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Induction of Hyporesponsiveness and Impaired T Lymphocyte Activation by the CD31 Receptor:Ligand Pathway in T Cells

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CD31 is a member of the Ig superfamily expressed on various cell types of the vasculature, including a certain subpopulation of T lymphocytes. Previous reports suggest that interaction of CD31 with its heterophilic ligand on T cells (T cell CD31 ligand) plays a regulatory role in T lymphocyte activation. Here we demonstrate that a soluble rCD31-receptorglobulin (CD31Rg) specifically down-regulated the proliferation of human peripheral blood CD31+ T lymphocytes stimulated via CD3 and CD28 mAbs. Notably, engagement of the T cell CD31 ligand by CD31Rg during primary stimulation also induced a prolonged unresponsive state in T cells. Retroviral transduction of CD31 into CD31+ Th clones resulted in a significant inhibition of their proliferative capacity. When cocultured with purified CD31− T lymphocytes, irradiated CD31-transduced Th clones counterregulated the CD3/CD28-mediated activation of these cells. Furthermore, primary stimulation in the presence of CD31-transduced Th clones induced a comparable state of hyporesponsiveness in the T cell responders as the soluble CD31Rg. Thus, by counterregulating the activation of cognate T lymphocytes, CD31-expressing T cells might contribute to the establishment and maintenance of peripheral tolerance. The Journal of Immunology, 2001, 166: 2364–2371.

For an immune response to occur, T lymphocytes must be exposed to two types of stimulus. The first signal, an Ag, ensures the specification of activation and is transduced via the T cell Ag receptor. Second signals for T cells are costimulators and cytokines that promote clonal expansion of the specific T cells and their differentiation into effector and memory cells (1). In addition, the immune system provides for control mechanisms, which maintain homeostasis after active immune responses to foreign Ags, and prevent or abort responses to self-Ags. Mechanisms in the periphery limiting ongoing immune responses and the reactivity to self include the following: 1) failure of lymphocyte activation (ignorance), 2) clonal anergy, 3) death by neglect, and 4) active termination of T cell activation (2, 3).

Multiple cell surface receptors contribute to the active down-regulation of T cell responses, in particular death receptors such as Fas (4) and other members of the TNF receptor family (5, 6), as well as CTLA-4 (7–9) and certain killer cell-inhibitory receptors (10–12). We recently identified a 120-kDa ligand for CD31 (platelet-endothelial cell adhesion molecule-1) expressed by human CD31− Th clones with a similar counterregulatory role in T lymphocyte activation (13). CD31 is a transmembrane glycoprotein of the Ig superfamily present on the surface of endothelial cells, platelets, monocytes, polymorphonuclear leukocytes (PMN), as well as a certain subpopulation of T lymphocytes (14, 15). A number of reports have indicated that CD31 can engage in both homophilic (CD31−CD31) (14, 15) and heterophilic binding to other cell surface molecules. Apart from the T cell CD31 ligand (T-CD31L; Ref. 13), the proposed heterophilic counterreceptors of CD31 include a heparin-containing proteoglycan (16, 17), the integrin αβ3 (18, 19), a molecule expressed by parasitized RBC (20), and the ADP-ribosyl cyclase CD38 (21).

The multitude of ligand interactions makes CD31 an ideal candidate for mediating a range of different functions. CD31 has been demonstrated to play a role in the transmigration of PMN, monocytes, and NK cells in both in vitro and in vivo models (15, 22). Recent evidence coming from experiments using CD31− mice and mice transgenic for a soluble CD31-receptorglobulin (CD31Rg) confirm the involvement of CD31 in the recruitment of leukocytes to inflammatory sites (23, 24). In vitro studies suggest that CD31 also contributes to endothelial tube formation, and CD31 mAbs have been demonstrated to interfere with murine angiogenesis in vivo (25). A putative role for CD31 in the activation of monocytes (26, 27) and neutrophils (28), as well as in platelet function (14), have also been proposed.

