CD31 Expressed on Distinctive T Cell Subsets
Is a Preferential Amplifier of β1 Integrin-mediated Adhesion

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Summary
The CD31 (platelet endothelial cell adhesion molecule-1 [PECAM-1]/endothelial cell adhesion
molecule [endoCAM]) molecule expressed on leukocytes, platelets, and endothelial cells is postulated
to mediate adhesion to endothelial cells and thereby function in immunity, inflammation, and
wound healing. We report the following novel features of CD31 which suggest a role for it
in adhesion amplification of unique T cell subsets: (a) engagement of CD31 induces the adhesive
function of β1 and β2 integrins; (b) adhesion induction by CD31 immunoglobulin G (IgG)
monoclonal antibodies (mAbs) is sensitive, requiring only bivalent mAb; (c) CD31 mAb induces
adhesion rapidly, but it is transient; (d) unique subsets of CD4+ and CD8+ T cells express
CD31, including all naive (CD45RA+ ) CD8 T cells; and (e) CD31 induction is selective,
inducing adhesive function of β1 integrins, particularly very late antigen-4, more efficiently than
the β2 integrin lymphocyte function-associated antigen-1. Conversely, CD3 is more effective
in inducing β2-mediated adhesion. Taken together, these findings indicate that unique T cell
subsets express CD31, and CD31 has the capacity to induce integrin-mediated adhesion of T
cells in a sensitive and selective fashion. We propose that, in collaboration with other receptors/
ligands, CD31 functions in an “adhesion cascade” by amplifying integrin-mediated adhesion of
CD31+ T cells to other cells, particularly endothelial cells.

Regulated adhesion is critical to virtually all the functions
of T lymphocytes. These functions include both antigen-
 independent processes such as lymphocyte recirculation/
homing and antigen-specific recognition events. Consequently,
evolution has provided multiple modes of regulation of T
cell adhesion. These include regulated expression of the T
cell adhesion receptors such as very late antigen (VLA)1 inte-

1 Abbreviations used in this paper: ELAM-1, endothelial leukocyte adhesion
molecule-1; FN, fibronectin; HEV, high endothelial venule; HSA, human
serum albumin; ICAM-1, intracellular adhesion molecule-1; LN, laminin;
PECAM-1, platelet endothelial cell adhesion molecule-1; VCAM-1, vascular
cell adhesion molecule-1; VLA, very late antigen.
cells, and naive (CD45RA+CD45RO -) CD8 T cells were pre-
cytes, and B cells (3-7, 10). Crosslinking of other receptors on the T cell surface, including
activated not only by CD3 crosslinking (and the similar activa-
tion stimulus provided by pairs of CD2 mAb), but also by
crosslinking of other receptors on the T cell surface, including
CD7, CD28, and CD44 (1, 15, 19, 20). These findings for
T cells have been both foreshadowed and complemented by a
variety of findings regarding regulated function of integrins
on other cell types including particularly platelets, granulo-
cytes, and B cells (3-7, 10).

We use the terms adhesion "inducer" or "amplifier" to refer
to a molecule on the T cell surface that augments integrin
function. The nature of regulated adhesion makes the amplifier
molecules as critical to the process as the adhesion molecule
itself. In particular, differential expression of such amplifier
molecules will be as important in T cell differentiation as
differential expression of the adhesion molecule. The present
report identifies CD31 as an amplifier molecule on unique
subsets of T cells, and characterizes novel features of that adhe-
sion induction. CD31 glycoprotein (also designated PECAM-1,
platelet endothelial cell adhesion molecule) is an Ig
superfamily member that is most similar in structure to classical
adhesion molecules such as ICAM-1, VCAM-1, and neural
adhesion molecule (NCAM) (21). It is expressed at high
density on endothelium, platelets, granulocytes, and mono-
cytes (22-24). It is also expressed by lymphocytes (23, 24).
It has been implicated in cell-cell adhesion by a variety of
findings. It accumulates at contact regions between endothelial
cells (22), transfection of CD31 into L cells causes them to
aggregate (25), and CD31 mAbs inhibit endothelial cell con-
tact, as well as transfected L cell aggregation (25). CD31 has
been postulated to bind to CD31 in homophilic interactions,
as well as to participate in heterophilic interactions involving
proteoglycans (22, 26). The present report demonstrates a
role for CD31 in adhesion induction on T cells, and pro-
poses a model of its potential involvement in an "adhesion
cascade" on T cells.

