Leukosialin (CD43)–Major Histocompatibility Class I Molecule Interactions Involved in Spontaneous T Cell Conjugate Formation

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Summary

Resting T cells spontaneously adhere in a selective manner to potent accessory cells, such as dendritic cells (DC) and lymphoblastoid B blasts (LCL). Here we demonstrate that leukosialin (CD43) and major histocompatibility complex class I molecules (MHC-I) might play a critical role in this process. T cell conjugate formation with monocyte-derived DC (md-DC) and LCL could be strongly inhibited by either preincubating T cells with Fab fragments of CD43 monoclonal antibody (mAb) 6F5 or by preincubating md-DC or LCL with MHC-I mAb W6/32. Intact CD43 mAb 6F5, in contrast to monovalent Fab fragments, enhanced T cell adhesiveness by transactivating CD2 binding to CD58 molecules. Interestingly, induction of this proadhesive signal via CD43 with intact 6F5 mAb was found to revert mAb W6/32-mediated inhibition of T cell conjugate formation. These observations indicated that CD43 cross-linkage mimics and monovalent mAb 6F5 inhibits interaction of T cell CD43 with a stimulatory ligand on opposing cells, presumably MHC-I. For the demonstration of direct physical interaction between CD43 on T cells and MHC-I–coated beads it was necessary, however, to ligate CD2 on T cells with a stimulatory pair of CD2 mAbs (VIT13 plus TS2/18). This suggests that CD2 ligation crosswise upregulates CD43 binding avidity for MHC-I and that both adhesion molecule pairs (CD43/MHC-I and CD2/CD58) act in concert to induce and mediate T cell conjugate formation with certain cell types.

Physical contact formation with other cells is an essential feature of T lymphocytes. It is required for the induction of antigen-specific T cell activation by APC (1–4) and represents an essential step in most effector functions mediated by activated T lymphocytes (5–9).

Antigen-induced T cell activation requires the formation of intimate contact between T cells and APC. This binding process can be divided into two events. The first step in this heteroadhesion process seems to be antigen independent and to precede antigen/MHC recognition by TCR (2, 5, 10–13). This initial, randomly occurring interaction allows T cells to screen the surface of an opposing cell for the expression of appropriate TCR ligands. Ligation of TCR molecules induces subsequent activation of T cell membrane adhesion molecules (14, 15), which further stabilizes conjugate formation between T cells and target cells or APC.

Resting T cells are much more selective than activated T cells in their capacity to physically interact and form stable conjugates with other cells (1, 4–6, 9, 16). This can possibly be explained by the observation that activated T cells a priori express high-avidity adhesion molecules, whereas the majority of adhesion molecules on resting T cells are in a low-avidity–binding state (17, 18). Yet, resting T cells can spontaneously form stable conjugates with certain cell types. This is particularly true for potent APC such as dendritic cells (DC)[1] and activated B blasts (1, 3, 6, 13, 19–21). In contrast, other leukocytes such as monocytes/macrophages (1, 22), resting B cells (1, 3, 6, 20), or granulocytes (9) do not spontaneously bind resting T cells.

Several adhesion molecule pairs on APC and T cells, respectively, have been identified that can contribute to such physical contact and conjugate formation. Among them are the adhesion molecule pairs CD2 (LFA-2)/CD58 (LFA-3), LFA-1 (CD11a/CD18)/ICAM-1, 2, 3, and CD28/B7 (CD80) (4, 12, 13, 21, 23, 24). However, none of them can fully explain the observed exquisite selectivity in the binding behavior of resting T cells to certain APC.

In an attempt to further analyze the molecular basis of the spontaneous adhesion of resting T cells to DC and B blasts,

[1] *Abbreviations used in this paper.* β_2m, β_2-microglobulin; CB, cord blood; DC, dendritic cells; EBV-LCL, EBV-transformed lymphoblastoid B cell line; md-DC, monocyte-derived DC; MHC-I, MHC class I molecules; O-SGP, O-sialoglycoprotease; PB, peripheral blood; rh, recombinant human

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respectively, we have searched for additional surface molecules potentially involved in this adhesion process. We could indeed demonstrate a new, as yet unreported adhesion pathway. It involves leukosialin (CD43) on T cells and MHC class I molecules (MHC-I) on DC and B blasts, respectively. Interaction between these two molecules can promote T cell binding to such cells in two ways: via direct CD43/MHC-I binding and via transduction of proadhesive signals leading to enhanced CD2-mediated CD58 binding.

Materials and Methods

Media, Reagents, and Chemicals. The cell culture medium RPMI 1640 (Gibco Ltd., Paisley, Scotland) was supplemented with 2 mM L-glutamine, 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin, except for EBV-transformed lymphoblastoid B cell lines (LCL) HB654-SN and XX224, which were cultured in serum-free DMEM/F-12 medium (catalog no. B-1004-BB; Hyclone, Logan, UT) to which insulin-transferrin-sodium selenit supplement (catalog no. 1074547; Boehringer-Mannheim, Penzberg, Germany), 0.002% human serum albumin (Behring Institute, Vienna, Austria) and further 50 μg/ml of L-cystein (Merk, Darmstadt, Germany) and 30 μg/ml of L-serin and L-methionin (Fluka Chemie, Vienna, Austria) was added.

Recombinant human (rh) GM-CSF and rhIL-4 were kindly provided by Sandoz Research Institute (Vienna, Austria). rhTNF-α was a kind gift from G. R. Adolf (Ernst Boehringer Institut für Arzneimittelforschung, Vienna, Austria). O-sialoglycoprotease (O-SGP) from *Pasteurella haemolytica* was obtained from D. Sutherland (Oncology Research, Toronto Hospital, Toronto, Canada). Neuraminidase from *Vibrio cholerae* was purchased from Behring Behringwerke AG, (Marburg, Germany).

Purified β_2-microglobulin (β_2m), cytochalasin B, EDTA, neuraminic acid (type VIII), sodium metaperiodate, sodium arsenite solution, thiobarbituric acid, cyclohexanone, and NaF were purchased from Sigma Chemie GmbH (Deisenhofen, Germany). Formaldehyde was obtained from Polysciences Inc. (Warrington, PA). In some experiments, either APC or T cells were pretreated with 10 mM EDTA for 30 min at room temperature and washed afterwards with calcium/magnesium-free PBS. Cytochalasin B (5 μg/ml) or NaF (30 mM) preincubation was carried out at 37°C for 30 min. Fixation of cells, with precooled 1% formaldehyde in PBS, was performed for 20 min strictly at 0°C before washing with PBS.

