CD43 Diminishes Susceptibility to T Lymphocyte-Mediated Cytolysis[1]

Thomas A. McFarland, Blair Ardman, N. Manjunath, Jessica A. Fabry, and Judy Lieberman[2]

Department of Medicine, Division of Hematology-Oncology, New England Medical Center Hospitals, Boston, MA 02111

CD43 is a major membrane sialoglycoprotein expressed by cells of hematopoietic origin. One property of CD43 is its ability to interfere with heterotypic and homotypic cellular adhesion. To determine whether CD43 expression can affect cell functions requiring intercellular adhesion, we compared a CD43-positive human T cell line (CEM) and its CD43-negative counterpart derived by gene targeting for susceptibility to cell-mediated lysis. CD43-negative CEM cells were more susceptible than CD43-positive cells to lysis by allospecific T cell lines derived from several donors. Induction of CD43 expression on transfected HeLa cells also imparted resistance to lectin-mediated lysis by a $CD8^+$ T cell clone. The effect of CD43 expression on reducing susceptibility to lysis was more pronounced in short-term cytotoxicity assays and tended to disappear as the time of contact between the effector cell and its target increased. The enhanced susceptibility of CD43-negative cells to lysis was not associated with increased expression of adhesion molecules known to mediate antigen-independent cellular adhesion. Sialic acid residues on CD43 contributed to the CD43 protective effect. These results suggest that either diminished CD43 expression or incomplete sialylation may render hematopoietic cells more susceptible to T lymphocyte-mediated cytolysis. *The Journal of Immunology,* 1995, 154: 1097–1104.

Susceptibility to lysis by T lymphocytes is enhanced by the interaction of Ag-independent adhesion receptors on effector cells with their corresponding counter-receptors on target cells (1). Cell surface proteins that interfere with the engagement of receptor-counter-receptor pairs could diminish cell susceptibility to lysis. CD43 (sialophorin or leukosialin), a cell surface sialoglycoprotein expressed by virtually all hematopoietic cells (2, 3), has structural characteristics that suggest it might interfere with intercellular adhesion. The extracellular domain of CD43 in humans and rodents is heavily *O*-glycosylated and sialylated (4–7), features that are predicted to result in a negatively charged, unfolded structure (8). Moreover, the extracellular domain of rat CD43 has been shown by electron microscopy to extend 45 nm from the cell surface (9), a distance greater than that predicted for intermembrane engagement of cell adhesion counter-receptors, such as CD2 and LFA-3 (<13 nm) or LFA-1 and ICAM-1[3] (<36 nm) (10).

Because heterotypic adhesion between effector and target cells is an early event in T lymphocyte-mediated cytolysis, interference with adhesion would be expected to inhibit cytolysis. Previous observations by us suggest that CD43 inhibits cellular adhesion. Elimination of CD43 expression in the human T cell line CEM by gene targeting enhances its homotypic adhesion (11). Conversely, induction of CD43 expression by transfection of HeLa cells diminishes their heterotypic adhesion to T cells (12). For this latter effect, sialic acid residues of CD43 were shown to be important. These data suggest that diminished CD43 expression or sialylation on target cells would increase their susceptibility to T lymphocyte-mediated cytolysis. However, our data that suggest a predominantly anti-adhesive role for CD43 are controversial. Evidence from Rosenstein et al. (13) actually suggests the reverse, i.e., that CD43 promotes heterotypic adhesion of cells by binding to ICAM-1. As CD43 is expressed on all T lymphocytes (2, 3) and ICAM-1 is expressed on a wide variety of cells

Received for publication June 17, 1994. Accepted for publication October 27, 1994.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by Grants AI33180 (B.A.) and CA01449 and AI30926 (J.L.) from the National Institutes of Health, a Pew Scholar Award (J.L.), and a grant from Biosource Genetics Corporation, Vacaville, CA (B.A.). T.A.M. is supported by Physician-Scientist Award AI01069 from the National Institutes of Health.

[2] Address correspondence and reprint requests to Dr. Judy Lieberman, New England Medical Center Hospitals, 750 Washington St., Box 245, Boston, MA 02111.

[3] Abbreviations used in this paper: ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated Ag.

0022-1767/95/$02.00

(10), CD43 binding to ICAM-1 might be expected to facilitate, rather than inhibit, formation of effector-target cell conjugates, thus leading to enhanced cytolysis.

In the present study we compared the susceptibility to cytolysis of genetically altered targets that differed in their CD43 expression. CEM cells, which normally express CD43, were rendered CD43-negative by gene targeting, and HeLa cells, which normally do not express CD43, were stably transfected to express levels of CD43 comparable with that in hematopoietic cells. We observed that CD43 expression by target cells makes them more resistant to T cell-mediated cytolysis and that sialic acid residues contribute to this resistance. These results suggest that the predominant effect of CD43 expression is to interfere with heterotypic cellular interaction.