Contrary to the involvement of CD31 in the recruitment of PMN and monocytes to sites of inflammation, its role in T lymphocytes homing remains less clear (23, 29, 30). However, several reports indicate that CD31 and a ligand on T cells contribute to the regulation of T lymphocyte activation. CD31 is preferentially expressed by the naive T cell subset, and down-regulated on the majority of CD4+ and about 50% of the CD8+ T lymphocytes upon their transition to the memory phenotype (31, 32). Furthermore, CD31 has been reported to be a marker for T cells of the suppressor lineage, and a CD31 mAb could inhibit the suppressor function of T lymphocytes in a B cell Ig synthesis system (33). Direct engagement of CD31 has been suggested to costimulate the activation of CD31+ T cells, but reports on this subject have been controversial (28, 34). A CD31-derived peptide interfered with T cell activation in vivo and delayed the onset of graft-vs-host disease in a mouse model (35, 36).
In a previous study, we have shown that a CD31Rg down-regulated the response of CD31+ Th clones via interaction with a heterologous 120-kDa ligand (13). To assess the function of this heterophilic T-CD31L in the activation of peripheral blood (PB) T lymphocytes and, in particular, to test the role of CD31 and its counterreceptor in T cell-T cell interaction, CD31 was introduced into CD31+ Th clones by retrovirus-mediated gene transfer. The CD31-transduced clones, as well as the soluble CD31Rg counterregulated the responses of CD31+ T lymphocytes and, notably, induced a state of prolonged hyporesponsiveness in these cells.

Materials and Methods

Abs and Rgs

Mouse mAb IB5 to CD31 was produced and characterized at our Institute. mAbs MEM-05 to CD31, MEM-18 to CD14, MEM-97 to CD20, MEM-154 to CD16, MEM-181 to CD25, MEM-188 to CD56, and MEM-M63 to CD147 were kindly provided by Dr. V. Hojfš̄i̊ (Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic). mAb OKT3 to CD31 was obtained from Ortho Pharmaceuticals (Raritan, NJ), mAbs Leu28 to CD28, BN13 to CTLA-4, and FN50 to CD69 from Becton-Dickinson (San Jose, CA). Human IgG1 was purchased from Sigma (St. Louis, MO). Expression and purification of soluble rCD31Rg and control M6Rg were performed as described previously (13). Briefly, the recombinant proteins were expressed in CHO cells, and isolated from the culture supernatants by protein A chromatography using 4 M imidazole (pH 8) as elution buffer. The resultant protein fractions were dialyzed against PBS and stored at −20°C.

Cells and cell lines

The amphotropic retroviral packaging cell line, Phoenix-ampho, was kindly provided by Dr. G. Nolan (Stanford University, Stanford, CA) and used to obtain and expand under the usual restimulation conditions. The amphotropic retroviral packaging cell line, Phoenix-ampho, was kindly provided by Dr. G. Nolan (Stanford University, Stanford, CA) and cultured in DMEM plus 10% FCS. Establishment and culture of the human Th clones (see Fig. 2) was determined by Ag-capture ELISA as described previously (13). Staining of CD31+ T cells that were activated via CD3 and CD28 in the presence of CD31Rg or the control Rg was performed using directly labeled mAbs. For cell cycle analysis, T lymphocytes or Th clones were activated in triplicate wells as described for proliferation assays. After 48 to 96 h, three identical wells were harvested and resuspended in DNA staining solution containing 0.1% Nonidet P-40 and 50 μg/ml propidium iodide (Sigma). Permeabilized cells were stored on ice and samples analyzed by flow cytometry. The number of apoptotic cells and the percentage of cells in the G0/G1 and S phase of the cell cycle were determined using CellQuest software (BD Biosciences).

Induction of T cell hyporesponsiveness

Purified CD31+ T lymphocytes were prestimulated by plate-bound CD3 and CD28 mAbs (immobilized at 1 μg/ml each) in the presence of immobilized Rgs or irradiated Th clones as described for proliferation assays. After overnight incubation, cells were removed from the plates, washed, and resuspended in RPMI 1640 medium supplemented with 10% FCS without stimuli. Following a resting period of 7 days, dead cells were removed by Ficoll-Hypaque (Pharmacia) centrifugation from the cultures, and T lymphocytes were restimulated by plate-bound CD3 and soluble CD28 mAbs or IL-2 as above. Alternatively, the cells were reactivated via 0.01 U/ml PHA (Wellcome, Beckenham, U.K.) plus 6 ng/ml PMA (Sigma). Proliferation of triplicate cultures was determined after 96 h following an 18-h pulse with [3H]thymidine (1 μCi/well).

Cytokine assays

Concentrations of IL-2, TNF-α, IFN-γ, and IL-10 were analyzed in CD31+ T lymphocyte cultures stimulated as described for proliferation assays. Supernatants were harvested 48 h following activation, and cytokine levels determined by Ag-capture ELISA as described previously (13, 38). To quantitate TGF-β production, T cell assays were performed in serum-free UltraCulture medium (BioWhittaker, Walkersville, MD). TGF-β levels were measured in supernatants collected after a 72-h stimulation period using the Quantikine TGF-β immunoassay (R&D Systems, Minneapolis, MN).