Antibodies. Antibodies were used at a final concentration of 10 μg/ml. The following murine mAbs were generated in our laboratory: VIAP (calf intestine alkaline phosphatase specific), VIT13 (CD2R), VIT3 (CD3), VIT4 (CD4), 5D7 (CD5), VIT8 (CD8), VIM13 (CD14), 6F5 and 10G4 (CD43), 6B6 (CD44), VIT200 and 562 (CD45), VIM3c (CD97), TA9 (CD99), LA-45 (MHC class I free α chain), AAA1 (M6 antigen). The CD2 mAb TS2/18–, CD11a mAb TS1/22–, HLA-DR mAb L243–, MHC class I mAb W6/32–, CD58 mAb TS2/9–, and β_2m mAb BBM.1–producing hybridomas were obtained from American Type Culture Collection (Rockville, MD). The following mAbs were kindly provided: CD14 mAb MEM18 and CD45R0 mAb UCHL1 were from An der Grub (Bio Forschungs GmbH, Kaumberg, Austria); CD26 mAb A24 (134-2C2) was from R. Vilella (Hospital Clinic i Provincial, Barcelona, Spain); CD48 mAb 5-4.8 was from M.S. Sandrin (Austin Research Institute, Heidelberg, Australia); CD43 mAb L10 was from E. Remold-O'Donnell (Harvard Medical School, Boston, MA); CD40 mAb G28-5 was from J.A. Ledbetter (Bristol-Meyers Squibb Pharmaceutical Research Institute, Seattle, WA); CD58 mAb 1A10 was from S. Meuer (German Cancer Research Center, Heidelberg, Germany); CD59 mAb P282 was from A. Bernard (INSERM Unite 29, Paris, France); CD8 mAb Campath8c was from G. Hale (University of Cambridge, UK); and MHC class I mAb Q1/28 was from S. Ferrone (NY Medical College, Valhalla, NY) The CD3 mAb OKT-3 was purchased from Ortho Diagnostics Systems Inc. (Raritan, NJ). CD28 mAb Leu28 and CD56 mAb Leu19 were obtained from Becton Dickinson & Co. (San Jose, CA), and CD80 mAb BB-1 was from Serotec Ltd. (Oxford, UK). The CD29 mAb K20 was from Immunotech S.A. (Marseille, France). CD54 mAb RR1/1 was from Bender AG (Vienna, Austria). CD86 mAb IT2.2 was purchased from PharMingen (San Diego, CA), and CD19 mAb HD37 was from Behring.

Fab fragments of mAb 6F5 (CD43) were prepared using papain and the recommended protocols from the Avid Chrom™ F(ab) kit (Bioprobe International, Inc., Tustin, CA). The preparation was further purified by affinity chromatography with protein A and size fractionation with FPLC superdex™ 200 gel filtration (Pharmacia, Uppsala, Sweden). Purity was checked using SDS-PAGE followed by silver staining.

Cell Lines. The following cell lines were used in this study: EBV-LCL HB654-SN, XX224 (kindly provided by H. Jungfer and U. Weidle, Boehringer-Mannheim, Penzberg, Germany), and OTMA (our laboratory) and the myeloid cell lines RC-2a and HL-60 (American Type Culture Collection).

Cell Preparation. PBMC were isolated from heparinized whole blood of normal healthy donors by standard density gradient centrifugation with Ficoll-Paque (Pharmacia). Subsequently, monocytes, T cells, B cells, and NK cells were separated by magnetic sorting using the MACS technique (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) as previously described (25). Monocytes were isolated by positive selection with CD14 mAbs VIM13 and MEM18. Purified T cells were obtained through negative depletion of CD11b, CD14, CD16, CD19, CD33, and MHC class II–positive cells with the respective mAbs. B cells and NK cells were isolated by positive selection with CD19 mAb and an NK cell isolation kit (Miltenyi), respectively.

The $CD4^+$ and $CD8^+$ T cell subpopulations were further isolated from purified T cells with CD8 mAb VIT8 and CD4 mAb VIT4, respectively, by negative selection with sheep anti–mouse IgG Dynabeads (Dynal, Hamburg, Germany). Purity of each subpopulation was regularly >90%. Monoclonal TCR-γ/δ^+ T cells were obtained from the mononuclear cell fraction of a patient with T cell leukemia. The mononuclear cell fraction of this patient consisted of 95% T cells ($CD3^+$), of which 93% were TCR-γ/δ^+ and 2% were TCR-α/β^+. Polymorphonuclear granulocytes were prepared as previously described (26). Sheep erythrocytes were purchased form Immuno AG (Vienna, Austria).

Generation of Monocyte-derived (md) DC. md-DC were generated according to Sallusto and Lanzavecchia (27) by culturing purified blood monocytes for 8 d with a combination of rhGM-CSF (50 ng/ml) and rhIL-4 (1,000 U/ml) followed by rhTNF-α (50 U/ml) and GM-CSF (50 ng/ml) treatment for 24 h.

Immunofluorescence Staining Procedures. For membrane staining, (10^7/ml) cells were incubated for 15 min at 0–4°C with conjugated (FITC or PE) or unconjugated mAb. For stainings using unconjugated mAbs, FITC-conjugated $F(ab')_2$ fragments of sheep anti–mouse Ig antibodies (An der Grub) were used as a second-step reagent as described previously (26).