Materials and Methods

Human T Cell Subsets. Highly purified CD4 T cells, CD8 T
cells, and naive (CD45RA+CD45RO-) CD8 T cells were pre-
pared from PBMC of volunteer research healthy donors by exhaus-
tive immunomagnetic negative selection, essentially as previously
described (27). We routinely use Advanced Magnetic Particles (Ad-
vanced Magnetics, Cambridge, MA) and Dynabeads (Dynal Inc.,
Fort Lee, NJ), and a cocktail of mAbs consisting of MHC class
II mAb IVA12, CD19 mAb FMC63, CD16 mAb VD2, CD11b
mAb NIH11b-1, CD14 mAb 63D3, antiligycophorin mAb 10F7,
and CD4 mAb OKT4 or CD8 mAb B9.8.4 with or without
CD45RA mAb FMC71 (to negatively isolate memory T cells),
or CD45RO mAb UCHL1 (to negatively isolate naive T cells)
(27). The anti-HLA-DR mAb IVA12 was included in the selec-
tion cocktail to exclude the normal low percentage of circulating
activated T cells. Furthermore, the CD8 dull (dim) population
which has NK-like features phenotypically and functionally (28),
was also excluded from the CD8+ population by the use of a separation
cocktail containing the CD16 mAb VD2 and the CD11b mAb
NIH11b-1. The purity of T cell subsets were >96% CD4+ or
>94% CD8+, and >99% CD45RA+ or >99% CD45RO+, as
determined by flow cytometric analysis.

Antibodies and other Reagents. The following mAbs were used
as purified Ig: CD31-specific mAb NIH31-1 and NIH31-2 were
generated and their specificity determined by binding to CD31-
transfectants (data not shown); CD31 mAb PECA1-1.2 (P. J.
Newman, unpublished observations); CD31 mAb 4G6 (S. M.
Albelda, unpublished observations); CD31 mAb SIG34 (29) (S.
Goyert, Cornell University Medical College, Manhasset, NY), CD31 mAb
LAK1 (30) (M. Zocchi, Laboratory of Adoptive Immunotherapy,
Milan, Italy), CD31 mAb L33 (D. Buck; Becton Dickinson & Co.,
San Jose, CA). Other mAb are as follows: CD11b mAb NIH11b-1,
CD49d mAb NIH49d-1, CD44 mAb NIH44-1 (31) and CD45
mAb NIH45-2 (generated locally), CD3 mAb OKT3, CD4 mAb
OKT4, CD14 mAb 63D3, class II mAb IVA12, CD7 mAb 3A1,
antiligycophorin mAb 10F7 (all from American Type Culture
Collection, Rockville, MD), CD2 mAb 95-5-49 (R. R. Quinones,
Children's Hospital Medical Center, Washington, DC), CD8 mAb
B9.8.4 (B. Malissen, Centre National de la Recherche Scientifique,
Marseille, France), CD19 mAb FMC63, CD45RA mAb FMC71
(H. Zola, Flinders Medical Center, Bedford Park, Australia),
CD45RO mAb UCHL1 (P. Beverley, Courtauld Institute of Bio-
chemistry, London, UK), CD16 mAb MHM23 (J. E. Hildreth,
Johns Hopkins Medical School, Baltimore, MD), CD49d mAb
L25 (D. Buck), CD29 mAb MAB13, CD49e mAb MAB16 (both K.
Yamada, National Institute of Dental Research, Bethesda, MD),
CD28 mAb CLB-28/1 (R. van Lier, Centraal Laboratorium van
de Bloedtransfusiedienst (CLB) Amsterdam, The Netherlands),
CD16 mAb VD2 (A. E. G. K. von dem Borne, CLB, Amsterdam,
The Netherlands). CD31 polyclonal Ab PECAM IgG was prepared
by Protein A affinity chromatography from polyclonal rabbit anti-
CD31 antisera (25, 32). IgG was present at 10 μg/ml final concen-
tration throughout the assays, except for CD28 mAb which was
used at a 1:1000 concentration which is functional in other assays.