Results

Down-regulation of PB T cell activation by CD31Rg

We previously demonstrated that a CD31Rg containing the whole extracellular part of CD31 down-regulated the activation of CD4+CD31+ Th clones by interaction with a 120-kDa ligand termed T-CD31L (13). Similar to its effect on Th effector cells, CD31Rg inhibited the proliferation of freshly isolated PB T lymphocytes, when the cells were stimulated via CD3 and CD28 mAbs (Fig. 1A, left). To confirm the heterophilic interaction of CD31 in this culture system, CD31+ T cells were depleted from the whole...
population of PB T lymphocytes. As shown in Fig. 1A (right), no difference in the inhibitory capacity of CD31Rg on the activation of unfractionated or CD3⁺ T cells was observed. This result emphasizes the heterophilic mechanism of the CD31Rg interaction with purified PB T cells.

Several members of the TNF receptor family of cell surface molecules have been demonstrated to down-regulate T lymphocyte activation by inducing apoptosis in T cells (5). Engagement of T-CD31L by CD31Rg, in contrast, preferentially interfered with the cell cycle progression of PB T lymphocytes, as shown in a propidium iodide staining of the cells at several time points following their activation (Fig. 1B). When CD3⁺ T lymphocytes were stimulated by CD3 plus CD28 mAbs in the presence of CD31Rg, the number of T cells in the S phase of the cell cycle was reduced by about 70% as compared with the number of cycling cells in the presence of a control fusion protein. However, CD31Rg treatment only insignificantly increased the number of apoptotic cells in these cultures, indicating that the fusion protein acts via a different mechanism than the cell death receptors.

Ectopic expression of CD31 in CD4⁺CD31⁻ Th clones
Concerning the CD4⁺ T lymphocyte subset, in particular, CD31 expression is restricted to naïve cells and completely downregulated upon repetitive stimulation (32). By contrast, about 50% of the CD8⁺ T cell population significantly up-regulate the molecule during their transition to the memory phenotype (31). To assess a potential inhibitory role of the CD31⁺ T cell subset(s) in T lymphocyte activation, the molecule was expressed ectopically in two different human CD4⁺ Th clones, which lack CD31 mRNA and protein expression (13). Following retrovirus-mediated introduction of the human CD31 cDNA into the Th clones, CD31-expressing cells were enriched by magnetic bead-separation until a homogeneous positive cell population was obtained (see Materials and Methods, Fig. 2).

The CD31-transduced Th clones showed a significantly reduced proliferation rate upon restimulation under standard conditions in the presence of exogenous cytokines (see Materials and Methods). In contrast to the parental cells, they could hardly be restimulated by CD3 and CD28 mAbs alone, and proliferation was still considerably inhibited in the presence of exogenous IL-2 (Fig. 3A). Resembling the down-regulatory effect of CD31Rg on the proliferation of CD3⁺ T lymphocytes, cell cycle progression of the CD31-transduced Th clones was reduced by about 70% in comparison to the untransduced control cells (Fig. 3B). Likewise, the number of apoptotic cells in the CD3⁺ and CD31⁻ Th cell cultures was comparable, indicating a similar mechanism of T cell down-regulation as promoted by CD31Rg.

**CD31-transduced Th cells counterbalance activation of CD31⁻ PB T lymphocytes**

The reduced growth rate of the CD31-transduced Th clones could be due to a negative signaling following the homophilic interaction of CD31 molecules expressed by adjacent cells or, alternatively, due to the heterophilic interaction of CD31 with T-CD31L. To distinguish between these possible scenarios, irradiated CD31-transduced and parental control clones were added to stimulation cultures of purified CD3⁺ T lymphocytes. Upon activation via CD3 mAb the proliferation of the responder T cells was significantly inhibited (up to 60%) in the presence of the irradiated CD31⁺ Th clones, as compared with cultures containing the parental clones (Fig. 4). The CD31-transduced Th clones inhibited the growth rate of the CD31⁺ T lymphocytes to a similar extent, when the stimulation was performed with CD3 and CD28 mAbs, or CD3 mAb plus IL-2 (Fig. 4). Thus, the CD31:T-CD31L interaction seems to interfere with both TCR/CD3- and costimulator-induced signaling pathways.

