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] integrins and L-selectin (LAM-1/Leu-8); regulated expression of the ligands such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin (endothelial leukocyte adhesion molecule-1 [ELAM-1]); and regulated function of the T cell adhesion receptors.

It is rapidly becoming apparent that this last mode of regulation, namely regulated function of the adhesion receptors, is powerful and widely used, not only by T cells, but by other cell types (1–10). Regulated function is a prominent characteristic of the integrin adhesion molecules, which are a diverse family of heterodimeric adhesion receptors used by virtually all cell types in adhesion to other cells and to extracellular matrix (11–14). Resting T cells express at least five integrins (11, 15). LFA-1 (αLβ2), the best known integrin on resting

[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.

T cells, mediates primarily cell–cell interactions via at least three distinct ligands: ICAM-1, ICAM-2, and ICAM-3 (12, 16). In addition, there are four β1 integrins (VLA-3, VLA-4, VLA-5, and VLA-6) that mediate adhesion both to extracellular matrix via fibronectin (FN) and laminin (LN) (15) and to other cells via VCAM-1 (17). It is remarkable that integrins on circulating ("resting") T cells do not mediate effective adhesion. Dustin and Springer (1) demonstrated that crosslinking of the CD3/TCR complex induces transient adhesion via LFA-1, and proposed that such regulated adhesion could account for many of the adhesive events that accompany antigen-specific T cell recognition. Thereafter, the concept of regulated integrin function on T cells has been generalized by extensions in a variety of directions: (*a*) the β1 integrins show similar regulated function on resting T cells (15); (*b*) antigen-specific recognition induces β1 integrin function on T cells (18); and (*c*) integrin function on T cells is regulated not only by CD3 crosslinking (and the similar activation 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, granulocytes, 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 adhesion 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 cell adhesion molecule (NCAM) (21). It is expressed at high density on endothelium, platelets, granulocytes, and monocytes (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 contact, 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 proposes 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 prepared from PBMC of volunteer research healthy donors by exhaustive immunomagnetic negative selection, essentially as previously described (27). We routinely use Advanced Magnetic Particles (Advanced Magnetics, Cambridge, MA) and/or 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, antiglycophorin 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 selection 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 documented by binding to CD31-transfectants (data not shown); CD31 mAb PECAM-1.2 (P. J. Newman, unpublished observations), CD31 mAb 4G6 (S. M. Albelda, unpublished observations), CD31 mAb SG134 (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, antiglycophorin 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, Marseilles, France), CD19 mAb FMC63, CD45RA mAb FMC71 (H. Zola, Flinders Medical Center, Bedford Park, Australia), CD45RO mAb UCHL1 (P. Beverley, Courtauld Institute of Biochemistry, London, UK), CD18 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 concentration 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). Collagen type I and fibrinogen type I as control were bought from Sigma Chemical Co., (St. Louis, MO). VCAM-1–transfected L cells and mock transfectant 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

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. L 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 ^{51}Cr-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.

Flow Microfluorometry. Staining and flow cytometric analysis were carried out by standard procedures (36) using FACS-II® (Becton Dickinson & Co., Mountain View, CA). The mAbs used were CD45RA mAb Leu-18-FITC and CD31 mAb SG134 (29). Amplification was provided by a three-decade logarithmic amplifier.