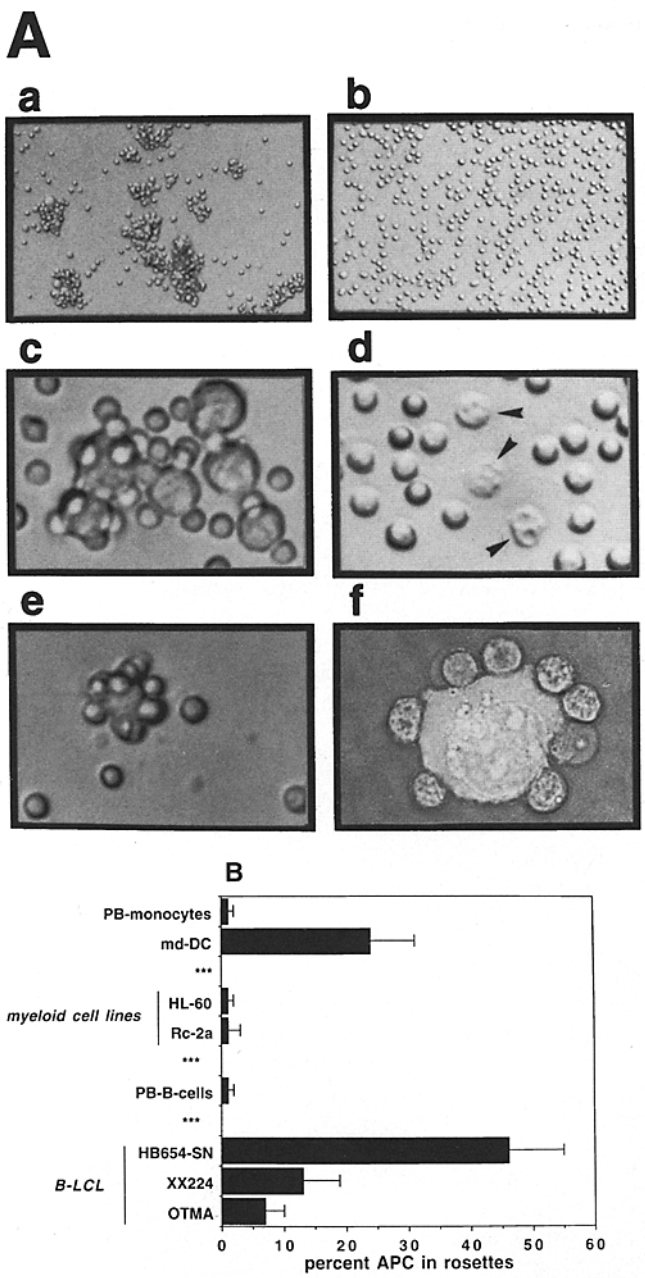


Figure 1. Spontaneous T cell conjugate formation with APC. (*A*) Resting PB T cells coincubated with md-DC without centrifugation. Clusters at 37°C with formation of large aggregates within 2 h (*a* and *c*). In contrast, freshly isolated PB monocytes from the same donor do not interact with resting T cells (*c* and *d*, *arrows* indicate monocytes). At 4°C, T cells formed typical rosettes with md-DC (*e*) or B-LCL HB654-SN cells (*f*). (*B*) Comparison of T cell rosetting capacity of different hemopoietic cells at 4°C. Results show mean values ± SD of four experiments.

Flow Cytometry. Flow cytometric analysis was performed using a FACScan® flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Antigen Loading of Dynabeads. 2×10^7 sheep anti-mouse IgG–coated Dynabeads of 4.5-μm diameter were labeled with CD58 mAb 1A10 (50 μg/ml) at 4°C for 2 h. The mAb is against an epitope on CD58 that is not involved in CD2 binding (28). After washing twice in PBS, the beads were incubated with irrelevant mouse IgG1 mAb VIAP (100 μg/ml) to block the free anti-mouse binding site and washed twice. Then, rhCD58 protein (kindly provided by U. Moebius and S.C. Meuer, German Cancer Research Center, Heidelberg, Germany) was added to the beads and incubated for 2 h at 4°C. Afterwards, the beads were washed two times. CD58 molecule loading of beads was controlled by staining them with CD58 mAb TS2/9, directed against an epitope on CD58 distinct from the capture mAb 1A10.

Coating of MHC-I to Dynabeads was done as described for CD58 beads by using the anti–MHC-I mAb Q1/28 (29) as capture antibody. After blocking residual free anti-mouse IgG binding sites on beads with irrelevant IgG1 mAb VIAP, MHC class I molecules from supernatants of densely growing LCLs (HB654-SN, OTMA, XX224) were bound. 2×10^7 mAb Q1/28–coated dynabeads were incubated for this purpose with 50 ml of an individual cell culture supernatant and gently shaken for 3 h at 4°C. After three washings, the loading of beads with MHC-I was assessed by staining with FITC-labeled anti-β_2m mAb BBM.1 and flow cytometric analysis. Clear reactivity of anti-β_2m mAb BBM.1 with all beads demonstrated effective labeling with MHC-I. Negative control staining with mAb 6F5 (CD43, IgG1) ascertained that all anti-mouse IgG binding sites of the beads were blocked and that CD43 molecules were absent (data not shown).

Dynabeads coated with free MHC-I α chains were prepared using mAb LA-45 as a capture antibody. This mAb specifically recognizes free, not β_2m associated MHC-I α-chains (30).

Heterotypic Adhesion Assay (Rosette Formation Assay). The heteroadhesion experiments were performed as previously described (31). APC were stained with the vital cell dye calcein-AM (Molecular Probes, Inc., Eugene, OR) at 37°C for 5 min and washed twice. Subsequently, 100-μl aliquots of APC (10^6/ml) and T cells (10^7/ml) were mixed in silicone-coated tubes (Becton Dickinson & Co.) and incubated without prior centrifugation for up to 3 h at 4°C and 37°C, respectively. Heterotypic cell adhesion of APC and T cells was analyzed every 30 min using a fluorescence microscope (Leitz, Aristoplan, Jena, Germany). By enumerating 100 fluorescence-labeled APC, heteroadhesion events were regarded as positive (rosette) when at least three T cells (nonfluorescent) adhered to a single APC. In some experiments NK cells, B cells, monocytes, granulocytes, or human erythrocytes were used instead of T cells.

SRBC, rhCD58-coated beads, or MHC-I–coated beads binding to T cells were analyzed by staining the T cells (10^6/ml) with calcein-AM and subsequent addition of a 20-fold excess of SRBC or a 10-fold excess of beads. Without centrifugation, the mixtures were incubated for 2 h at 4°C, and rosette formation between T cells and SRBC or beads was analyzed as described above.