Materials and Methods

Cell lines

The CD43-positive CEM cell line A3.01 was obtained from the AIDS Research and Reference Reagent Program. A CD43-negative CEM subline was derived from the A3.01 CEM cells by gene targeting and negative immunophenotypic selection as previously described (11). Stable CD43-positive and -negative HeLa cell transfectants were generated respectively by co-transfection with the CDM8-CD43 and pSV2neo plasmids or by transfection with the pSV2neo plasmid alone, as described previously (12). The CD43-positive and -negative CEM cell lines and the HeLa cell transfectants were maintained in RPMI 1640 media supplemented with 10% FCS (HyClone, Logan, UT), 2 mM L-glutamine, 100 mg/ml penicillin and streptomycin, and 10 mM HEPES). Anti-CEM human T cell lines were generated from PBMC obtained by Ficoll-Hypaque density centrifugation of heparinized blood from normal donors, obtained after informed consent. PBMC were cultured at 5×10^5 cells/ml with irradiated (4000 centiGray) CEM cells at a ratio of 3:1 in T cell medium with 15% FCS and 200 U/ml recombinant human IL-2 (Cetus Corp., Emeryville, CA). T cell lines were fed biweekly for up to 6 wk without restimulation. JL89 is an IL-2-dependent, human $CD3^+$, $CD8^+$, TCR^+ T cell clone the target specificity of which is unknown. It has been demonstrated to be clonal by the presence of a single rearranged TCR β-chain on Southern blot (unpublished data).

mAbs

mAbs to CD3, CD4, CD16, CD20, CD43 (anti-Leu 22), CD56, CD57, and HLA-DR were purchased from Becton Dickinson (Mountain View, CA). mAbs to CD43 (L10, 84–3C1), β1-integrin subunit (4B4), CD45 (GAP 8.3), ICAM-1 (RR1/1), ICAM-2 (CBR1C2/2), ICAM-3 (CBR1C3/3), β_2-integrin subunit (TS1/18), LFA-1 (TS1/22), CD2 (TS2/18), LFA-3 (TS2/9) and integrins $\alpha_1\beta_1$ (TS2/7), $\alpha_2\beta_1$ (5E8), $\alpha_3\beta_1$ (J143), $\alpha_4\beta_1$(B-5G10), and $\alpha_5\beta_1$ (16) have been described previously (3, 14–21).

Cytotoxicity assay

Effector T cells were added in 100 μl of T cell media to triplicate wells of U-bottom microtiter plates. Target cells were labeled with 200 μCi of $Na_2{}^{51}CrO_4$ at 37°C for 1 h and washed before adding 5×10^3 to 10×10^3 cells/well in 100 μl of media to microtiter wells. After incubation at 37°C for 20 min, 40 min, or 4 h, the plates were centrifuged for 5 min at $600 \times g$ and 75 μl of supernatant was counted in a gamma counter. For inhibition assays, effector cells were preincubated with Ab for 30 min at room temperature (final concentration of 1:100 for ascites or 10 μg/ml for purified Ab) and added without washing to the corresponding target cells. For lectin-mediated cytotoxicity assays, Con A (5 μg/ml, final concentration) was added to each well.

Neuraminidase treatment

Radiolabeled target cells were pelleted and resuspended in 200 μl of RPMI 1640 media with 0.1 U of neuraminidase (CalBiochem, La Jolla, CA) and incubated for 1 h at 37°C. Control cells were incubated with media in the absence of neuraminidase. The cells were then washed three times before using as targets in the cytotoxicity assay.

Immunophenotyping

For indirect immunofluorescence, cells (2.5×10^5 to 5×10^5) were incubated for 30 min at 4°C with 50 μl of purified Ab or ascites diluted 1:100 in PBS containing 1% BSA and 0.02% sodium azide (staining buffer). After washing twice in staining buffer, the cells were incubated for 30 min at 4°C with 50 μl of FITC-conjugated goat anti-mouse IgG $F(ab')_2$ (Tago, Burlingame, CA) diluted 1:60 in staining buffer. For direct immunofluorescence, cells were incubated as above with FITC- or phycoerythrin-conjugated Abs diluted 1:10 in staining buffer. Cells were washed twice in staining buffer, fixed in 0.37% formalin and analyzed on an EPICS 541 flow cytometer (Coulter, Hialeah, FL). Normal mouse serum diluted 1:100 or an isotype-matched Ig served as negative controls for indirect and direct staining respectively.

Cell depletion

Depletion of $CD4^+$ or $CD8^+$ T cell subsets or both from an anti-CEM T cell line was accomplished by co-incubating cells with immunomagnetic beads coated with either mouse anti-human CD4 or anti-CD8 mAbs (Dynal, Great Neck, NY) with a bead-to-cell ratio of 3:1 at 4°C for 30 min. Two serial bead/cell co-incubations were performed for each depletion experiment and the efficacy of the depletion was verified by flow cytometry. Depleted T cell subsets constituted less than 0.5% of resultant cell lines tested subsequently in cytotoxicity assays.

Results

Anti-CEM effector T cell lines

Irradiated CEM cells ($CD43^+$), rather than the CD43-negative CEM subline, were used to generate allospecific T cell lines to insure that none would recognize determinants possibly unique to the CD43-negative CEM cells. Allospecific T cell lines were used in cytotoxicity experiments after 3 to 5 wk in culture. Two representative lines were immunophenotyped (Table I). Both lines contained greater than 90% $CD2^+$, $CD3^+$ cells that were predominantly $CD4^+$.

