hypotheses are not mutually exclusive; they need to be subjects of future investigation and obviously do not apply to mCEACAM1a isoforms that express the short cyt tail.

The function of CEACAM1 on T cells remains controversial from studies in human T lymphocytes that have ascribed both stimulatory and inhibitory properties to this molecule (27–29). In this report we have shown that an anti-CEACAM1a-specific mAb, CC1, inhibits the activation of mouse T cells in vitro. Whether these results are due to blockade of a costimulatory signal provided by mCEACAM1a or stimulation of an inhibitory signal delivered by mCEACAM1a cannot be concluded. In addition, it must be noted that signaling through Ab-mediated ligation is not physiologic such that firm conclusions about the function of mCEACAM1a on T cells must await studies with native ligands. In the allo-MLR, it is possible that the CC1 mAb is blocking an activation signal provided by interactions between mCEACAM1a on the activated T cell and the counterligand, presumably mCEACAM1a (49, 50), on the APC. Alternatively, cross-linking of mCEACAM1a by the CC1 mAb may directly stimulate an inhibitory pathway, as suggested by our results showing that CC1 mAb inhibits the response of purified spleen T cells to anti-CD3- and anti-CD28-stimulated proliferation. Regardless, these observations are likely to be biologically relevant, because we observed that the mCEACAM1a-specific mAb, CC1, inhibited a DTH response to oxazolone in vivo. Interestingly, and potentially consistent with the fact that mCEACAM1a was observed to be an early activation Ag, the inhibition of the DTH response was observed only during the phase of T cell priming and not the effector phase in vivo as shown in this work. Arguably, these results are more consistent with blockade of a crucial positive signal delivered by an APC such as a dendritic cell (49) to an mCEACAM1a-bearing T cell rather than to either direct stimulation of an inhibitory signal by the mAb on an effector T cell or blockade of T cell recruitment into the affected tissues through interactions between T cells and endothelial cells (9). Future studies must be aimed at defining the specific mechanism by which mCEACAM1a is capable of regulating T cell responses in vivo.

In summary, in contrast to previous studies that have concluded that mouse T cells do not express mCEACAM1a, we have observed that mouse T cells are capable of expressing mCEACAM1a on the cell surface under conditions of T cell activation. Cell surface expression of mCEACAM1a is likely to be physiologically important in regulating T cell responses during the early phases of T cell activation in vivo. These studies thus mark mCEACAM1a as an important immunoregulatory molecule on mouse T cells.

References

11. Rojas, M., L. DeMarle, R. S. Sreeratun, and C. P. Olds. 1996. Radical differ-