To exclude that the observed inhibitory effect was mediated by a soluble factor produced by the CD31-transduced Th clones, in parallel experiments the CD31⁺ and control clones were separated from the CD31⁺ responder T cells by a semipermeable membrane.

**FIGURE 1.** Effect of CD31Rg on proliferation and cell cycle progression of purified T lymphocytes. A. Unfractionated or CD3⁻ PB T lymphocytes (10⁶) were activated via 1 μg/ml immobilized CD3 and 500 ng/ml soluble CD28 mAbs or 200 U/ml IL-2 in the presence of immobilized CD31Rg or control M6Rg. [3H]Thymidine incorporation was determined after 72 h. Data are representative of five independent experiments performed with T cells from different donors. Results are expressed as mean cpm of triplicate determinations. B. CD3⁺ T lymphocytes were stimulated for 72 h via CD3 and CD28 mAbs as above, after which triplicate wells were collected and analyzed by propidium iodide staining and flow cytometry. The percentage of apoptotic (subdiploid) and cycling (S phase) cells are indicated. Data are representative of three independent experiments performed at 48 h as well as 72 h following activation.
Under these stimulation conditions no difference in the [3H]thymidine incorporation was observed, indicating that the counterbalancing function of the CD31-transduced Th clones requires direct cell-cell contact to the responder population (Fig. 5B). Furthermore, the CD31 mAb IB5 dose-dependently reversed the counterregulatory effect of the CD31-transduced Th clones on the growth of the CD31+ T lymphocytes, confirming that the inhibition was indeed CD31-specific, and not due to an unspecified alteration in the cell surface phenotype of the Th clones (Fig. 5A).

CD31Rg and CD31-transduced Th clones induce an anergy-like state in CD31+ T lymphocytes

Suppression of T cell activation by cell surface molecules such as CTLA-4 (39), or soluble factors such as IL-10 (40) can result in the induction of T cell tolerance. To determine whether the engagement of T-CD31L induces such a long-term state of unresponsiveness in PB T lymphocytes, CD31+ responder T cells were preactivated via CD3/CD28 in the presence of CD31Rg or control Rg. As a positive control for anergy induction, T lymphocytes were prestimulated with immobilized CD3 mAb alone. After a resting period, live cells were collected and reactivated by CD3 and CD28 mAbs. The secondary response of T lymphocytes treated with CD31Rg in the primary cultures was reduced by about 90%, as compared with cells preactivated in the presence of control Rg. Thus, the CD31Rg-pretreated cells were anergized to a similar extent as T lymphocytes prestimulated via immobilized CD3 mAb alone (Fig. 6A). However, unresponsiveness was partially reversed when the secondary stimulation was performed in the presence of IL-2, and was completely abrogated by restimulation with PHA and PMA (Fig. 6A). A comparable state of hyporesponsiveness was induced in CD31+ T lymphocytes when the responder cells were preactivated in the presence of the CD31-transduced Th clones. Under these conditions, the proliferation of PB T cells upon secondary stimulation with CD3 and CD28 mAbs was reduced by about 50% as compared with the control response (Fig. 6B). Restimulation in the presence of PHA plus PMA again reversed the anergy-like state of the responder population induced by the CD31+ Th clones (Fig. 6B).

CD31Rg and CD31+ Th clones counterregulate the production of cytokines by PB T cells

Although there are differences in the pathways of anergy induction in T cells, at the molecular level the key event in the induction of the unresponsive state is the inability of the T lymphocyte to produce the growth factor IL-2 (41, 42). In accord with this definition, when purified CD31+ T cells were stimulated via CD3 and CD28 in the presence of CD31Rg or CD31-transduced Th clones the accumulation of IL-2, as well as TNF-α and IFN-γ in the culture supernatants was specifically blocked (Fig. 7). However, interaction of CD31Rg or CD31+ Th clones with PB T cells did not result in the production of the growth-inhibiting cytokines IL-10 and TGF-β (Fig. 7). These results suggest that CD31Rg/CD31-expressing T cells act via a different mechanism or signaling pathway to down-regulate T cell activation than the previously characterized Th3 and Tr1 clones (43, 44).