Effect of eliminating CD43 expression on CEM cell susceptibility to cytolysis

The parental CD43-positive CEM cell line and its CD43-negative counterpart derived by gene targeting were tested for their susceptibility to lysis by an anti-CEM T cell line derived from a normal donor in ^{51}Cr release assays of 20

Table I. *Phenotype of anti-CEM T cell lines from two individuals*

		% Positive Cells	
Ag	Ab	Cell line E	Cell line H1
CD2	Leu 5b	100	99
CD3	Leu 4	96	93
CD4	Leu 3a	91	69
CD8	Leu 2a	9	37
CD16	Leu 11c	1	3
CD20	Leu 16	0	0

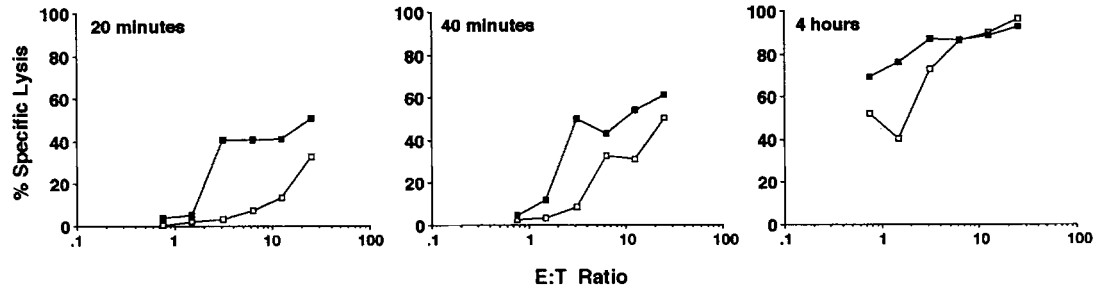

FIGURE 1. Cytolysis of CEM cells by an anti-CEM T cell line. Susceptibility of CD43-positive (open squares) and -negative (solid squares) CEM cells to cytolysis by an anti-CEM T cell line was tested in a 20-min, 40-min, and 4-h cytotoxicity assay at varying E:T cell ratios.

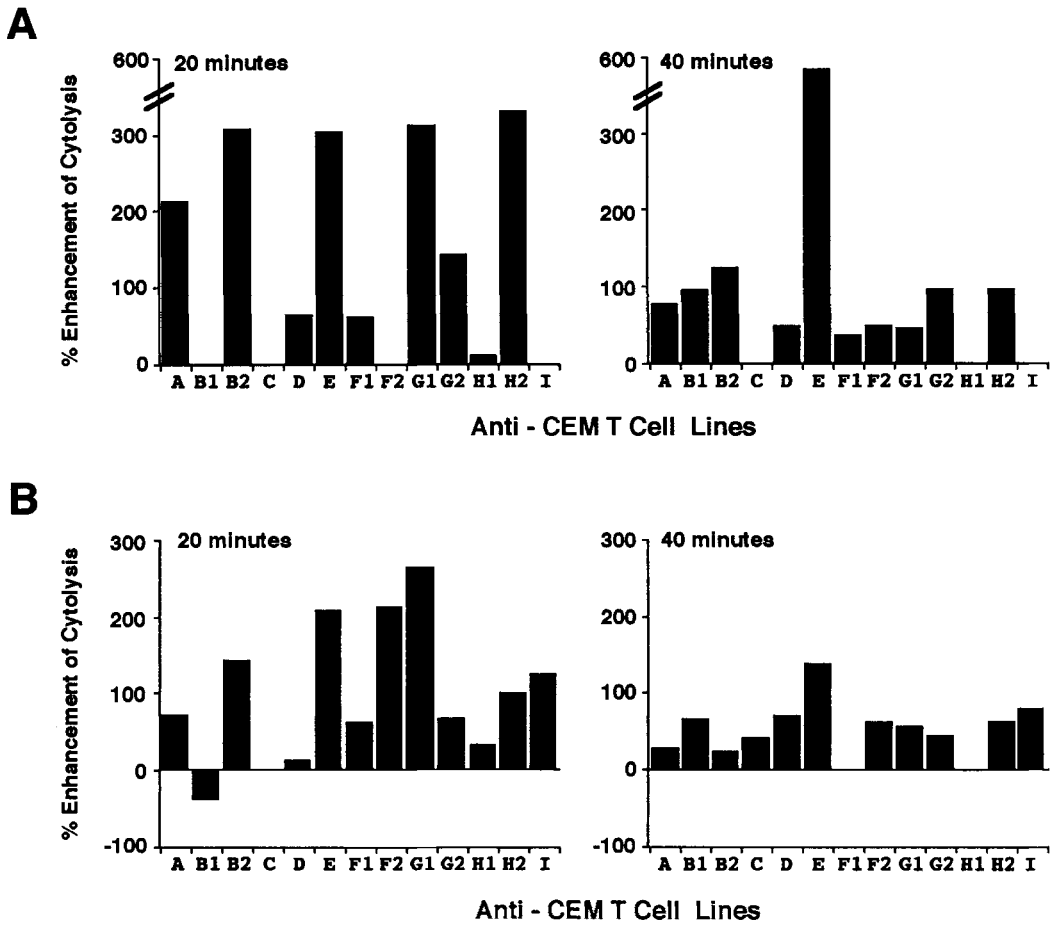

FIGURE 2. Enhanced susceptibility of CD43-negative CEM cells to cytolysis by different anti-CEM T cell lines. CD43-positive and -negative CEM cells were tested for susceptibility to cytolysis by 13 different anti-CEM T cell lines (A through I) in 20-min and 40-min cytotoxicity assays at an E:T ratio of 12.5:1 (*A*) and 25:1 (*B*). Each bar represents the percent specific lysis of the CD43-negative cells divided by the percent specific cytolysis of the CD43-positive cells multiplied by 100. All assays were performed in triplicate.

min, 40 min, and 4 h duration. At 20 and 40 min, the CD43-negative targets were more susceptible to lysis than the CD43-positive cells at several E:T cell ratios (Fig. 1). The difference in susceptibility tended to disappear at the highest E:T ratios by 4 h. These data suggest that CD43 expression made cells resistant to cytolysis, but this effect was reduced when the effector and target cells were in contact longer.