Evaluation of Sialic Acid Cell Surface Density. Cell surface–associated sialic acid was released from freshly isolated PB monocytes or md-DC (5×10^6) from the same donors with *V. cholerae* neuraminidase treatment. The amount of sialic acid in the supernatants was then determined using the thiobarbituric acid assay as described by Warren (32). As a reference standard, we used pure neuraminic acid from sheep submaxillary glands (Sigma Chemie GmbH).

The amount of releasable cell surface sialic acid for both cell types was related to cell surface area. For this purpose, cell size was measured with a cytometer (Multisizer II; Coulter, Luton, U.K.), and the minimal surface area was calculated from cell diameter. Given the villous surface structure and numerous projections of DC, this method underestimates the surface area of md-DC in relation to the surface area of freshly isolated monocytes.

Table 1. *Rosette Formation Is a Selective Process*

HB654-SN cells cultured with	Rosette formation
T cells	+
CB T cells	+
B cells	−
NK cells	−
Monocytes	−
Granulocytes	−
Erythrocytes	−

Identical results were obtained in two independent experiments.
+ = ≥40% of HB654 cells in rosettes; − = <5% of HB654 cells in rosettes.

Table 2. *T Cell Rosette Formation Represents an Active Process*

Agent added	T cell rosette formation with md-DC	T cell rosette formation with HB654-SN cells	T cell rosette formation with HB654-SN cells: Preincubation of T cells	T cell rosette formation with HB654-SN cells: Preincubation of HB654-SN cells
cytochalasin B (5 μg/ml)	−	−	−	+
NaF (30 mM)	−	−	−	+
formaldehyde (1%)	−	−	−	−
EDTA (10 mM)	+	+	+	+
Medium control	+	+	+	+

Identical results were obtained in three independent experiments.
−, <5% APC in rosettes; +, ≥30% APC in rosettes.

Results

Spontaneous T Cell Conjugate Formation Is a Selective Process. For the assessment of spontaneous T cell conjugate formation, freshly isolated peripheral blood (PB) T cells were coincubated without centrifugation with a panel of various types of hemopoietic cells. The binding of T cells or aggregate formation was microscopically evaluated.

Upon coincubation for 2 h at 37°C, T cells formed large aggregates with md-DC (Fig. 1 *A*, *a* and *c*). Variable degrees of aggregate formation were also observed with the EBV-LCL HB654-SN, OTMA, and XX224. No aggregate formation whatsoever was observed with the myeloid cell lines HL-60 and RC-2a or with purified PB B cells or monocytes (Fig. 1 *A*, *b* and *d*).

At the single-cell level, more clearly visible and distinguishable was heterotypic adhesion at 4°C. Upon coincubation at 4°C, T cells formed typical rosettes with single md-DC (Fig. 1 *A*, *e*) and LCL HB654-SN cells (Fig. 1 *A*, *f*). An average of 24 ± 7% of md-DC and 46 ± 9% of

Table 3. *Antibody Inhibition Study on HB654-SN/T Cell Rosette Formation*

mAbs against APC antigens		Rosette formation	mAbs against T cell antigens		Rosette formation
5D7	CD5	++	TS2/18	CD2	+
G28-5	CD40	++	VIT3	CD3	++
L10	CD43	++	VIT4	CD4	++
6B6	CD44	++	5D7	CD5	++
VIT200	CD45	++	VIT8	CD8	++
5-4.8	CD48	++	Campath8c	CD8	++
RR1/1	CD54	++	TS1/22	CD11a	++
TS2/9	CD58	−	TS1/18	CD18	++
P282	CD59	++	A24	CD26	++
BB1	CD80	+*	VIT14	CD27	++
IT2.2	CD86	++	Leu28	CD28	+*
VIM3c	CD97	++	K20	CD29	++
W6/32	MHC-I	−	6F5	CD43	++
BBM.1	β_2m	−	6B6	CD44	++
L243	MHC-II	++	UCHL-1	CD45RO	++
AAA1	M6	++	TA9	CD99	++

Indentical results were obtained in three independent experiments.
++ = ≥40% LCL–HB654-SN cells in rosettes (medium control was 50 ± 6% rosettes); + = <20% LCL–HB654-SN cells in rosettes; − = <10% LCL–HB654-SN cells in rosettes.
*Transient inhibition.

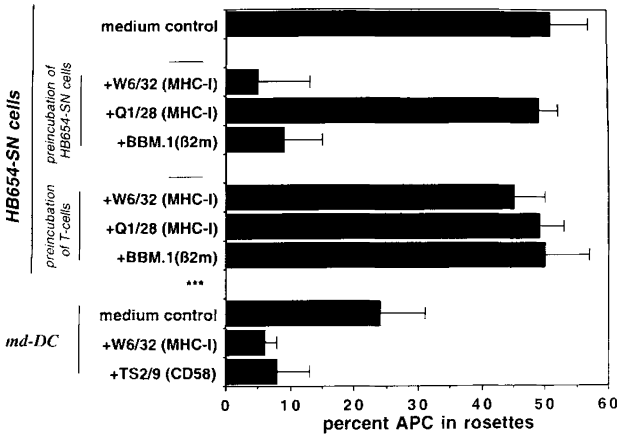


Figure 2. Inhibition of rosette formation with mAbs against MHC-I on APC. Preincubation of HB654-SN cells, but not T cells, with anti-MHC-I mAb W6/32 and mAb BBM.1 (β_2m) inhibits T cell rosette formation, whereas preincubation with MHC-I mAb Q1/28 of either HB654-SN or T cells had no effect. Addition of mAb W6/32 or TS2/9 (CD58) was also inhibitory for md-DC–T cell interaction. Results indicate mean values ± SD of three experiments

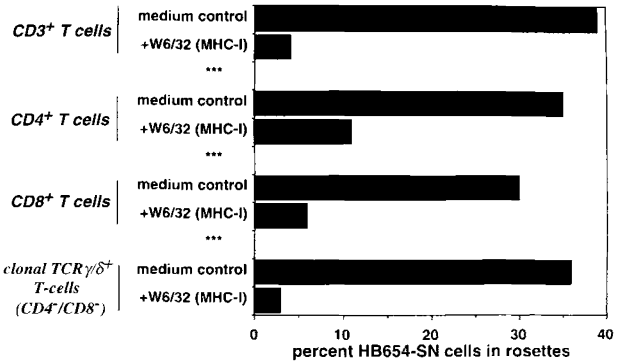


Figure 3. MHC-I–dependent APC–T cell interaction does not require CD8/TCR-α/β molecules on the T cell side. MHC-I–dependent rosette formation of T cells with HB654-SN cells was observed with purified $CD4^+CD8^-$ as well as with $CD8^+CD4^-$ or unfractioned T cells. This formation also occurred with $CD4^-CD8^-$ clonal γ/δ T cells from a patient with T cell leukemia. Results show mean values of two experiments.