Purified soluble VCAM-1 was prepared as previously described
(33). Human FN was obtained from New York Blood Center, New
York. ICAM-1 was purified by affinity chromatography from the
Reed-Sternberg cell line L428 as previously described (34). Col-
gen type I and fibrinogen type I as control were bought from Sigma
Chemical Co., (St. Louis, MO). VCAM-1-transfected L cells and
mock transfected L cells were also prepared as previously
reported (25, 35).

Adhesion Assay. Adhesion assays were performed essentially as
previously described (15). Purified VCAM-1 (80 ng/well), FN (1
μg/well), ICAM-1 (6 ng/well), collagen type I (1 μg/well), fibrinogen type I (1 μg/well), and control BSA (3% solution) were
applied to 96-well microtiter plates (Costar, Cambridge, MA) in

246 CD31 Is an Adhesion Amplifier of T Cells
Ca/Mg-free PBS at 4°C overnight. Binding sites on plastic were subsequently blocked with Ca/Mg-free PBS/3% BSA for 2–3 h at 37°C to reduce nonspecific attachment. Cells were plated onto 96-well plates (Costar) and cultured to confluence. Plates were washed three times with PBS before the addition of 50,000 ³¹Cr-labeled T cells to each well in a final volume of 100 µl PBS/0.5% human serum albumin (HSA). mAbs (1 µg/well) were added to relevant wells. After a settling phase of 30 min at 4°C, which also allowed mAb binding, plates were rapidly warmed to 37°C for 15 min, and nonadherent cells were washed off. Well contents were lysed with 1% Triton X-100, and γ emissions of well contents determined. Background binding of T cells to BSA or collagen was 1–7%. Data were expressed as mean percentage and SE of binding of T cell subsets from representative individuals. Crosslinking of CD3 and CD31 on T cells or T cell subsets was performed as described (15) by 30 min preincubation with relevant mAbs at 4°C and washing before addition to triplicate wells containing 0.05 µg goat anti-mouse Ig. When not being crosslinked, CD31 mAb was added at the beginning of the settling phase.

**Results and Discussion**

CD31 is Expressed on Unique Subsets of T Cells. The complexities of T cell migration/homing are most readily understood in terms of regulated adhesion of different T cell subsets to different apposing surfaces, particularly endothelial cells. Our previous studies have emphasized differential regulation of adhesion molecules on different T cell subsets, and their relevance to cell–cell adhesion (2). Our interest in CD31 was first stimulated by observing that CD31 was differentially expressed on subsets of circulating T cells. Our comparisons between CD31 and other markers of T cell subsets indicate that CD31 is expressed on unique subsets of T cells (Fig. 1). CD31 heterogeneity does not correlate precisely with either of the two best understood dichotomies within T cells: CD4 vs. CD8 and CD45RA (“naive”) vs. CD45RO (“memory”). Nevertheless, there are biases towards higher frequency of CD31+ cells among CD8+ cells and among CD45RA+ cells. The conclusions from combined analysis of CD4/8 and CD45RA are that: among CD8+ cells, typically 90% express CD31, all of the naive (CD45RA+) cells and about half of the memory (CD45RA−) cells (Fig. 1 B); and among CD4 cells, typically 20% express CD31, about half of the naive (CD45RA+) cells and few of the memory (CD45RA−) cells (Fig. 1 A). This heterogeneity does not
correspond to reactivity of any of the more than 50 molecules whose expression we have examined on T cells (data not shown). The bias toward CD31 expression on CD8+ cells and naive (CD45RA+) cells is remarkable since most adhesion molecules are similarly expressed on CD4 vs. CD8 cells (Y. Tanaka, unpublished observations), and of the many adhesion molecules differentially regulated on T cells, most are preferentially expressed on memory (CD45RA-) cells (2, 36-38).

CD31 mAbs Induce Integrin-mediated Adhesion of Resting Peripheral Human T Cells. Preliminary studies demonstrated that CD31 mAbs induced integrin-mediated adhesion. As expected, the induction of adhesion by CD31 is seen only in purified T cell fractions which include CD31+ T cells (data not shown). Since CD31 is uniformly positive on naive (CD45RA+) CD8 cells (Fig. 1), we undertook the most systematic analysis of adhesion induction on that subset of cells. Purified naive (CD45RA+) CD8 cells show augmented adhesion to the integrin ligands FN and VCAM-1 when CD31 mAbs are present during the assay (Fig. 2). The uniqueness of CD31 mAb-induced adhesion to each ligand is illustrated by the comparison with six different control mAbs, four of which (CD7, CD28, CD3, and CD44) have been described to be inducers of integrin-mediated adhesion of T cells. None of these control mAbs cause marked induction of adhesion in the absence of additional crosslinking (see below). In contrast, adhesion is augmented by most of the CD31 mAbs without additional crosslinking. The two CD31 mAbs that are least effective in induction of adhesion are the two mAbs that bind to the most membrane-proximal domains of CD31 (S. M. Albelda, unpublished observations).

