

Crosslinking of the T Cell-specific Accessory Molecules CD7 and CD28 Modulates T Cell Adhesion

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Summary

Regulated adhesion enables T cells to migrate through tissue and transiently interact with an endless succession of cells. Monoclonal antibody (mAb) engagement of the CD3/T cell receptor (TCR) complex results in a rapid and transient augmentation of the adhesion function of LFA-1 and VLA integrin molecules on human T cells. We show in this study that mAb crosslinking of the T cell-specific accessory molecules CD7 and CD28, or treatment with the Ca^{2+} ionophore A23187, results in the rapid induction of integrin-mediated adhesion to three distinct ligands: the extracellular matrix protein fibronectin, and the cell surface molecules ICAM-1 and VCAM-1. Like CD3 crosslinking, increased adhesion via CD7 and CD28 crosslinking appears to involve both protein kinase C (PKC) and cAMP-dependent protein kinases. In contrast, A23187 induction of adhesion is unaffected by PKC inhibitors. CD7 is preferentially expressed on naive T cells and is unique in being a potent inducer of naive T cell adhesion. Enhanced expression/function of adhesion-inducing molecules thus overcomes relative deficits in adhesion receptor expression.

Lymphocytes express a multitude of cell surface receptors that are capable of mediating adhesion to other cells and proteins (1, 2). Regulation of the functional activity of these adhesion molecules is critical to both T cell migration and recognition, since T cells must transiently interact with cells and proteins found in the extracellular environment. One important element in the regulation of adhesion is the rapid increase in the adhesive function of integrin molecules that occurs after crosslinking of the CD3/TCR complex by mAb (3–6) or foreign antigen (7). Integrins mediate T cell adhesion to other cells via interactions such as LFA-1 binding to its ligands ICAM-1 and ICAM-2, and VLA-4 binding to VCAM-1, and also to extracellular matrix (ECM)¹ components via interactions such as VLA-4 and VLA-5 to fibronectin (FN) (1, 2, 8). Activation of other lymphoid cell types by mAb crosslinking of functionally relevant cell surface molecules also results in increased LFA-1-dependent adhesion. Such molecules include HLA class II (9) and CD14 (10) on monocytes, and surface immunoglobulin (11), CD19 (12), and CD40 (13) on B cells. These results suggest that increased adhesion via integrin molecules represents one of the earliest responses of lymphoid cells to the delivery of various activation signals.

We reasoned that the functional activity of integrin molecules on human T cells might also be increased by mAb crosslinking of other molecules besides the CD3/TCR. In this study we report the ability of three different activation signals to increase integrin-mediated adhesion: the Ca^{2+} ionophore A23187, and mAb crosslinking of the CD7 and CD28 molecules, both of which have been implicated as T cell activation molecules (14–16). These activation signals can coordinately increase T cell adhesion to three different ligands: the LFA-1 ligand ICAM-1, the VLA-4 endothelial cell surface ligand VCAM-1, and the VLA-4 and VLA-5 ligand FN. We demonstrate similarities in the biochemical signals generated by CD3, CD7, and CD28 crosslinking that distinguish receptor-mediated activation from the effects of A23187. The differential expression of CD7 on functionally important T cell subsets suggests unique functional roles for these molecules in regulating T cell adhesion.

Materials and Methods

Human T Cells. CD4^+ T cells were isolated by negative magnetic immunoselection as previously described (4, 17). Briefly, PBMC were isolated from either leukapheresis packs or buffy coats by Ficoll-Hypaque density-gradient centrifugation. Resting CD4^+ T cells were subsequently isolated from PBMC using Advanced Magnetic Particles (Advanced Magnetics, Inc., Cambridge, MA),

¹Abbreviations used in this paper: ECM, extracellular matrix; FN, fibronectin; HSA, human serum albumin.

Dynabeads (Dynal, Inc., Great Neck, NY), and a cocktail of mAbs consisting of: HLA class II mAb IVA12, CD19 mAb FMC63, CD16 mAb 3G8, CD11b mAb NIH11b-1, CD14 mAb 63D3, CD8 mAb B9.8.4, and antiglycophorin mAb 10F7. CD4⁺CD45RA⁺ naive T cells and CD4⁺CD45RO⁺ memory T cells were isolated by adding the CD45RA mAb G1-15 (to negatively isolate memory T cells) or the CD45RO mAb UCHL1 (to negatively isolate naive T cells) to the mAb cocktail. The T cell populations were >99% CD3⁺ and >96% CD4⁺. Naive and memory T cell purity was >95% as determined by flow cytometric analysis.