HB654-SN cells bound three or more T cells (Fig. 1 *B*). Rosette formation, albeit weaker, was also observed with LCL XX224 and OTMA cells, but not with PB B cells, monocytes, or the myeloid cell lines HL-60 and RC-2a (Fig. 1 *B*).

Spontaneous conjugate formation with HB654-SN cells seems to be a selective feature of T cells. As shown in Table 1, both PB and cord blood (CB) T cells strongly bound, whereas PB B cells, NK cells, monocytes, granulocytes, and red blood cells showed no rosette formation.

T cell rosette formation at 4°C with md-DC and HB654-SN cells seems further to represent an active process (Table 2). Addition of the metabolic inhibitor NaF or cytochalasin B to the coculture or pretreatment of T cells with NaF or cytochalasin B prevented rosette formation. Fixation of either cell type with formaldehyde before coincubation abolished conjugate formation. In contrast, chelation of divalent cations with EDTA had no inhibitory effect. T cell adhesion to md-DC and HB654-SN cells thus seems to require metabolic energy and an intact cytoskeleton, but is obviously not dependent on extracellular divalent cations.

MHC Class I Molecules Are Involved in APC–T Cell Interaction. To elucidate which molecules might be involved in the observed rosette formation of resting T cells with md-DC and LCL HB654-SN cells, we selected a panel of mAbs against molecules expressed on md-DC as well as on HB654-SN cells. Also included was a panel of mAbs against T cell antigens. All were tested for their ability to inhibit rosette formation of T cells with the "model-cell" HB654-SN.

As shown in Table 3, CD58 mAb TS2/9 was the most efficient inhibitor among the analyzed mAbs against cell surface molecules on the APC side. Inhibition, although only within the first 90 min of incubation, was also observed with the CD80 (B7) mAb BB-1. mAbs against other molecules on the APC side were not inhibitory. At the T cell side, this inhibition pattern was reflected by a clear-cut inhibitory effect of CD2 mAb TS2/18 and moderate inhibition with CD28 mAb Leu28. mAbs against other prominent T cell antigens were not inhibitory (Table 3).

Suprisingly, very pronounced inhibition of T cell rosette formation was also observed with mAb W6/32 directed against a monomorphic epitope on the MHC-I α chain and with mAb BBM.1 directed against β_2m. Addition of mAb Q1/28 directed against a different monomorphic epitope of MHC-I α chain than mAb W6/32 (29) or purified β_2m molecules (data not shown) had no inhibitory effect. Subsequently performed separate preincubation experiments of HB654-SN or T cells revealed that both mAbs (W6/32 and BBM.1) mediated inhibitory effects only when HB654-SN cells were pretreated with the respective mAbs before setting up the rosette formation assay (Fig. 2). MHC-I molecules also seem to be critical for md-DC/T cell rosette formation. T cell binding to md-DC can also be efficiently inhibited with MHC-I mAb W6/32 (Fig. 2).

Search for Candidate Counterreceptors on T Cells for MHC-I. The two prototype MHC-I counterreceptors on T cells, the monomorphic receptor structure CD8 and the clonal MHC-I/peptide receptor structure TCR-α/β (33–35) do not seem to be responsible for the observed adhesion phenomena.

Purified $CD8^-CD4^+$ T cells bind to the same extent to HB654-SN cells as do $CD8^+CD4^-$ cells, and adhesion is equally inhibitable by mAb W6/32 (Fig. 3). mAb Campath8c, shown before to inhibit MHC-I–CD8 interactions (34), has no inhibitory effect in our test system (Table 3). For TCR-α/β to be responsible, one would have to postulate that up to 50% of the added PB T cells recognize antigenic epitopes presented by md-DC or HB654-SN cells, respectively. This is rather unlikely. In addition, we could demonstrate that a clonal population of TCR-γ/δ cells ($CD4^-CD8^-$) from a patient with T cell leukemia also avidly binds to HB654-SN cells (Fig. 3).

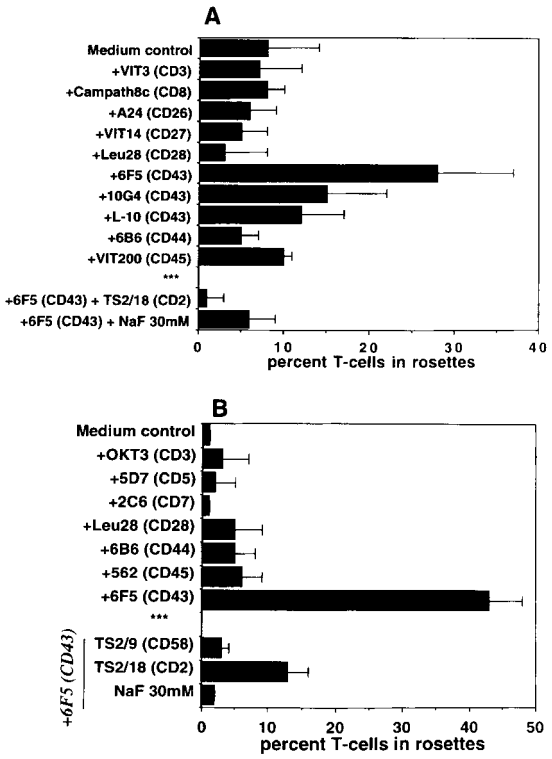


Figure 4. Activation of the CD2 adhesion pathway upon ligation of CD43 with mAb 6F5. (*A*) Engagement of CD43 on T cells with mAb 6F5 induces CD2-dependent sheep erythrocyte rosetting. (*B*) Engagement of CD43 on T cells with mAb 6F5 triggers binding of rhCD58 protein–coated beads. In both experimental settings, the T cells were coincubated with targets at 4°C in the presence of the indicated mAbs or NaF (30 mM) without centrifugation. Results show mean values ± SD of three experiments for each condition.