Table II. *Phenotype of CD43-positive and -negative CEM cells*

Ag	Ab[b]	Specific Linear Fluoresence Intensity[a] CD43-Positive	CD43-Negative
CD43	Leu 22	82	0
CD43	L10	176	0
CD45	GAP 8.3	390	350
CD3	Leu 4	0	0
CD4	Leu 3a	20	19
CD8	Leu 2a	0	0
HLA-DR	L234	0	0
ICAM-1	RR1/1	9	8
ICAM-2	CBR1C2/2	68	62
ICAM-3	CBR1C3/3	115	70
β_2 integrin subunit	TS1/18	26	9
LFA-1	TS1/22	29	10
LFA-2	TS2/18	0	0
LFA-3	TS2/9	36	41
β_1 integrin subunit	A1-A5	62	39
$\alpha_1\beta_1$	TS2/7	6	2
$\alpha_2\beta_1$	5E8	3	2
$\alpha_3\beta_1$	J143	10	13
$\alpha_4\beta_1$	B-5G10	46	18
$\alpha_5\beta_1$	16	5	4

[a] Fluorescence of cells after subtraction of background fluorescence observed with either normal mouse serum or isotype control antibody.
[b] Leu 4, Leu 3a, Leu 2a, and clone L234 were used as directly conjugated Abs. Binding of all other mAbs was detected with a FITC-conjugated, goat anti-mouse IgG.

To verify this result, a total of 13 anti-CEM T cell lines derived from 9 different donors were tested for their cytolytic activity against the CD43-positive and -negative targets. At an E:T ratio of 12.5:1, 9 of 13 CTL lines at 20 min and 10 of 13 lines at 40 min demonstrated enhanced lysis of the CD43-negative targets (Fig. 2*A*). Analysis of all CTL lines at this E:T ratio showed that the mean percent specific cytolysis (MPSC) of the CD43-negative CEM cells was almost twice that of the CD43-positive targets at 20 min (18.3 ± 3.9% vs 9.4 ± 2.1%) and was 62% greater than that of the CD43-positive targets at 40 min (26.5 ± 4.9 vs 16.4 ± 3.3%). The degree of enhancement of cytolysis of CD43-negative compared with CD43-positive targets correlated inversely with the percent specific lysis of the CD43-positive targets (correlation coefficient = −0.623 at an E:T ratio of 12.5:1) and tended to be greatest when the percent specific lysis of the CD43-positive cells was less than 15%.

At an E:T ratio of 25:1, 11 of 13 T cell lines at 20 min and at 40 min demonstrated enhanced cytotoxicity against the CD43-negative targets (Fig. 2*B*). At this E:T ratio, the MPSC of the CD43-negative CEM cells was 54% greater than that of the CD43-positive CEM cells at 20 min (25.1 ± 4.2% vs 16.3 ± 3.1%) and was 35% greater than that of the CD43-positive cells at 40 min (37.3 ± 4.9 vs 27.7 ± 4.7%). At 4 h, the MPSC of the CD43-negative targets at E:T ratios of 12.5:1 and 25:1 was similar to that of the CD43-positive CEM cells (not shown). These data indicate that the CD43-negative target cells were more susceptible to cell-mediated lysis and that the effect was diminished with increased duration of cell co-incubation.

Table III. *Cytotoxicity of T cell subset-depleted effector cell line B2*

Cell Line	Surface Phenotype CD4	CD8	CD16	% Specific Cytolysis of CEM Targets[a] CD43-negative	CD43-positive
Unmanipulated	65	23	8	29	12
CD4-Depleted	<1	74	13	36	29
CD8-Depleted	90	<1	4	12	7
CD4/8-Depleted	<1	<1	55	31	21

[a] Cytotoxicity was measured in a 40-min ^{51}Cr release assay at an E:T ratio of 25:1.

CD43 expression diminishes susceptibility to cytolysis by different effector T cell subpopulations

Although the anti-CEM T cell lines used in this study are predominantly $CD4^+$ (Table I, data not shown), the lack of Class II MHC cell expression on the CEM cell targets (Table II) suggested that their cytolysis was secondary to TCR-specific CD8 and/or nonspecific NK and NK-like killing. To identify cell subpopulation(s) capable of mediating CEM cell cytolysis in these heterogeneous anti-CEM T cell lines and to determine whether CD43 expression inhibited susceptibility to cytolysis by both specific and nonspecific effectors, we studied one anti-CEM T cell line (B2) in more detail. Line B2 is predominantly $CD4^+$ (65%) but also contains 23% $CD8^+$ and 8% $CD16^+$ $CD4^-CD8^-$ subpopulations. Enriched subpopulations of B2 effector cells were isolated by immunomagnetic bead depletion of CD4 and/or CD8-bearing cells. All of the selected B2 lines lysed the CD43-negative CEM targets better than the CD43-positive targets (Table III). As expected, the CD8-depleted, predominantly CD4-expressing cell line had markedly reduced cytolytic activity. The residual cytolysis by this line may have been due to the NK or NK-like activity by the small subset of $CD16^+CD4^-CD8^-$ cells.