Antibodies and Other Reagents. The following mAbs were used as purified Ig: CD3 mAb OKT3, CD7 mAb 3A1, CD5 mAb OKT1, CD58 mAb TS2/9 (all American Type Culture Collection, Rockville, MD), CD2 mAb 95-5-49. The following mAbs were used as dilutions of ascites fluids: HLA class II mAb IVA12, CD14 mAb 63D3, CD28 mAb OKT10, anti-glycophorin mAb 10F7 (all ATCC), CD19 mAb FMC63 (Dr. H. Zola, Flinders Medical Centre, Bedford Park, Australia), CD16 mAb 3G8 (Dr. D. Segal, National Cancer Institute, Bethesda, MD), CD11b mAb NIH11b-1 (18), CD8 mAb B9.8.4 (Dr. B. Malissen, Centre d'immunologie de Marseille-Luminy, Marseilles, France), CD45RA mAb G1-15, and CD28 mAb 9.3 (Dr. J. Ledbetter; Oncogen, Seattle, WA), CD45RO mAb UCHL1 (Dr. P. Beverley, Imperial Cancer Research Fund, London, UK), CD43 mAb 84-3C1, CD26 mAb 5/9, CD59 mAb BRIC 229 (Dr. D. Anstee, Southwest Regional Blood Transfusion Centre, Bristol, UK). E2-specific mAb TU12 (A. Ziegler, Institute of Experimental Immunology, Tubingen, Germany), and GD-3 mAb C281 (Dr. D. Cheresch, Scripps Clinic, La Jolla, CA).

ICAM-1 was purified by affinity chromatography from the Reed-Sternberg cell line L428 as previously described (19). Recombinant, soluble VCAM-1 was isolated from the culture supernatants of Chinese hamster ovary cells expressing a truncated form of the full-length VCAM-1 cDNA as previously described (20, 21). Human FN was obtained from the New York Blood Center. Cytochalasin B was purchased from Sigma Chemical Co. (St. Louis, MO), and staurosporin was purchased from Boehringer Mannheim Diagnostics, Inc. (Indianapolis, IN). Both were dissolved in DMSO before use. Dibutyryl cAMP (Calbiochem-Behring Corp., San Diego, CA) was dissolved in PBS before use. The Ca²⁺ ionophore A23187 (Sigma Chemical Co.) was dissolved in ethanol.

Adhesion Assays. Adhesion assays were performed as described (4). Briefly, 96-well microtiter plates (Costar Data Packaging Corp., Cambridge, MA) were incubated overnight at 4°C with the indicated concentrations of FN, ICAM-1, or VCAM-1, and unbound binding sites subsequently blocked with PBS/2.5% BSA. Each well contained 50,000 ⁵¹Cr-labeled T cells in a final volume of 0.1 ml PBS/0.5% human serum albumin (HSA). For A23187 activation, cells were added to wells containing 1 μg/ml A23187. For PMA activation, cells were added to wells containing 10 ng/ml PMA (Sigma Chemical Co.). For CD3, CD7, and CD28 activation, T cells were incubated with 10⁶ μg/ml of purified mAb, or a 1:5,000 dilution of ascites for 30 min at 4°C, washed twice, and added to wells containing 0.5–1 μg/ml goat anti-mouse Ig (Organon Teknica, Malvern, PA). After 1 h settling at 4°C, plates were rapidly warmed to 37°C for 10 min, nonadherent cells washed off, and the percentage of bound cells was determined by lysing the well contents with detergent and counting γ emissions. All data are expressed as the mean percent of cells binding from three replicate wells plus SEM.

Flow Microfluorometry. Staining and flow cytometric analysis was performed as previously described (22) using sequential incubations with: saturating concentrations of test mAb; Texas red-labeled anti-Ig sandwich; irrelevant excess IgG to block free sites on bound

sandwich; and CD45RA mAb Leu-18-FITC (Becton Dickinson and Co., Mountain View, CA). Logarithmic amplification was provided by a three-decade logarithmic amplifier.