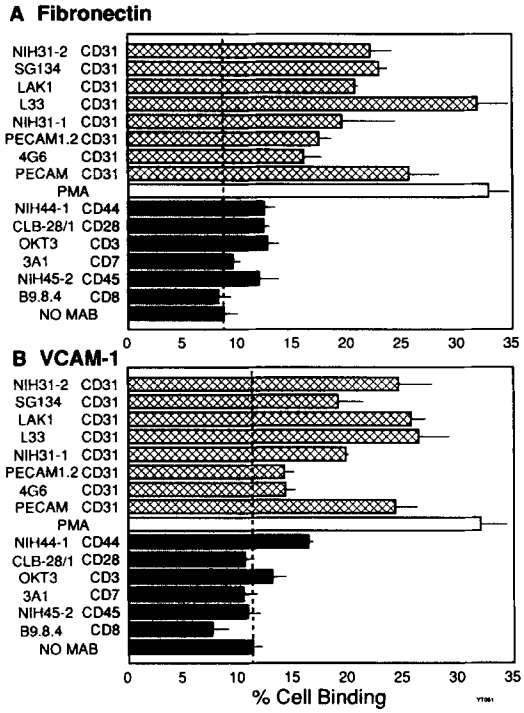

Figure 2. Multiple CD31 mAbs induce adhesion of T cells but control mAbs do not. Binding of ^{51}Cr-labeled naive (CD45RA$^+$) CD8 T cells to purified FN (*A*) and VCAM-1 (*B*) was assessed in the presence of the following stimuli: CD31 mAb or PECAM polyclonal anti-CD31 IgG (*cross-hatched bars*), 10 ng/ml PMA (*open bars*), and control mAb (*filled bars*). No additional crosslinking agent was added. Data are expressed as mean percentage and SE of binding of T cells from three replicate wells. (*Dotted line*) Visual aid indicating binding of T cells to each ligand without mAb stimulus.

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

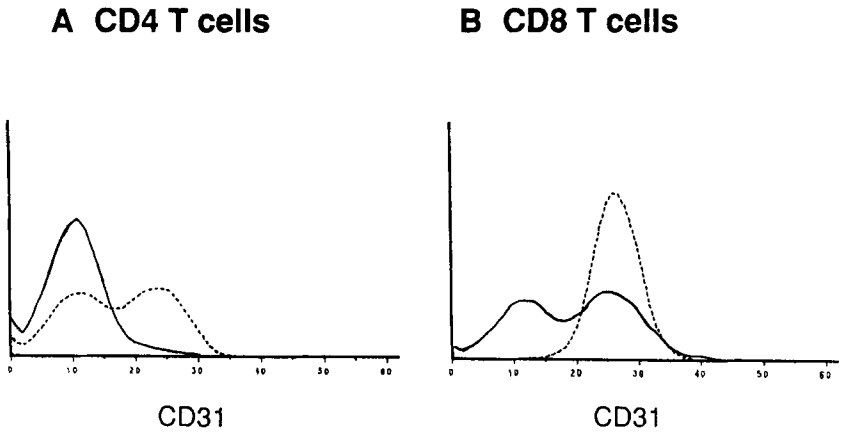

Figure 1. Unique expression of CD31 antigens on resting peripheral T cell subsets. Histograms for CD31 on naive (CD45RA$^+$) subpopulations (*dotted line*) and memory (CD45RA$^-$) subpopulations (*solid line*) of purified resting peripheral CD4$^+$ cells (*A*) and CD8$^+$ T cells (*B*). Analyses were carried out with the CD45RA mAb Leu-18-FITC and the CD31 mAb SG134. The fraction of CD45RA$^+$ cells is 34% for the CD4 and 65% for the CD8 preparation.

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 nonspecifically 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 $\beta 1$ family, and binding to ICAM-1 was mediated by integrins of the $\beta 2$ family. More specifically, the VCAM-1 binding was mediated by VLA-4 and the FN binding

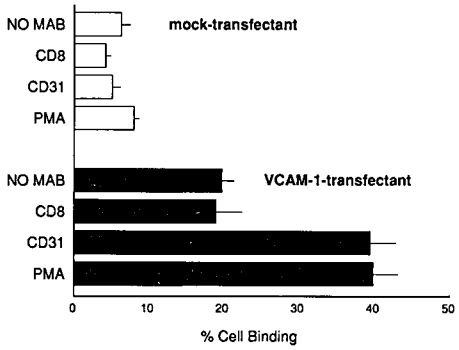

Figure 3. CD31 mAbs induce adhesion of T cells to VCAM-1–transfected L cells. Binding of $CD8^+$ T cells to VCAM-1–transfected L cells LVE3 (*filled bars*) and mock-transfectant NED2-L cells (*open bars*) was assessed in the presence of CD31 mAb SG134, CD8 mAb B9.8.4, or PMA. Data are expressed as mean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