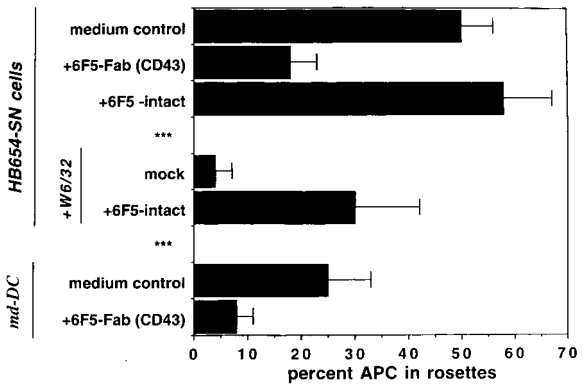


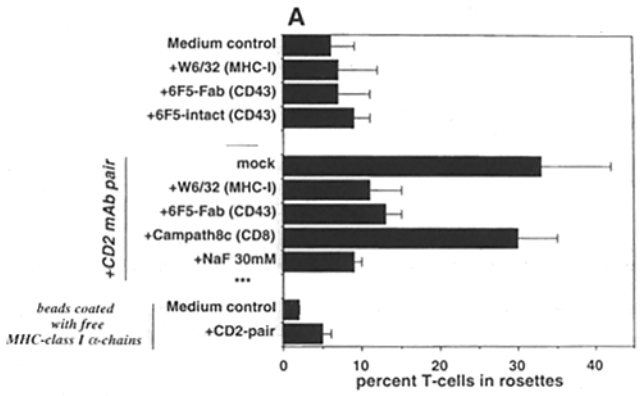
Figure 5. Involvement of CD43 on T cells in APC/T cell rosette formation. Addition of Fab fragments of CD43 mAb 6F5 inhibited rosette formation of T cells with HB654-SN cells, which do not express the 6F5-defined epitope. Inhibition was also shown with md-DC. Intact 6F5 mAb, however, enhanced APC/T cell rosette formation and was capable of reconstituting rosette formation after inhibition with MHC-I mAb W6/32. Results represent mean values ± SD of three experiments.

Other as yet undefined MHC-I ligands on T cells must, therefore, be considered. They could be classical adhesion molecules, which directly mediate T cell binding and/or represent signaling structures that, upon ligation, transactivate heterologous adhesion molecules. To us, the latter possibility appeared more likely. Such a mechanism could, for instance, induce enhanced adhesiveness of the T cell–specific receptor structure CD2, shown above to be critically involved in spontaneous T cell conjugate formation in our test conditions (Table 3). We therefore screened for T cell surface molecules that are able to transactivate the adhesiveness of CD2 under our test conditions at 4°C.

CD43 Ligation Transactivates CD2. As a system for the detection of potential CD2 avidity upregulating effects, we used rosette formation of T cells with SRBC under suboptimal conditions (no cocentrifugation) and T cell binding of rhCD58-coated beads. Fig. 4 summarizes the effects of various antibodies to T cell surface antigens on the rosette-forming capacity of T cells with SRBC (*A*) and rhCD58 coated beads (*B*), respectively. By far, the most striking stimulatory effect was observed with CD43 mAb 6F5. This mAb clearly enhanced rosette formation with both targets. The effect was specific and could be inhibited with blocking CD2 and CD58 mAbs. It required metabolic energy and did not occur in the presence of NaF. Engagement of CD43 and, in particular, of the CD43 epitope recognized by mAb 6F5 thus transactivates the adhesiveness of CD2.

CD43 Molecules on T Cells Are Involved in T Cell Conjugate Formation. To evaluate whether CD43 molecules on T cells are indeed involved in the observed spontaneous adhesion of T cells to HB654-SN cells or md-DC, we analyzed the effects of intact CD43 mAb 6F5 and Fab fragments of this mAb on T cell rosette formation. As can be seen from Fig. 5, the two mAb preparations gave discrepant effects. Fab fragments strongly inhibited rosette formation of T cells with HB654-SN cells or md-DC. Inhibition of APC–T cell interactions with Fab fragments of 6F5 and W6/32 mAb was found at 4°C as well as at 37°C (data not shown). Because HB654-SN cells do not express the 6F5-defined epitope, Fab fragments of mAb 6F5 seemed to compete at the T cell side with an adhesion-promoting ligand on APC. In contrast, intact 6F5 enhanced T cell rosette formation and seemed to imitate signaling properties of such a presumed ligand. If MHC-I were this ligand, then proadhesive signals induced by intact CD43 mAb 6F5 should reverse anti–MHC-I mAb W6/32–caused inhibition of T cell rosette formation. As can be further seen from Fig. 5, this was indeed observed. Reconstituted rosette formation was then completely inhibited with CD58 mAb TS2/9 (data not shown).

Engagement of CD2 Transactivates CD43 and Induces MHC-I Binding. The results obtained so far demonstrate that CD43 on T cells and MHC-I on md-DC and HB654-SN cells play critical roles in spontaneous T cell conjugate formation. They further suggest, but do not yet formally prove, that these two molecules physically interact with each other in this process. We therefore attempted to demonstrate more directly MHC-I binding to CD43 on T cells. For this purpose, we coincubated purified T cells with MHC-I–coated beads and checked for MHC-I bead binding to T cells. As can be seen from Fig. 6, only insignificant



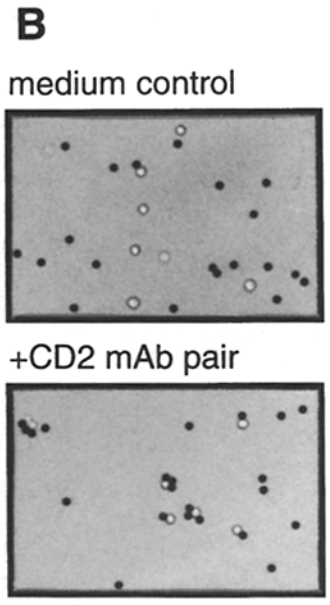


Figure 6. Binding studies of MHC-I protein–coated Dyna beads to T cells. (*A*) MHC-I protein–labeled beads showed only little and unspecific binding to resting T cells. However, upon addition of a stimulatory CD2 mAb pair (VIT13, TS2/18), ~30% of the T cells bound at least three beads. Binding to T cells could be specifically inhibited with mAb W6/32 and Fab fragments of mAb 6F5 (CD43), but not with CD8 mAb Campath8c. (*B*) Illustration of the low frequency of MHC-I–loaded beads bound to T cells and the appearance of bead rosetting with T cells activated via CD2.

and nonspecific inhibited binding of beads can be observed. Affinity for MHC-I, if it exists at all, thus seems to be too low to be detectable in this test system.