Results and Discussion

Multiple Activation Signals Upregulate T Cell Adhesion to VCAM-1, FN, and ICAM-1. We and others have previously shown that the phorbol ester PMA, mitogenic pairs of CD2 mAbs, or mAb crosslinking of the CD3/TCR results within minutes in increased adhesion via LFA-1 to ICAM-1 (3, 5, 6), via the VLA integrins VLA-4 and VLA-5 to FN, and via VLA-6 to LN (4, 5). The data in Fig. 1 show that three additional activation signals can similarly increase integrin-mediated adhesion: the Ca²⁺ ionophore A23187, and mAb crosslinking of the T cell-specific molecules CD7 and CD28. In addition to increasing T cell adhesion to FN and ICAM-1 (Fig. 1 B), A23187, CD7, and CD28 crosslinking also increase adhesion to the VLA-4 ligand VCAM-1 (Fig. 1 A), extending our earlier findings on VCAM-1 adhesion using PMA activation (21, 23). These results suggest that these activation signals act coordinately on multiple T cell integrins, enabling T cells to adhere to a variety of cell surface and ECM ligands. mAb blocking studies demonstrated that adhesion to these ligands after activation occurs via the expected integrin receptors (data not shown).

The percentage of cells binding after CD7 crosslinking is generally similar in magnitude to that induced by CD3 crosslinking. In contrast, CD28-mediated activation often results in the adhesion of fewer cells (Fig. 1 A and Fig. 2) and shows more donor-dependent variation in magnitude than does CD3- or CD7-mediated activation (data not shown). Crosslinking of T cell surface molecules by mAb is not a general stimulus for increasing adhesion since mAb crosslinking of 12 other cell surface molecules expressed on resting CD4⁺ T cells fails to increase T cell adhesion to FN (Fig. 1 B and data not shown).

Signalling Pathways Involved in A23187-, CD7-, and CD28-mediated Induction of Adhesion. Studies in a variety of systems have shown that adhesive interactions involving the integrin family of adhesion molecules require an intact cytoskeleton (24). The data in Fig. 2 A also show that increased integrin function induced by the various activation stimuli can all be inhibited by treatment of T cells with the cytoskeleton disrupting agent cytochalasin B, further illustrating the importance of the cytoskeleton to these activation-dependent changes in adhesion.

Activation of PKC by the phorbol ester PMA appears to be sufficient on its own to induce integrin-mediated adhesion (Fig. 2) (3–5, 23), and PKC has been implicated in induction of adhesion via CD3/TCR crosslinking (3). Furthermore, activation of cAMP-dependent protein kinases may play a negative regulatory role in adhesion, since treatment of T cells with dibutyryl cAMP has been shown to inhibit CD3-mediated activation of LFA-1 (3). To assess the signaling pathways involved in these newly described modes of inducing T cell adhesion, we have analyzed the ability of PKC inhibitors and dibutyryl cAMP to inhibit adhesion to FN induced