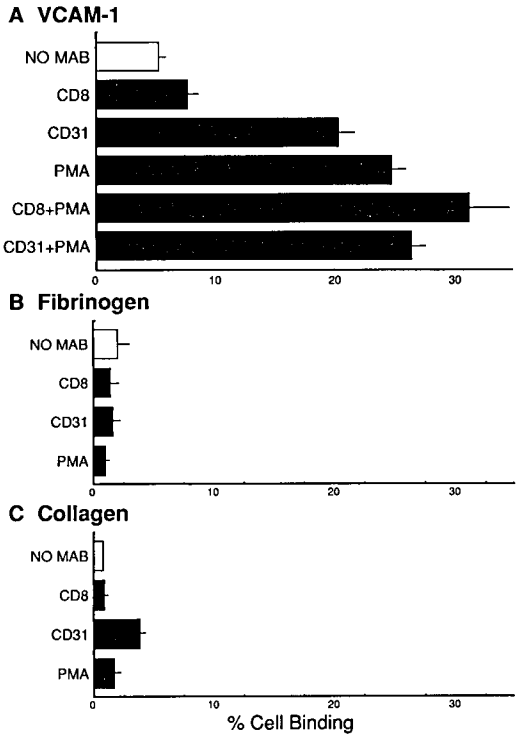

Figure 4. CD31 mAbs induce adhesion of T cells to VCAM-1, but not fibrinogen and collagen. Binding of $CD8^+$ T cells to purified VCAM-1 (*A*), fibrinogen (*B*), and collagen (*C*) was assessed in the presence of the following stimuli: CD31 mAb SG134, CD8 mAb B9.8.4, PMA, or a combination of PMA with CD31 mAb. Data are expressed as mean percentage and SE of binding of T cells from three replicate wells.

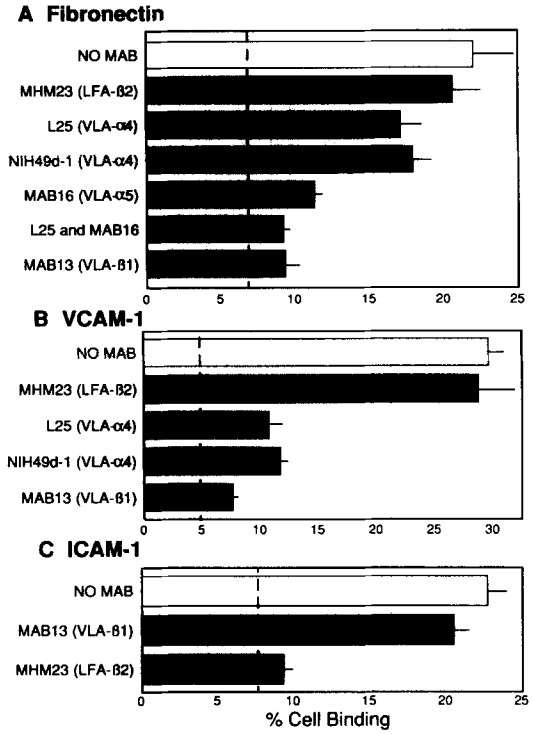

Figure 5. mAb inhibition of CD31-induced adhesion. Binding of naive (CD45RA$^+$) CD8 T cells to FN (*A*), VCAM-1 (*B*), and ICAM-1 (*C*) induced by the CD31 mAb NIH31-1 and SG134 was assessed in the absence (*open bars*) or presence (*filled bars*) of the indicated purified mAb at 10 μg/ml. Data are expressed as mean percentage and SE of binding of T cells from three replicate wells. (*Dashed line*) Background binding of naive (CD45RA$^+$) CD8 T cells to control BSA.

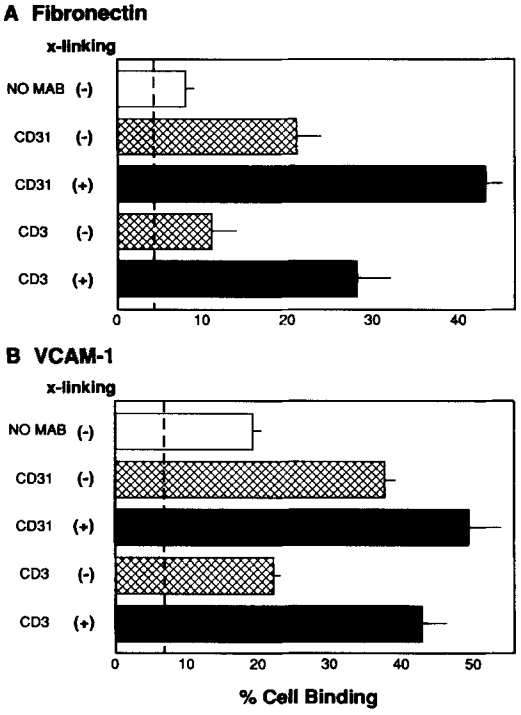

Figure 6. Effect of additional crosslinking on CD31- or CD3-induced adhesion of T cells. Binding of naive (CD45RA$^+$) CD8 T cells to FN (*A*) and VCAM-1 (*B*) induced by CD31 mAb SG134 or CD3 mAb OKT3 was assessed in the absence (*crosshatched bars*) or presence (*filled bars*) of additional crosslinking with goat anti-mouse Ig. Data are expressed as mean percentage and SE of binding of T cells from three replicate wells. (*Dashed line*) Background binding of naive (CD45RA$^+$) CD8 T cells to control proteins (collagen in *A* or BSA in *B*).