To confirm that T cell binding to purified immobilized ligand is a valid model of integrin-mediated adhesion, the critical features of CD31-induced adhesion were reproduced for T cell binding to an L cell transfected with VCAM-1 (Fig. 3). About 20% of the resting CD8 naive cells bound to the VCAM-1 transfectant, and this binding was doubled by pretreatment with CD31 mAbs. Since VCAM-1 is expressed on the transfected cells at a level severalfold lower than on activated endothelial cells (data not shown), these data indicate that CD31-induced adhesion could be relevant to T cell adhesion to VCAM-1 expressing endothelium.

It is noteworthy that the adhesion induced by the best CD31 mAbs approaches or equals that of PMA (Figs. 2 and 3), which is generally the strongest pharmacologic inducer of T cell adhesion. To determine whether PMA and CD31 mAbs might activate cells in a complementary fashion, cells were activated by both CD31 and PMA (Fig. 4 A). The lack of demonstrable additive induction provided no evidence for distinct signaling pathways or activation of distinct subsets within this relatively homogenous population of CD8 naive (CD45RA+) cells. Additional controls in that experiment demonstrate that CD31-mediated induction does not non-specifically alter adhesion of T cells to otherwise irrelevant extracellular matrix proteins (Figs. 4, B and C).

mAb blocking studies were performed to confirm that CD31-induced adhesion to the three ligands (FN, VCAM-1, and ICAM-1) was mediated by the integrin receptors on T cells which normally bind these ligands (Fig. 5). As expected, T cell binding to VCAM-1 and FN was mediated by integrins of the β1 family, and binding to ICAM-1 was mediated by integrins of the β2 family. More specifically, the VCAM-1 binding was mediated by VLA-4 and the FN binding

Figure 3. CD31 mAbs induce adhesion of T cells to VCAM-1-transfected L cells. Binding of CD8+ T cells to VCAM-1-transfected L cells LVE3 (filled bars) and mock-transfectant NED2-L cells (open bars) was assessed in the presence of CD31 mAb SG134, CD8 mAb B9.8.4, or PMA. Data are expressed as mean percentage and SE of binding of T cells from three replicate wells.

Figure 4. CD31 mAbs induce adhesion of T cells to VCAM-1, but not fibrinogen and collagen. Binding of CD8+ T cells to purified VCAM-1 (A), fibrinogen (B), and collagen (C) was assessed in the presence of the following stimuli: CD31 mAb SG134, CD8 mAb B9.8.4, PMA, or a combination of PMA with CD31 mAb. Data are expressed as mean percentage and SE of binding of T cells from three replicate wells.
was mediated primarily by VLA-5, with a small contribution from VLA-4. Functional inhibition by our newly generated NIH49d-1 mAb was identical to that of the reference VLA-4 mAb L25. Thus, the interactions of VLA-4/VCAM-1, VLA-4/FN, VLA-5/FN, and LFA-1/ICAM-1 induced by CD31 mAbs are consistent with those observed with other T cell populations and other inducing stimuli (19).

The capacity of CD31 IgG mAbs to induce adhesion in the absence of additional crosslinking by a polyvalent anti-Ig reagent (Fig. 2) seems to be a fundamental characteristic of CD31 IgG mAbs, which distinguish them from CD3 IgG mAbs, the prototypic inducers of T cell adhesion (1). When the issue of crosslinking is explored (Fig. 6), the results confirm that CD31 mAbs can induce in the absence of additional crosslinking, while CD3 IgG mAbs (and the other adhesion inducer molecules shown in Fig. 2) do not. CD31-induced adhesion is often augmented by crosslinking, but is almost always observed without it (Fig. 6, and data not shown). Thus, the CD31 "trigger" of adhesion appears to be a uniquely sensitive one requiring only dimer formation, since most CD31 IgGs tested induce adhesion (Fig. 2). Since Fab fragments of CD31 mAbs induce little adhesion (data not shown), the minimal stimulus in this system seems to be CD31 dimer formation, not receptor occupancy.