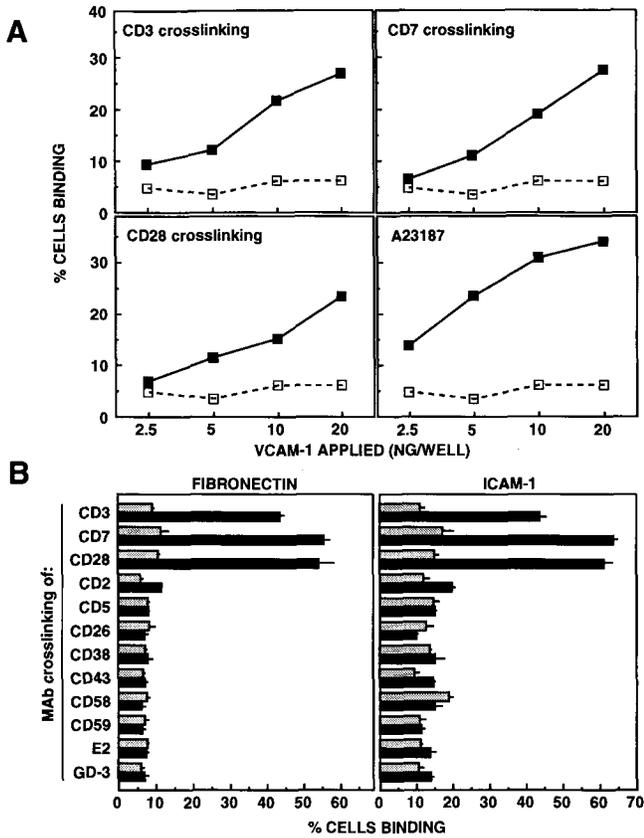


Figure 1. Multiple activation stimuli rapidly increase CD4⁺ T cell adhesion to VCAM-1, FN, and ICAM-1. (A) Binding of ⁵¹Cr-labeled CD4⁺ T cells to purified VCAM-1 (applied at the indicated concentrations) after no activation (□), or after activation for 10 min at 37°C (■) with one of the following stimuli (as indicated): 1 μg/ml A23187, the CD3 mAb OKT3 crosslinked by goat anti-mouse Ig (GAMIg), the CD7 mAb 3A1 crosslinked by GAMIg, and the CD28 mAb 9.3 crosslinked by GAMIg. (B) Binding of ⁵¹Cr-labeled T cells to FN (left) or purified ICAM-1 (right) after coating of T cells with mAbs specific for the indicated molecules in the presence (solid bars) or absence (shaded bars) of 0.5 μg/ml GAMIg. Purified FN was applied at 1 μg/well and purified ICAM-1 was applied at 3 ng/well. Data are representative of a minimum of three experiments with three different donors.

by each of these modes of activation. First, as previously described, incubation of T cells with the PKC inhibitor staurosporin was shown to completely inhibit T cell adhesion induced by PMA (Fig. 2 B). In contrast to earlier results (3), CD3-mediated activation of binding was also completely inhibited by staurosporin. Both CD7- and CD28-mediated activation of adhesion also appear to involve the PKC pathway, since staurosporin completely inhibits adhesion induced by these stimuli. In contrast, A23187-mediated activation is unaffected by the same concentrations of staurosporin, indicating that: the concentration of staurosporin used was not generally inhibitory; and the changes in intracellular Ca²⁺ caused by A23187 that lead to increased adhesion appear not to require PKC activation. These results suggest that A23187-induced adhesion may involve a biochemical mechanism distinct from the other modes of activation.

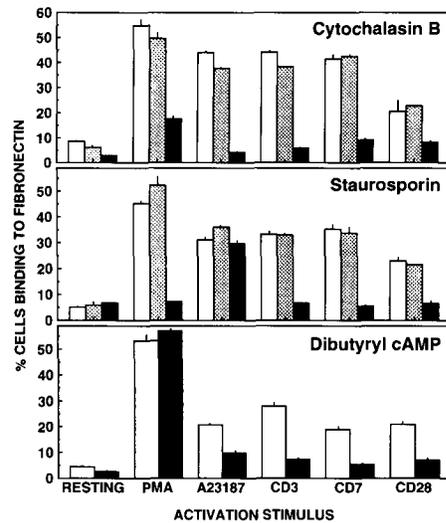


Figure 2. Effects of cytochalasin B, the protein kinase C inhibitor staurosporin, and dibutyryl cAMP on CD4⁺ T cell adhesion to FN induced by the indicated activation stimuli. Binding was assessed as described in Materials and Methods. For cytochalasin B (top) and dibutyryl cAMP (bottom), T cells were preincubated with inhibitor for 20 min at 37°C. In all experiments, inhibitor was present throughout the adhesion assay. Open bars indicate adhesion in the absence of inhibitor, solid bars indicate adhesion in the presence of inhibitor, and crosshatched bars indicate adhesion in the presence of an equivalent amount of DMSO. Dibutyryl cAMP was dissolved in PBS. Cytochalasin B was used at a final concentration of 5 μg/ml, staurosporin at 1 μM, and dibutyryl cAMP at 3 mM. FN was applied at 1 μg/well. Data shown are representative of a minimum of three experiments with different donors. Results shown reflect independent experiments done on different days with different donors.