CD31Rg inhibits induction of CTLA-4 (CD152) expression in CD31+ T cells

Interaction of T-CD31L with CD31Rg resulted in cell cycle arrest and inhibition of IL-2 production, ultimately leading to the induction of anergy events that are also observed in activated T cells after engagement of the negative regulatory molecule CTLA-4 (39, 45, 46). Therefore, we assessed whether T-CD31L may be linked to the CTLA-4 pathway; i.e., induction of T cell anergy by CD31Rg may be an indirect effect mediated by its up-regulation of CTLA-4 expression. However, interaction of CD31Rg with activated CD31+ T cells did not induce or enhance CTLA-4 surface expression. On the contrary, stimulation of CD31+ T cells in the presence of CD31Rg completely prevented the up-regulation of CTLA-4. In agreement with the effect of CD31Rg on cell cycle progression, the expression of other T cell activation markers, the
IL-2 receptor α-chain (CD25), and CD69 were also blocked (Fig. 8). Thus, while the CTLA-4 and T-CD31L pathways both restrict the transition of T lymphocytes to an activated state, the mechanism whereby these molecules act appears to be different.

**Discussion**

Several reports have implicated the cell adhesion molecule CD31 and/or a T cell-ligand of the molecule in the regulation of T lymphocyte responses (see Introduction). In our previous work we characterized a 120-kDa heterophilic CD31-counterreceptor expressed on the surface of CD31⁺ Th clones, which inhibited CD3/CD28-mediated activation of these cells. To define the role of CD31 and this ligand in the responses of human PB T lymphocytes and in T cell-T cell interactions, we used a soluble CD31Rg and, in parallel studies, two different Th cell clones genetically modified to express CD31 on their surface. Our results provide evidence pressed on the surface of CD31⁺ Th clones, which inhibited CD3/CD28-mediated activation of these cells. To define the role of CD31 and this ligand in the responses of human PB T lymphocytes and in T cell-T cell interactions, we used a soluble CD31Rg and, in parallel studies, two different Th cell clones genetically modified to express CD31 on their surface. Our results provide evidence

**FIGURE 6.** Induction of hyporesponsiveness in purified CD31⁺ T lymphocytes by CD31Rg and CD31-transduced Th clones. Purified CD31⁺ T cells (10⁵) were activated with CD3 mAb alone, or CD3 plus CD28 mAbs (each immobilized at 1 µg/ml) in the presence of CD31Rg or control Rg (A), as well as in the presence of irradiated CD31-transduced or parental Th clone EP 27.1 and O A.1 (5 × 10⁴). Proliferation was determined after 72 h. Results are shown as mean ± SE of triplicate cultures. Data are representative of five separate experiments performed with T lymphocytes from different donors.
that CD31-transduced Th cells and the rCD31Rg counteract the activation of PB CD31^2 T lymphocytes by a similar mechanism. Most importantly, both CD31Rg and the CD31-expressing Th cells can induce a state of prolonged hyporesponsiveness in the T responder population.

Recent investigations have suggested that direct CD31 engagement by mAbs can also influence the activation of T lymphocytes (28, 34). Our observations do not rule out an additional direct function of CD31 in the regulation of T cell activation. However, CD31 is predominantly expressed by the naive and CD8^+ T cell population, while the majority of T cells that lack CD31 on their surface are found among the CD4^+ T lymphocytes (31, 32). Therefore, CD31 mAbs and CD31Rg may target different, although overlapping, T cell subpopulations. To exclude a potential homophilic interaction of CD31 in our assays, we used CD31^- T lymphocytes as responders, and either CD31Rg or irradiated CD31-transduced Th clones as regulatory/accessory cells in an APC-free system. The similarity in the mechanism of down-regulation by CD31Rg and the CD31^- Th cells suggested that both exert their dampening effect via interaction with the same T cell surface-ligand as follows: 1) the counterbalancing function of the CD31^+ clones is CD31-specific and dependent on cell contact to the responder population; 2) CD31Rg and CD31-transduced Th clones both block cell cycle progression and induce an anergy-like state in the responder T lymphocytes; 3) CD31Rg and CD31-expressing Th cells inhibit IL-2, TNF-α, and IFN-γ production by PB T lymphocytes; and 4) in both cases inhibition is not mediated by the induction of the known T cell suppressor cytokines IL-10 and TGF-β.