The kinetics of CD31 induction of adhesion to VCAM-1 were analyzed (Fig. 7). Induction by CD31 was rapid, regardless of the presence or absence of additional crosslinking. Induction by crosslinked CD3 was similar. The induced adhesion was gone by 60 min. Thus, the adhesion induced by CD31 resembles the rapid onset and decay of CD3-induced adhesion. This time course is consistent with models in which CD31-induced adhesion, like CD3-induced adhesion, plays a transient role in a coordinated sequence of events mediating T cell adhesion.

Differential Induction of Adhesion by CD31 vs. CD3. The fact that multiple surface molecules, including CD3, CD2, CD7, CD28, CD44, and now CD31 can regulate T cell adhesion, suggests that regulation of adhesion is a fundamental role served by a variety of cell surface molecules. Adhesion regulation would be most adaptive if different adhesion-inducing molecules preferentially regulated different adhesion receptors. We tested this possibility by comparing the ligand specificity of adhesion induced by crosslinked CD3 and CD31 in multiple donors and experiments (Fig. 8). Each point represents the differential binding of a particular preparation of T cells to two different ligands.
when CD31 is used as the inducer (O) the T cells bind preferentially to VCAM-1 and to FN. Differential induction was not due to differences in kinetics of response (Fig. 7, and data not shown).

These findings of preferential induction have two important implications. First, they imply that there is more than one biochemical mechanism for integrin regulation. This complements the findings of Hermanowski-Vosatka and Wright (39) who have identified a unique lipid which augments adhesive function of β2 integrins, but not β1 integrins (S. Wright, personal communication). Our previous studies using pharmacologic inhibitors also indicate that there is more than one biochemical pathway for inducing β1 and β2 integrin function (19). Taken together, these results suggest a rich diversity of mechanisms for selectively regulating adhesion via different integrins. This complexity is fully consistent with the growing conviction that adhesion regulation is very important physiologically. In addition, these data on differential induction imply that CD31 and CD3 have specialized roles in adhesion induction. CD3 is the critical adhesion inducer in antigen-specific recognition; β2 integrin-mediated binding to ICAM-1 on apposing cells may be particularly critical for this process. On the other hand, CD31 may be critical to T cell–endothelial cell interactions, as discussed below. β1 integrin-mediated binding to VCAM-1, FN, and other ligands may be particularly important for T cell–endothelial cell adhesion, and subsequent migration of T cells.

For these studies we have used CD8 + CD45RA + cells. A distinct, but equally interesting potential explanation is that despite their uniform expression of CD3 and CD31, CD3 preferentially induces LFA-1 function on some of these cells, and CD31 induces VLA-4 function in others.

Previous studies have fostered the concept of CD31 as an adhesion molecule per se. The present studies add a new perspective by showing that CD31 is an adhesion-inducing molecule. We favor the concept that CD31 mediates both weak and potent adhesion-induction, allowing it to serve as an adhesion amplifier. This kind of molecule would be the missing element in our understanding of T cell–endothelial cell adhesion as a cascade consisting of a coordinated series of receptor/ligand interactions which includes: (a) initial tenuous adhesion (tethering); (b) triggering; (c) integrin-mediated strong adhesion (glue); and (d) subsequent detachment (8). Studies of granulocytes indicate that the initial tethering is usually served by molecules of the selectin family (40). L-selectin may function in that role for many T cells (41). Strong adhesion is most likely mediated by the integrins LFA-1 and VLA-4 (2, 35, 42), however, this cannot occur on resting T cells in circulation until the integrins become functionally activated. The intervening step by which the integrins become activated is not understood. Obviously, the CD3/T cell receptor would not be expected to be involved in T cell–endothelial cell interactions. L-selectin is a good candidate, but has not been shown to induce integrin function.