Adhesion molecule expression and involvement in cytolysis

To determine whether the enhanced susceptibility of the CD43-negative CEM subline to T cell-mediated lysis could be due to factors other than the absence of CD43 expression, we compared the expression of known cell surface adhesion molecules and other relevant Ags in the CD43-positive and -negative CEM cells. For all Ags examined, the CD43-negative CEM cells expressed levels that were either comparable with or less than those expressed by the CD43-positive CEM cell line (Table II, Fig. 3). These data indicate that the enhanced susceptibility of the CD43-negative targets did not result from increased expression of adhesion molecules. Rather, the data support

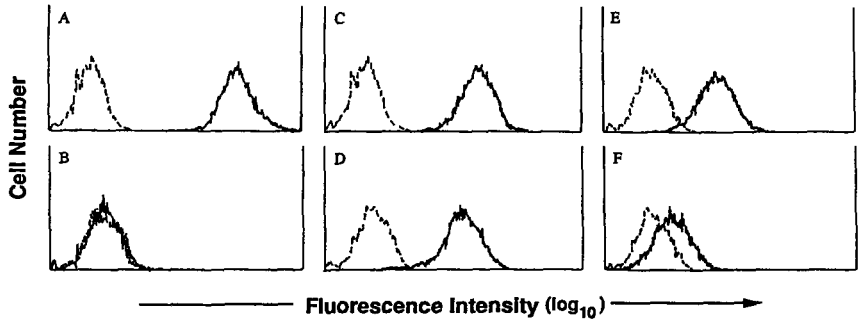

FIGURE 3. Sample flow cytometry histograms of CD43-positive (*panels A, C,* and *F*) and CD43-negative (*panels B, D,* and *F*) CEM cells stained with mAbs to CD43 (mAb L10; *A* and *B*); β_1 integrin subunit (mAb A1-A5; *C* and *D*); and β_2 integrin subunit (mAb TS1/18; *E* and *F*). The hatched lines represent background fluorescence obtained with normal mouse serum staining.

Table IV. *Inhibition of cytolysis by mAbs*

Ag	Ab	% Inhibition of CEM Cell Lysis	
		CD43-positive	CD43-negative
LFA-1	TS1/22	65.4	47.1
β_2 integrin subunit	TS1/18	58.0	54.7
CD2	TS2/18	2.0	0
ICAM-1	RR1/1	2.2	3.3
LFA-3	TS2/9	1.3	8.0

the notion that CD43 expression was responsible for the interference with cytolysis by significantly inhibiting heterotypic adhesion.

We also examined whether the difference in susceptibility to T cell-mediated lysis could have resulted from different adhesion pathways facilitating the binding of the anti-CEM T cell lines to the CD43-positive and -negative cells. Because intercellular adhesion mediated by LFA-1 (CD11a) and CD2 is known to enhance cytolysis (1), the cytoxicity assay was performed in the presence of blocking Abs to LFA-1 or CD2. Preincubation of anti-CEM T cell lines with the anti-LFA-1 mAb TS1/22 resulted in a moderately greater reduction in lysis of the CD43-positive cells than the CD43-negative ones (65 vs 47%, respectively; Table IV). A mAb (TS1/18) against the β_2 integrin subunit of LFA-1 showed a similar inhibitory effect on cytolysis of the CD43-positive and -negative cells (58 vs 55% respectively), confirming the importance of LFA-1 binding for cytolysis of both CD43-positive and -negative CEM cells. Preincubation of the effector cells with a blocking Ab to CD2 (TS2/18) did not inhibit lysis of either the CD43-positive or -negative targets. The relatively equivalent contribution of LFA-1-mediated adhesion to the cytolysis of the wild-type and gene-targeted CEM cells suggests that the enhanced lysis of the CD43-negative targets did not result from unique adhesive interactions.

Effect of CD43 expression on susceptibility of nonhematopoietic cells to cytolysis

To determine whether the resistance of CD43-expressing targets to T cell-mediated lysis was restricted to hematopoietic cells, we compared the susceptibility of CD43-positive and -negative HeLa cell transfectants for their susceptibility to Con A-mediated lysis by the $CD3^+$ $CD8^+$ TCR^+ human T cell clone JL89. We have previously shown that these stable CD43-positive and -negative HeLa cell transfectants express comparable amounts of ICAM-1 and that the binding of JL89 to both HeLa cell lines is predominantly mediated by LFA-1 (11). At 40 min, no significant killing by JL89 of either HeLa cell transfectant was detected (data not shown). At 4 h, however, the CD43-positive HeLa cells were substantially less susceptible to lysis than the CD43-negative HeLa cells at all E:T ratios tested (3.7- to 3.9-fold reduction in percent specific lysis) (Fig. 4). These data demonstrate that CD43 expression alone is sufficient to diminish susceptibility to T cell-mediated lysis and that its effect is not cell lineage restricted.

Effect of sialic acid residues on CD43-related resistance to cytolysis

We have observed previously that sialic acid residues on CD43 contribute to its anti-adhesive effect (11). To determine whether sialic acid residues of CD43 contributed to its protection of target cells from cytolysis, we tested HeLa cell transfectants treated with neuraminidase for their susceptibility to lysis by the JL89 CTL clone. Removal of sialic acid residues was verified by the complete elimination of the sialic acid-dependent CD43 epitope Leu 22 (data not shown). Neuraminidase treatment of the CD43-positive cells rendered them significantly more susceptible to lysis than the untreated CD43-positive cells (2.3-fold increase; Fig. 5). By contrast, neuraminidase treatment of the CD43-negative cells showed little effect. These data indicate that sialic acid residues on CD43 contribute substantially to its ability to confer resistance to lysis.