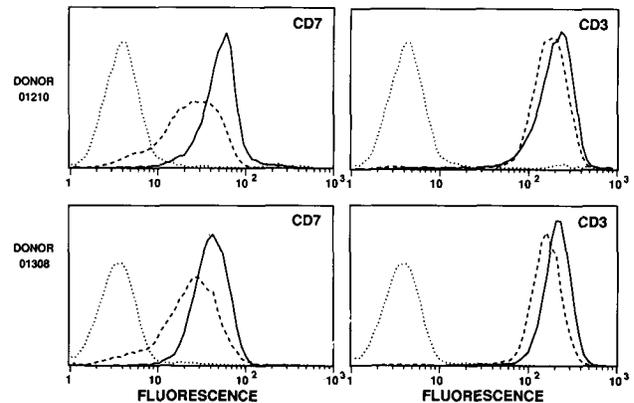


Figure 3. Differential expression of CD7 on CD4⁺ CD45A⁺ naive T cell and CD4⁺ CD45RO⁺ memory T cells. Naive T cells are represented by a solid line and memory cells by a dashed line. Staining of both populations with negative control mAb is comparable, and the naive pattern is shown (dotted line). Results shown are for two donors whose cells have been extensively characterized for differential expression of multiple cell surface molecules (data not shown). Median expression of CD7 was 1.5-fold higher on naive cells than memory cells for donor 1308, and 2.1-fold higher for donor 1210. Test mAb used were CD7 mAb 3A1, and CD3 mAb OKT3.

Elevation of cAMP levels in T cells using dibutyryl cAMP was also shown to dramatically inhibit adhesion induced via CD3, CD7, and CD28 (Fig. 2 C). There was no effect on PMA-mediated activation of adhesion, as previously reported (3). Partial but consistent inhibition by dibutyryl cAMP of A23187-mediated induction of adhesion was also observed. The simplest interpretation of these findings is that at least two pathways of regulation are available, one of which is inhibited by cAMP, and the other by staurosporin. Although sufficient signal (i.e., A23187 or PMA) via either is enough on its own to induce adhesion, weaker cooperative signals via both pathways may be activated by CD3, CD7, and CD28.

Preferential Expression of CD7 on CD4⁺ Naive T Cells. Peripheral T cells are a heterogeneous mix of phenotypically distinct subsets that migrate, adhere, and respond differently (25, 26). Two major subsets of CD4⁺ T cells functionally designated as naive and memory T cells can be identified by differential expression of isoforms of the CD45 molecule (22). There is generally concordant increased expression of a number of adhesion molecules on memory T cells, including a one- to twofold greater expression of LFA-1 and a three- to fourfold greater expression of VLA-4, VLA-5, and VLA-6 (4, 5, 22). Phenotypic analysis by two-color flow cytometry shows that in contrast to integrin expression, CD7 is expressed at 1.5–2.6-fold higher levels on CD4⁺ naive T cells than memory cells (Fig. 3). Greater CD7 expression on naive T cells than memory cells is also unique when compared with other molecules involved in regulating integrin function, such as CD2 and CD28 (5, 22) (data not shown). CD3 expression is compared with CD7 in Fig. 3 because: of the foregoing molecules, CD3 is the only one that has greater expression on naive cells than memory cells (rather consistently

1.2-fold higher); and CD3- and CD7-mediated augmentation of adhesion is compared below.

Regulation of Differential Naive and Memory T Cell Adhesion by Differential Expression of Adhesion-inducing Molecules. Greater expression on memory compared with naive T cells is generally associated with greater binding of memory cells to ICAM-1, FN, and laminin using the previously described activation stimuli (PMA, CD3 crosslinking, and CD2 pairs) (4, 5). CD28 crosslinking similarly induces preferential adhesion of memory T cells from the donor shown in Fig. 4, and the majority of other donors' cells tested (data not shown). In contrast, each of five donors' cells tested showed naive T cell adhesion to integrin ligands at a level equal to or greater than comparable memory T cells after CD7 crosslinking (Fig. 4 and data not shown). Thus, CD7 may be particularly critical in regulating integrin function on naive T cells, consistent with its modest preferential expression on those cells. Our results demonstrate that, despite lower integrin expression on naive T cells, the adhesiveness of naive T cells can be efficiently induced by a compensatory mechanism of greater expression/function of adhesion-inducing molecules such as CD7.