Having observed that ligation of CD43 upregulates CD2 adhesiveness, we postulated that engagement of CD2 might crosswise activate CD43 and induce detectable MHC-I binding. Results presented in Fig. 6 demonstrate that this is indeed the case. Engagement of T cell CD2 molecules with the stimulatory CD2 pair mAb TS2/18 plus mAb VIT13 (36) induced significant binding of MHC-I–coated beads (33 ± 9% of T cells bound three or more MHC-I–coated beads). Binding was found to be specific, because it is inhibited by anti–MHC-I mAb W6/32. It seemed to involve CD43 on T cells, because it was also significantly reduced by Fab fragments of CD43 mAb 6F5 but not by CD8 mAb Campath8c. Binding was not only obtained with beads coated with MHC-I from culture supernatants of HB654-SN cells, but also with MHC-I from two other LCLs (OTMA, XX224) (data not shown). In all instances, CD2 triggered binding of T cells to MHC-I beads was reduced to background in the presence of NaF, indicating energy dependence. CD2 mAbs were required for induction of binding of MHC-I beads. Other mouse mAbs (W6/32, 6F5) had no such effect. Intact MHC-I α chain–β_2m heterodimers were required for binding to occur. Beads coated with free MHC-I α chains did not bind (Fig. 6 *A*).

Given the evidence that CD43 molecules on T cells can be induced to physically bind MHC-I, we postulated that this "adhesion molecule pair" can also per se mediate T cell binding to HB654-SN cells. To visualize such binding, we first had to "knock out" the normally occurring CD2/CD58 adhesion pathway by adding inhibitory CD58 mAb TS2/9 to our T cell/HB654-SN cell coculture system. This completely prevented spontaneous T cell conjugate formation. However, when subsequently adding the stimulatory CD2 mAb pair, significant rosette formation could be reobserved (Fig. 7). This CD2 mAb–induced conjugate formation in the presence of CD58 mAb TS2/9 could be significantly inhibited by CD43 mAb 6F5 Fab fragments and by intact MHC-I mAb W6/32, but not by CD5 mAb 5D7. The direct involvement of CD43 molecules on T cells in this type of T cell conjugate formation is further supported by our finding that O-SGP treatment of T cells, leading to CD43 molecule cleavage (37), prevents CD2 mAb pair–inducible T cell rosette formation (Fig. 7).

Discussion

Several studies have shown that freshly isolated resting T cells are extremely selective in their capacity to bind and

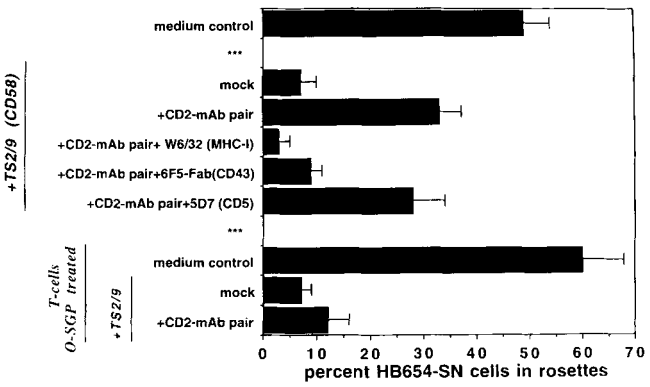


Figure 7. MHC-I and CD43 function as adhesion molecules in APC–T cell interaction. Rosette formation of T cells with HB654-SN cells was blocked with CD58 mAb TS2/9 and subsequently restored through signaling via CD2. APC–T cell interaction under this condition seems to be mediated via MHC-I–CD43 interactions, because it is inhibited with the respective mAbs W6/32 and 6F5 (Fab fragments). Reconstitution of rosette formation via CD2 was not observed when CD43 was removed from T cells upon O-SGP treatment. Results indicate mean values ± SD of three experiments.

form stable conjugates with other cells. The two most prominent cell types to which resting T cells were shown to spontaneously adhere are DC (1–4, 10, 13, 23, 24) and activated B blasts (3, 6, 12, 19–21).

In this study, we addressed the question which surface molecules, in addition to the already known adhesion molecule pairs such as LFA-1/ICAM-1,2,3, CD2/LFA-3, and CD28/B7 (4, 13, 21, 23, 24) might play a critical role in such spontaneous T cell conjugate formation with DC and activated B blasts. We used for this purpose DC generated from monocytes (md-DC) and an EBV-LCL (HB654-SN) observed to be particularly efficient in T cell binding.

To our surprise, we found two surface molecules, already known for other reasons, to be critically involved in this T cell conjugate formation process: sialomucin (CD43) on T cells and MHC-I on md-DC and HB654-SN cells, respectively. T cell conjugate formation could be strongly inhibited by either preincubating T cells with Fab fragments of CD43 mAb 6F5 or by preincubating APC (md-DC, HB654-SN) with MHC-I mAb W6/32 or β_2m mAb BBM.1.

The mode of action of CD43 molecules on T cells turned out to be particularly interesting in this context. Using intact bivalent CD43 mAb 6F5 instead of Fab fragments, we could demonstrate that ligation of CD43, under cross-linking conditions with intact mAb, enhances rather than inhibits T cell binding. This, together with the observation that Fab fragments inhibit, suggests that the molecular region of CD43 recognized by mAb 6F5 represents a signal transducing recognition site for a stimulatory ligand on the investigated target cells. Blockage of this site with Fab fragments obviously inhibits ligand interaction. Cross-linkage of this site on CD43 with intact mAb seems to mimic ligand interaction and to induce a signaling cascade, leading to enhanced T cell adhesiveness.