Discussion

The principal finding of this study is that CD43 expression by human cells renders them relatively resistant to T cell-mediated lysis. The effect of CD43 expression was observed with a cell line that normally expresses CD43 and with one in which CD43 expression was stably induced by

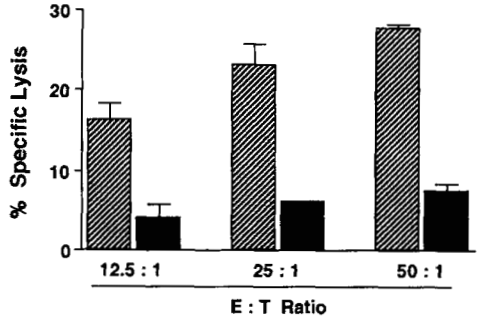

FIGURE 4. Cytolysis of HeLa cell transfectants by an allospecific T cell clone. Cytolysis of CD43-negative (hatched bars) and -positive (solid bars) HeLa cells by the JL89 T cell clone in a Con A-mediated, 4-h cytotoxicity assay.

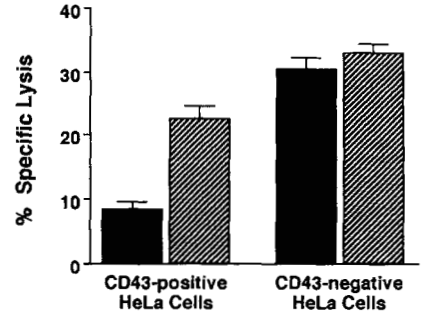

FIGURE 5. Effect of neuraminidase treatment on HeLa cell cytolysis. Untreated (solid bars) or neuraminidase-treated (hatched bars) HeLa cell transfectants were tested for susceptibility to cytolysis by the JL89 T cell clone at an E:T ratio of 50:1 under conditions described in Figure 4 legend.

transfection. The effect was observed with allospecific effector T cell lines derived from different individuals and with a $CD3^+$, $CD8^+$, TCR^+ human CTL clone. Although many of the allospecific anti-CEM T cell lines used in this study contained a high percentage of $CD4^+$ cells (Table I and III; data not shown), the negligible effect of depleting $CD4^+$ cells from an effector cell line on its cytoxicity (Table III) and the lack of HLA-DR expression on the surface of the CEM cells (Table II) suggest that the $CD4^+$ cells did not contribute significantly to cytolysis of the CEM cells. Susceptibility to both $CD8^+$ TCR-mediated cytolysis and $CD8^-$ NK or NK-like cytolysis appear to be inhibited by CD43 expression on targets. The anti-CEM effector T cell lines consistently killed the CD43-negative targets more efficiently even though they were generated against CD43-positive targets.

The resistance to cell lysis was related specifically to CD43 expression and this effect was observed in target cells of both hematopoietic and epithelial lineage. Both gene targeting to eliminate CD43 expression and stable transfection to induce CD43 expression demonstrated that CD43 expression on target cells made them relatively resistant to lysis. The CD43-negative CEM cells expressed equivalent or diminished levels of cellular adhesion molecules compared with their CD43-positive counterparts, eliminating the possibility that increased expression of these molecules by the CD43-negative targets enhanced their susceptibility to lysis. Moreover, the enhanced lysis of CD43-deficient cells was not secondary to their binding of effector cell adhesion molecules different from those bound by the CD43-positive cells. LFA-1 appeared to facilitate an equivalent degree of cytolysis of the CD43-positive and -negative CEM cells whereas CD2 facilitated virtually none. We have previously shown that adhesion of the JL89 T cell clone to both the CD43-positive and -negative HeLa cells is largely LFA-1-mediated (12). Taken together, these data suggest that the enhanced susceptiblity to lysis of CD43-negative cells resulted only from their lack of CD43 expression.

We have shown earlier that sialic acid residues on CD43 contribute to, but are not wholly responsible for, its antiadhesive effect (12). In the present experiments, neuraminidase treatment of CD43-positive HeLa cell transfectants significantly increased their sensitivity to lysis. These data suggest that inhibiting adhesion and conferring resistance to lysis are related functions and that they are properties of the CD43 extracellular domain. Two observations with granulocytes also support the notion that the extracellular CD43 domain is functionally important for limiting interactions of cells. Rieu et al. (22) found an association between proteolytic-induced shedding of CD43 triggered by phorbol myristate treatment and a rapid induction of granulocyte aggregation. Nathan et al. (23) showed a correlation between TNF-α-induced down-regulation (proteolysis) of CD43 and increased granulocyte spreading on serum precoated surfaces.

CD43 is one of several cell surface mucins naturally expressed by mammalian cells, all of which have similar features, i.e., they exist as large, extended molecules and contain a substantial amount of *O*-linked glycans (24). The epithelial cell mucin episialin (MUC1) previously has been shown to diminish susceptibility to cytolysis when expressed by a transfected melanoma cell line (25). In tumor cells, however, the level of MUC1 expression is augmented severalfold compared with levels seen in normal tissue (26–28). By contrast, the level of CD43 expression on the CEM cells and HeLa cells used in our experiments is comparable with that on normal hematopoietic cells (11, 12). These data suggest that naturally occurring levels of CD43 on lymphocytes and other hematopoietic cells may affect their in vivo susceptibility to cytolysis.