Active mechanisms that prevent or terminate T lymphocyte responses in principle fall into two different categories: pathways counterregulating the initial phase of T cell activation, and regulatory feedback systems whose primary function is to control the late stages of T cell proliferation and differentiation (3). Active down-regulation during the initial triggering of a T cell is mediated by signals transmitted via cell surface molecules such as CTLA-4 (8, 9) and the killer cell-inhibitory receptors (10, 12). In the late stage of a T cell response, molecules that induce activation-induced cell death in T lymphocytes, such as Fas and regulatory suppressor cytokines (2) terminate proliferation and effector functions. Interaction of CD31 with the T cell counterreceptor characterized in our studies does not significantly trigger apoptotic death in PB T lymphocytes, but predominantly blocks cell cycle progression of the cells in the G_0/G_1 stage. Thus, T-CD31L would be a candidate molecule for a negative regulator transmitting signals that increase the threshold for T cell activation, and prevent undesired stimulation by low-strength TCR signals, a role similar to that suggested for CTLA-4 (7, 8). T-CD31L as well as other inhibitory pathways described (47, 48) thereby might have preferential effects on different subsets of T cells, become operative after certain types of Ag exposure, or contribute to the development of different effector functions.

**FIGURE 7.** Effect of CD31Rg and CD31-transduced Th cells on cytokine production by CD31^- T lymphocytes. Purified T cells were stimulated via CD3 and CD28 mAbs in the presence of CD31Rg or control (A), and CD31-transduced Th clone O.A.1 or control (B) as described in Fig. 1 and 4. After 48 h, levels of IL-2, TNF-α, IFN-γ, and IL-10 were determined in culture supernatants by ELISA; TGF-β concentrations were measured after 72 h of culture. Results are expressed as mean pg/ml ± SE of duplicate determinations. Data are representative of five (A) or two (B) separate experiments performed with T lymphocytes from different donors.

**FIGURE 8.** Effect of CD31Rg on the expression of CTLA-4, CD25, and CD69 on activated CD31^- T lymphocytes. Purified T cells were stimulated via CD3 and CD28 mAbs in the presence of CD31Rg or control Rg as described in Fig. 1. After 36 h, cell surface expression of CTLA-4, CD25, and CD69 was determined by immunostaining and FACS analysis. Results are representative of three separate experiments.
The signaling thresholds of Ag receptors and coreceptors determine positive or negative cellular responses (i.e., immunity or tolerance to self-molecules) (49). Changes in costimulatory pathways can lead to enhanced activation of T lymphocytes and give rise to autoimmunity. Both central and peripheral tolerance mechanisms act in parallel to prevent such inappropriatereactivity against foreign and self-Ags (2). One major mechanism of peripheral tolerance is the induction of anergy in mature T lymphocytes, a form of unresponsiveness characterized by the inability of the T cell to produce and/or respond to proliferative signals (42). T cells can be anergized in various ways, including TCR occupancy in the absence of costimulation (42), triggering of the TCR with altered peptide ligands/partial agonists (50), or activation in the presence of IL-10 (40). However, recent studies demonstrate that the induction of T cell tolerance or functional inactivation of T lymphocytes is not a passive mechanism, but in vivo requires engagement of negative regulators such as CTLA-4 (39). Our results suggest that triggering of T-CD31L on activated T lymphocytes, concomitantly with optimal stimulation via CD3 and CD28, promotes the induction of a similar hyporesponsive state in T cells as CTLA-4. However, the T-CD31L and CTLA-4 pathways do not appear to be interconnected. The fact that triggering of activated T cells by CD31Rg completely down-regulated CTLA-4 surface expression argues against the hypothesis that it is induced CTLA-4 expression, which accounts for the suppressive effect of CD31Rg. The function of negative regulatory molecules can be restricted to certain T cell subsets (12, 51–53). Therefore, CTLA-4 might be important for some pathways to anergy, but alternative mechanisms such as the CD31-T-CD31L pathways might become operative in other settings of T cell activation. Consistent with this assumption, in some in vitro and in vivo models CTLA-4 appears not to be required for anergy induction (54–56). In addition, the role of CTLA-4 in self-tolerance may be more complex than previously assumed; recent reports suggest that costimilation of regulatory CD25+ T cells as opposed to suppression of reactive T cells accounts for some of the inhibitory effects of CTLA-4 (57, 58).

There is now compelling evidence that immune responses to foreign and self-Ags can also be down-regulated by specific suppressor T lymphocytes (59–61). A subset of these regulatory T cells function by producing cytokines whose net effect is immune suppression. T cell-derived inhibitory cytokines such as IL-10 (40). However, recent studies demonstrate that the induction of T cell unresponsiveness and autoimmunity is best illustrated in animal models (44) or TGF-β (62). Soluble domain 1 of platelet/endothelial cell adhesion molecule-1 (CD31) modulates the function of lymphocytes, monocytes and neutrophils. J. Biol. Chem. 268:16037.

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