In summary, the present studies demonstrate that T cell adhesion is regulated not only by CD3/TCR engagement but also by engagement of two other cell surface molecules, CD7 and CD28. Both CD7 and CD28 are T cell-specific molecules that have been demonstrated to facilitate T cell activation (14, 16), and thus might be expected to play some role in modulating T cell-adhesive interactions. Recent studies have shown that the molecule B7/BB-1 on activated B cells is a ligand for CD28 (27). We have not observed induction of adhesion with a soluble B7-Ig fusion protein (28), but this could be due to the very low affinity of the B7 fusion protein for CD28 on resting human T cells compared with the CD28-specific mAb used in this study (Y. Shimizu and P. Linsley, unpublished observations). We are currently using B7⁺ cell transfectants to address this issue further. While a ligand for CD7 has not been identified, CD7 is one of the earliest T cell-specific markers in the thymus (29, 30). Its absence has been reported in one case of severe combined immunodeficiency (31), and CD7 mAb crosslinking has been reported to induce changes in intracellular Ca²⁺ (15), as well as enhancing T cell proliferation (14).

The physiologic role of CD7 and CD28 in upregulation of T cell adhesion remains undefined. Since more is known about CD28, it is easiest to speculate on its potential role. For example, initial contact between a T and a B cell bearing the relevant antigen would result not only in augmented T cell adhesion due to CD3/TCR engagement, but also in induction of B cell B7 expression due to the engagement of MHC class II (32). Subsequent interaction between T cell CD28 and B cell B7 would result in activation via CD28 and longer cell-cell contact, ultimately resulting in functional responses such as cytokine production, proliferation, and differentiation. This model implicates CD28 in modulating the duration of the adhesive interactions accompanying TCR engagement. This is supported by preliminary findings that

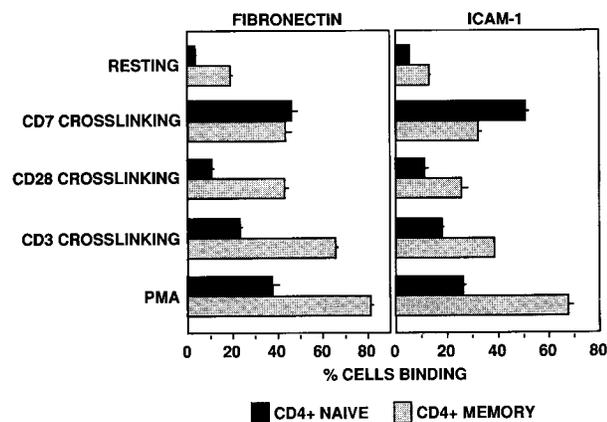


Figure 4. CD7 crosslinking results in comparable adhesion of CD4⁺ naive and memory T cells to FN and ICAM-1. Binding of purified CD4⁺CD45RA⁺ naive (solid bars) and CD4⁺CD45RO⁺ memory (shaded bars) human CD4⁺ T cells to FN (left) and ICAM-1 (right) after the indicated activation stimulus was assessed. FN was applied at 1 μg/ml and ICAM-1 was applied at 1.5 ng/well. Results are shown for a representative donor with differential adhesion of naive and memory cells to integrin ligands after PMA- and CD3-mediated activation as previously described (4, 5).

co-crosslinking of either CD7 or CD28 with CD3 results in prolongation of the transient adhesion via integrins induced by CD3 alone (E. Ennis and Y. Shimizu, unpublished observations). Alternatively, it is possible that CD7 and CD28 may sometimes modulate adhesion independently of CD3 engagement or preceding that engagement. This would require the ligand to be expressed on the apposing cell prior

to T cell contact, as would occur with previously activated B cells, activated monocytes, or certain thymic stromal cells (32–34). Progress in understanding CD7 will depend on definition of its natural ligands. We would expect that a CD7 ligand, which remains to be defined, is also expressed on a functionally unique cell type that is efficient in antigen presentation.

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