It is plausible that alterations in CD43 expression on effector cells also could have functional consequences. Although conditions that specifically diminish CD43 expression on activated T cells have not been described, activated cells do express a CD43 isoform that contains a branched, *O*-linked hexasaccharide that increases its molecular mass significantly and is not contained in CD43 on

resting lymphocytes (29). Such a substantial post-translational modification might result in an altered adhesive phenotype without a significant quantitative change in surface CD43 expression. CD43 also has been reported to provide co-stimulatory signals that optimize T cell activation (30). This finding suggests that qualitative or quantitative changes in CD43 expression might influence T cell responses to Ag, such as activation-induced up-regulation of integrin expression (1, 21). Although not strictly analogous, the CD43-negative CEM cell line used in this study was found to express lower levels of the β_1 and β_2 integrin chains than its nontargeted, CD43-positive counterpart (Table II).

In contrast to our results that suggest CD43 is an impediment to cell adhesion, Rosenstein et al. (13) have demonstrated that when expressed by a murine hybridoma, human CD43 can mediate cell binding to ICAM-1. We have not observed CD43-mediated binding of CEM cells to ICAM-1. The CD43-positive and -negative CEM cells used in our study express low levels of LFA-1 and ICAM-1 (Table II) and both bind negligibly to ICAM-1 (11; unpublished data). The low level of ICAM-1 expression by the CD43-positive and -negative cells, the inability of the anti-ICAM-1 Ab RR1/1 to block cytolysis (Table IV) and the diminished susceptibility of the CD43-positive cells to cytolysis all suggest that potential CD43-ICAM-1 interactions did not promote cytolysis in our experiments.

Previous clinical studies have implicated CD43 in two unrelated immunodeficiency syndromes. Decreased or abnormal CD43 expression on T lymphocytes has been observed in the Wiskott-Aldrich syndrome (14, 31), an X-linked immunodeficiency associated in part with progressive T cell depletion and opportunistic infections (32). IgG autoantibodies against a partially sialylated CD43 form naturally expressed on thymocytes have been detected in a large fraction of HIV-1 seropositive individuals (33). Although the specific consequences of these CD43-related clinical abnormalities remain uncertain, in vitro studies suggest that both may result in perturbations in lymphocyte adhesiveness. Decreased CD43 expression can result in increased lymphocyte adhesion (11) and anti-CD43 Abs can trigger lymphocyte and monocyte adhesion (34, 35). The results of the present study provide additional evidence that CD43 is involved in the regulation of cell adhesion and suggest a novel function for this cell surface glycoprotein that is expressed on hematopoietic cells.

Acknowledgments

We thank E. Remold O'Donnell, M. Hemler, T. A. Springer, F. Lozano, and M. Streuli for providing several mAbs used in this study, and M. Perales for assistance with flow cytometry experiments.

References

1. Springer, T. A., M. L. Dustin, T. K. Kishimoto, and S. D. Marlin. 1987. The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. *Annu. Rev. Immunol. 5:223.*
2. Remold-O'Donnell, E., C. Zimmerman, D. Kenney, and F. S. Rosen. 1987. Expression on blood cells of sialophorin, the surface glycoprotein that is defective in Wiskott-Aldrich syndrome. *Blood 70:104.*
3. Borche, L., F. Lozano, R. Viella, and J. Vives. 1987. CD43 monoclonal antibodies recognize the large sialoglycoprotein of human leukocytes. *Eur. J. Immunol. 17:1523.*
4. Gahmberg, C. G., P. Hayry, and L. C. Andersson. 1976. Characterization of surface glycoproteins of mouse cells. *J. Cell. Biol. 68:642.*
5. Brown, W. R. A., A. N. Barclay, C. A. Sunderland, and A. F. Williams. 1981. Identification of a glycophorin-like molecule at the cell surface of rat thymocytes. *Nature 289:456.*
6. Remold-O'Donnell, E., A. E. Davis III, D. Kenney, K. R. Bhaskar, and F. S. Rosen. 1986. Purification and chemical composition of gpL115, the human lymphocyte surface sialoglycoprotein that is defective in Wiskott-Aldrich syndrome. *J. Biol. Chem. 261:7526.*
7. Carlsson, S. R., and M. Fukuda. 1986. Isolation and characterization of leukosialin, a major sialoglycoprotein on human leukocytes. *J. Biol. Chem. 261:12779.*
8. Jentoft, N. 1990. Why are proteins *O*-glycosylated? *Trends Biochem. Sci. 15:291.*
9. Cyster, J. G., D. M. Shotton, and A. F. Williams. 1991. The dimensions of the T lymphocyte glycoprotein leukosialin and identification of linear protein epitopes that can be modified by glycosylation. *EMBO J. 10:893.*
10. Springer, T. A. 1990. Adhesion receptors of the immune system. *Nature 346:425.*
11. Manjunath, N., R. S. Johnson, D. E. Staunton, R. Pasqualini, and B. Ardman. 1993. Targeted disruption of CD43 gene enhances T lymphocyte adhesion. *J. Immunol. 151:1528.*
12. Ardman, B., M. A. Sikorski, and D. E. Staunton. 1992. CD43 interferes with T-lymphocyte adhesion. *Proc. Natl. Acad. Sci. USA 89: 5001.*
13. Rosenstein, Y., J. K. Park, W. C. Hahn, F. S. Rosen, B. E. Bierer, and S. J. Burakoff. 1991. CD43, a molecule defective in Wiskott-Aldrich syndrome, binds ICAM-1. *Nature 354:233.*
14. Remold-O-Donnell, E., D. M. Kenney, R. Parkman, L. Cairns, B. Savage, and F. S. Rosen. 1984. Characterization of a human lymphocyte surface sialoglycoprotein that is defective in Wiskott-Aldrich Syndrome. *J. Exp. Med. 159:1705.*
15. Berger, A. E., J. E. Davis, and P. Cresswell. 1981. A human leukocyte antigen identified by a monoclonal antibody. *Hum. Immunol. 3:231.*
16. Rothlein, R., M. L. Dustin, S. D. Marlin, and T. A. Springer. 1986. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J. Immunol. 137:1270.*
17. de-Fougerolles, A. R., S. A. Stacker, R. Schwarting, and T. A. Springer. 1991. Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. *J. Exp. Med. 174:253.*
18. de-Fougerolles, A. R., X. Qin, and T. A. Springer. 1994. Characterization of the function of intercellular adhesion molecule (ICAM)-3 and comparison with ICAM-1 and ICAM-2 in immune responses. *J. Exp. Med. 179:619.*
19. Sanchez-Madrid, F., J. A. Nagy, E. Robbins, P. Simon, and T. A. Springer. 1983. A human leukocyte differentiation antigen family with distinct α-subunits and a common β-subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J. Exp. Med. 158:1785.*
20. Sanchez-Madrid, F., A. M. Krensky, C. F. Ware, E. Robbins, J. L. Strominger, S. J. Burakoff, and T. A. Springer. 1982. Three distinct antigens associated with human T lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. *Proc. Natl. Acad. Sci. USA 79:7489.*
21. Hemler, M. E. 1990. VLA proteins in the integrin family: structures, functions and their role on leukocytes. *Annu. Rev. Immunol. 8:365.*
22. Rieu, P., F. Porteu, G. Bessou, P. Lesavre, and L. Halbwachs-Mecarelli. 1992. Human neutrophils release their major membrane sialoprotein, leukosialin (CD43), during cell activation. *Eur. J. Immunol. 22:3021.*
23. Nathan, C., Q.-W. Xie, L. Halbwachs-Mecarelli, and W. W. Jin. 1993. Albumin inhibits neutrophil spreading and hydrogen peroxide