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 CD3 is used as the inducer (●), the T cells preferentially adhere to ICAM-1, as indicated by their position above the diagonal. Conversely,

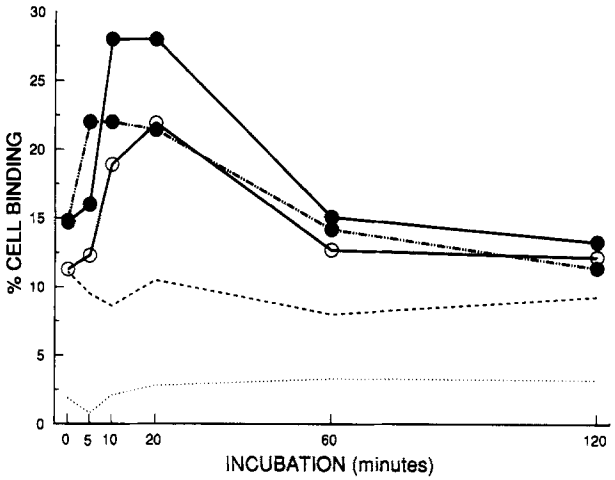

Figure 7. The kinetics of CD31-induced adhesion to VCAM-1. Binding of naive ($CD45RA^+$) CD8 T cells to purified VCAM-1 was assessed after the indicated duration (*x axis*) of incubation at 37°C with the following stimuli: continuous presence of the bivalent CD31 mAb SG134 (*open circle* and *solid line*); the crosslinked CD31 mAb SG134 (*filled circle* and *solid line*); the crosslinked CD3 mAb OKT3 (*filled circle* and *dotted-dashed line*); and without any simulation (*dashed* line). (*Dotted line*) Background binding of naive ($CD45RA^+$) CD8 T cells to BSA. Note that in this and all experiments, the cells have been allowed to settle for 30 min at 4°C before warming to 37°C. The inducing stimulus is present during this 4°C preincubation.

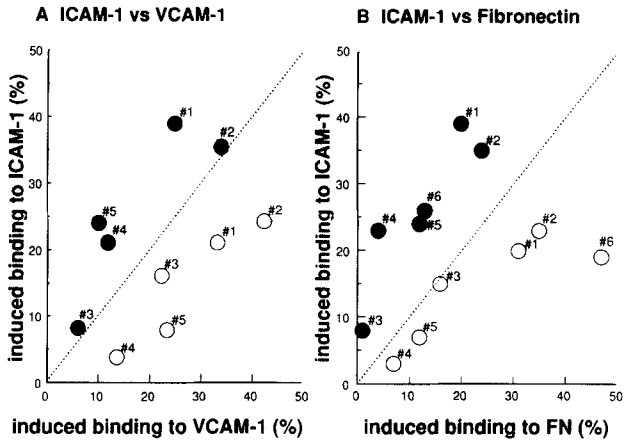

Figure 8. Crosslinked CD3 and CD31 differentially induce the adhesion of T cells to ligands for $\beta 1$ and $\beta 2$ integrins. Adhesion of naive ($CD45RA^+$) CD8 T cells to ICAM-1, VCAM-1, and FN was assessed after coating of T cells with mAbs specific for CD3 or CD31 followed by crosslinking by GAMIg. (*A*) and (*B*) show scatter plot analysis of induced percentage of cell binding to ICAM-1 vs. VCAM-1 (*A*), and ICAM-1 vs. FN (*B*) by crosslinked CD3 mAb (*filled circle*) or CD31 mAb (*open circle*). Symbols represent data from five (*A*) or six (*B*) representative individuals, distinguished by numbers adjacent to the circles. Data are expressed as (percent binding of mAb-induced cells − percent binding of uninduced cells).

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 $\beta 2$ integrins, but not $\beta 1$ integrins (S. Wright, personal communication). Our previous studies using pharmacologic inhibitors also indicate that there is more than one biochemical pathway for inducing $\beta 1$ and $\beta 2$ 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; $\beta 2$ 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. $\beta 1$ 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 will induce integrin-mediated adhe-

sion. 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 glycocalyx, 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 ($\alpha4\beta1$), there is an $\alpha4$-containing integrin ($\alpha4\beta7$) 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 for 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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