CD31 is an excellent candidate for an adhesion amplifier in T cell–endothelial cell interactions, given the adhesion-inducing capacity of CD31 shown in the present studies, and its demonstrated role in cell interactions with endothelial cells (22). If analogous CD31 engagement occurs when CD31 + T cells bind to endothelium, then our results predict that such CD31 crosslinking would induce integrin-mediated adhe-
The suitability of CD31 for an amplifying role is emphasized by the finding that dimer formation may be sufficient for triggering (Figs. 2 and 6) and that the adhesion induction is transient (Fig. 7). Furthermore, the relatively large number of Ig domains (six) in CD31 may allow it to protrude beyond much of the other glycosylaryx, and thereby make its distal parts readily available early in cell-cell interaction. Our studies indicate that CD31 is particularly effective in inducing the function of VLA-4 integrin (even more so than VLA-5 and -6 integrins; data not shown). This finding fits well with concept of CD31 as an amplifier in T cell-endothelial cell interactions, since it is increasingly apparent how important VLA-4 is in T cell interactions with endothelium in vitro, and ultimately in T cell recirculation in vivo (17, 35, 43–48).

We are currently testing the hypothesis that CD31 contributes to T cell–endothelial cell interactions.

Recent in vivo studies in the rat indicate effects of an anti-VLA-4 mAb on T cell movement into various sites, but most dramatically into gut (48). Until now, there has been no explanation why there is such a predominance of CD8 rather than CD4 cells (90% CD8) among intraepithelial lymphocytes of the gut mucosa (49). We propose that the preferential expression of CD31 on CD8 cells rather than CD4 cells (typically 80 vs. 20%), together with its selective capacity to induce VLA-4 function, may contribute to the preferential movement of CD8 cells into gut epithelium. In addition to the conventional VLA-4 molecule (α4β1), there is an α4-containing integrin (α4β7) on gut-homing T cells which mediates interactions with a ligand on specialized gut endothelium, Peyer’s patch high endothelial venule (HEV) (50, 51). It remains to be determined whether this integrin is also activated functionally by engagement of CD31.

In the foregoing description of T cell–endothelial cell interactions, the postulated adhesion-amplifying role played by CD31 is analogous to the adhesion–amplifying role played by CD3 in antigen-specific T cell interactions (1). We view these as distinct but generally homologous adhesion cascades. More generally, we expect that there will be multiple T cell adhesion cascades. Each T cell will have the potential for many adhesion cascades in its repertoire for use in interactions with different cells or extra cellular matrix. Different T cell subsets will have different specialized repertoires of adhesion cascades.

Obviously, CD31 can be an inducer of adhesion only for those unique subsets of T cells which express it. It is also apparent that there must be and are other adhesion-inducing molecules on resting T cells.

Although the foregoing data prompt us to propose a special role for CD31 in T cell–endothelial cell interactions in the gut, we suspect that it will also be important for T cells in other contexts. The preferential expression of CD31 on naive cells raises the possibility that it may contribute to the process of migration of naive cells into lymph node (52). Three lines of evidence indicate that VLA-4 ligands may exist in lymph node HEV, and therefore are consistent with this hypothesis. First, VCAM-1 can be expressed on endothelium in lymph nodes draining from sites of antigen stimulation (46). Second, VLA-4 mAb inhibits migration of several categories of T cells to peripheral lymph node (48). Finally, inhibition studies with a peptide sequence from the III-CS site of FN indicate that it inhibits lymphocyte binding to cultured lymph node HEV (44). This suggests that VLA-4 may be involved in binding to a ligand on these HEVs. Thus, CD31 and VLA-4 may be important for migration of some T cells through lymph node HEV. Furthermore, given data that CD31 may participate in homophilic interactions with CD31 (25), T cell CD31 may contribute to T cell interaction with not only CD31+ lymph node HEVs, which express CD31 better than endothelial cells (53), but also with CD31+ APCs. Finally, Stockinger et al. (23) have demonstrated that CD31 mAbs induce reactive oxygen metabolites from monocytes. CD31 thus appears capable of contributing to the regulation of cellular processes in addition to adhesion.

It is evident that CD31 does not act alone, but rather in the context of other molecules which constitute the cascade. T cells floating in a sea of CD31+ cells in circulation do not have their integrins activated. Furthermore, only some of the CD31+ T cells become detectably adhesive when exposed to CD31 mAbs. Therefore, a permissive role for a suitable tethering molecule and potentially other cofactors is likely to be a prerequisite for physiologic CD31 triggering. We predict a role for CD31 in T cell subset adhesion to specialized endothelial cells, where the combinatorial requirements of tether, trigger, and glue offer enormous flexibility in an adhesion cascade.

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251  Tanaka et al.
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