release by blocking the shedding of CD43 (sialophorin, leukosialin). *J. Cell. Biol. 122:243.*

24. Carraway, K. L., and S. R. Hull. 1991. Cell surface mucin-type glycoproteins and mucin-like domains. *Glycobiology. 2:131.*
25. E. van de Wiel-van Kemenade, M. J. L. Ligtenberg, A. J. de Boer, F. Buijs, H. L. Vos, C. J. M. Melief, J. Hilkens, and C. G. Figdor. 1993. Episialin (MUC1) inhibits cytotoxic lymphocyte-target cell interaction. *J. Immunol. 151:767.*
26. Boland, C. R., C. K. Montgomery, and Y. S. Kim. 1982. Alterations in human colonic mucin occurring with cellular differentiation and malignant transformation. *Proc. Natl. Acad. Sci. USA 79:2051.*
27. Zaretsky, J. Z., M. Weiss, I. Tsarfaty, M. Hareuveni, D. H. Wreschner, and I. Keydar. 1990. Expression of genes coding for pS2, c-*erb*B2, estrogen receptor and H23 breast tumor-associated antigen. a comparative analysis in breast cancer. *FEBS Lett. 265:46.*
28. Irimura, T., D. A. Carlson, J. Price, T. Yamori, R. Giavazzi, D. M. Ota, and K. R. Cleary. 1989. Differential expression of a sialoglycoprotein with an approximate molecular weight of 900,000 on metastatic human colon carcinoma cells growing in culture and in tumor tissues. *Cancer Res. 48:2353.*
29. Piller, F., V. Piller, R. I. Fox, and M. Fukuda. 1988. Human T-lymphocyte activation is associated with changes in *O*-glycan biosynthesis. *J. Biol. Chem. 263:15146.*
30. Park, J. K., Y. J. Rosenstein, E. Remold-O'Donnell, B. E. Bierer, F. S. Rosen, and S. J. Burakoff. 1991. Enhancement of T-cell activation by the CD43 molecule whose expression is defective in Wiskott-Aldrich syndrome. *Nature 350:706.*
31. Parkman, R., E. Remold-O'Donnell, D. M. Kenney, S. Perine, and F. S. Rosen. 1981. Surface protein abnormalities in lymphocytes and platelets from patients with Wiskott-Aldrich syndrome. *Lancet ii: 1387.*
32. Rosen, F. S., M. D. Cooper, and R. J. Wedgewood. 1984. The primary immunodeficiencies. *N. Engl. J. Med. 311:300.*
33. Ardman, B., M. A. Sikorski, M. Settles, and D. E. Staunton. 1990. HIV-1-infected individuals make autoantibodies that bind to CD43 on normal thymic lymphocytes. *J. Exp. Med. 172:1151.*
34. Axelsson, B., R. Youseffi-Etemad, S. Hammarstrom, and P. Perlmann. 1988. Induction of aggregation and enhancement of proliferation and IL-2 secretion in human T cells by antibodies to CD43. *J. Immunol. 141:2912.*
35. Nong, Y. H., E. Remold-O'Donnell, T. W. LeBien, and H. G. Remold. 1989. A monoclonal antibody to sialophorin (CD43) induces homotypic adhesion and activation of human monocytes. *J. Exp. Med. 170:259.*