Signaling via CD43, upon ligation with specific mAbs, is a well-documented phenomenon. It involves phosphorylation of a specific protein substrate (38), promotes T cell proliferation with particular mAbs (39, 40), and, at 37°C, is regularly accompanied by enhanced cellular adhesiveness and homoaggregate formation (41–43). CD43-mediated β_1- and β_2-integrin activation seems to play a predominant, although perhaps not an exclusive, role in all adhesion phenomena described so far (41). However, such an involvement of integrins is quite unlikely in our assay system. As observed by us, CD43-regulated T cell adhesiveness also occurred at 4°C and was independent of divalent cations. Cellular signaling at 4°C is not unique and has been shown before for several receptor structures (44, 45). However, integrin-mediated adhesion is known to be temperature sensitive and divalent cation dependent (5). Other temperature-insensitive and divalent cation–independent adhesion molecule systems must, therefore, be taken into consideration.

The best known T cell adhesion molecule fulfulling these requirements is certainly CD2 (5). We therefore evaluated the influence of CD43 ligation on CD2 function and could indeed show that cross-linkage of CD43 with intact CD43 mAb 6F5 induces significantly upregulated adhesiveness of CD2 for its ligand LFA-3 (CD58). The adhesive state of CD2 thus seems to be under regulatory control of the T cell surface molecule CD43.

Given the evidence for a signal-receiving and -transmitting function of CD43, the question of course arises, what is the nature of the presumed CD43 ligand on target cells? Is it perhaps MHC-I, shown by us to play a critical role on the target cell side? The first evidence in this direction was our observation that the anti–MHC-I mAb W6/32–caused inhibition of T cell conjugate formation can be reversed by T cell CD43 ligation with intact CD43 mAb 6F5. However, attempts to demonstrate more directly binding with MHC-I–coated beads coincubated with resting $CD43^+$ T cells were initially unsuccessful.

Having seen before a functional linkage between the two T cell surface molecules CD43 and CD2, we postulated that signaling also occurs in the reverse direction, leading to upregulated ligand binding of CD43 upon ligation of CD2. Signaling via CD2 is also a well-documented phenomenon (36, 46) and has been shown before to occur at 4°C (45). Regulated adhesiveness of CD43 has not as yet been reported. However, hyperphosphorylation of CD43 upon T cell activation was described (47). Using a stimulatory pair of CD2 mAbs, we could indeed demonstrate that ligation of CD2 on T cells induces CD43-dependent binding of MHC-I–coated beads and MHC-class I–positive HB654-SN cells, respectively.

Taken together, these findings suggest that CD43 molecules on T cells can, under certain conditions, directly interact with MHC-I on other cells. Such interaction and cross-linkage of CD43 on T cells subsequently induces upregulated CD2 adhesiveness for CD58. Crosswise engagement of CD2 with its ligands seems to activate CD43 and to upregulate its avidity for MHC-I.

Such a scenario does not occur, however, between T cells and every other MHC-I– and/or CD58-expressing cell type. Several $MHC\text{-}I^+LFA\text{-}3^+$ cells studied by us did not form stable conjugates with freshly isolated T cells. Among them are the myeloid cell lines HL-60 and RC-2a. PB monocytes and B cells, which express MHC-I and are at least weakly $CD58^+$, also do not spontaneously form T cell conjugates.

Additional criteria must obviously be fulfilled in order to allow the described interactions between CD43 and MHC-I and the consequent transregulations between CD43 and CD2 to be initiated. One such criterion might be reduced charge-based repulsion between T cells and target cells (48). The degree of cell surface molecule sialylation is a major denominator of the overall negative surface charge of human leukocytes (49, 50) and represents an important parameter for cell–cell contact (51–53).

The leukocyte-specific surface molecule leukosialin (CD43) represents one of the most highly sialylated glycoproteins known on human hemopoietic cells (50, 54–56). From its structure one can predict that it has a rod-like configuration, which protrudes much further from the surface than most other glycoproteins (56). As a result of this extended

conformation and the high degree of sialylation, CD43 is likely to contribute significantly to the overall negative surface charge of human leukocytes. As such it might represent a repulsion molecule that prevents hemopoietic cells, including T cells, to firmly associate with other cells, in particular, with other negatively charged cells. Experimental evidence for that has indeed been presented (53, 57, 58).

In the case of prominent charge-based repulsion between a T cell and an adjacent cell, CD43 molecules on T cells should not come into close and prolonged contact with potential ligand molecules on the opposing cell. MHC-I on a highly negatively charged cell should, thus, be unable to sufficiently engage and polymerize CD43 molecules on T cells to induce effective signaling.

In contrast, reduction of net cell surface charge should allow and promote interaction. Such charge reduction can, for instance, be achieved by enzymatic removal of cell surface sialic acid residues. Indeed, neuraminidase treatment of different cell types such as B cells (19, 20, 59, 60), macrophages (22), erythrocytes (51), or ICAM-1–transfected HeLa cells (53) has been shown to promote T cell adhesion. Reduced or altered sialylation might also be involved in the preferential binding of resting T cells to DC and activated B cells. Both cell types were shown to constitutively express hyposialylated cell surface molecules (20, 60–62).

The degree or nature of target cell surface sialylation seems also to play a role in our model system. md-DC, which efficiently bind T cells, show a clearly lower density of sialic acid on their surface than freshly isolated, non–T cell–binding monocytes (0.9 vs. 1.6 fM/mm^2, respectively). The B-LCL found to be superior in T cell binding (HB654-SN cells) is constitutively hyposialylated and lacks, for instance, the sialylation-dependent CD43 epitope recognized by mAb 6F5 (Majdic, O., unpublished observation). The other B-LCLs tested (XX224 and OTMA), normally sialylated, showed only moderate spontaneous T cell binding before and strongly upregulated binding after neuraminidase treatment (OTMA from 7 ± 4% to 26 ± 9%, XX224 from 13 ± 6% to 54 ± 7%).

Taken together, our findings show that certain cell types, in particular, potent accessory cells such as md-DC and lymphoblastoid B cells, have the capacity to interact with resting T cells via surface molecules MHC-I and CD43, respectively. We have shown this interaction to occur in a model system and have analyzed its functional consequences. The conditions we had to use to clearly visualize the individual steps in this process are certainly not physiological. We nevertheless believe that the demonstrated CD43/MHC-I adhesion pathway and its functional interaction with the well-established CD2/LFA-3 system might also under in vivo conditions play a potentially important role in T cell activation and/or mediation of effector functions.

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