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Research Article

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Requirements for the Construction of Antibody Heterodimers for the Direction of Lysis of Tumors by Human T Cells

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Abstract

We constructed a series of MAb heterodimers consisting of the J5 (anti-common acute lymphoblastic leukemia antigen [CALLA]) antibody and antibodies to a variety of structures present on the surface of activated human T cells, including CD3 antigen (T cell receptor-associated glycoproteins), CD2 antigen (T11/E-rosette receptor), CD25 antigen (IL-2 receptor), and the transferrin receptor. We tested the ability of these heterodimers to direct a CD2+CD3+CD8+CD4−CD25+ transferrin receptor+ MHC-restricted human cytolytic T lymphocyte (CTL) clone to lyse a CALLA+ human tumor in vitro. Only heterodimers containing an anti-CD3 antibody or activating antibodies to CD2 could direct the clone to lyse these human tumor targets, even when the clone was additionally activated with anti-CD3 or anti-CD2 antibodies. Our findings may have implications in the design of strategies for the use of such reagents in the treatment of human neoplasia.

Introduction

Human T cells with cytolytic function or potential can be activated through a number of cell-surface structures, including the T cell receptor-T3 associated glycoprotein complex (TCR-T3, CD3 antigen) (1–4), the T11-E rosette receptor (CD2 antigen) (5, 6) and certain other structures, including T_p 103 (7), T44 (8, 9), and 2H1 (10). Activated T cells also express a variety of surface structures upon activation, including the $T11_3$ epitope (6), the transferrin receptor (6, 11), and the IL-2 receptor (6, 12–14). Recent interest has focused on using some of these antigens to direct the lysis of tumor cells by human cytolytic cells; specifically, several workers have constructed antibody heterodimers in which one end of the dimer recognized the CD3 antigen, and the other half of the dimer recognized a tumor cell surface determinant. These antibody heterodimers were shown to be able to direct activated T cells to lyse tumor targets relevant for the antitumor antibody in vitro (15–20). Such heterodimers were also shown to mediate rejection of human xenografts in nude mice when infused with human PBL (21). We also demonstrated that heterodimers composed of an antitumor antibody and certain antibodies to the CD2 antigen could mediate tumor lysis by human T cells (22). In this report, we investigate this phenomenon further by comparing the efficiency of tumor lysis by a human cytolytic T lymphocyte (CTL)[1] clone in the presence of a variety of heterodimers composed of an anti-common acute lymphoblastic leukemia antigen (CALLA) antibody and antibodies to a number of different structures on activated T cells. We find that only certain heterodimers can actually mediate this type of lysis, even when the clone is additionally activated by anti-CD3 and anti-CD2 antibodies. The implications of these findings for the design of clinically useful heterodimers are discussed.

Methods

Antibodies. The following antibodies were used in these studies: 1 OLD 24Cl (anti-$T11_2$, IgG_{2a}); 1 MONO 2Ab, (anti-$T11_3$, IgG_3); RW24B6, (anti-T3, IgG_{2b}); and 1HT4, (anti-IL-2 receptor IgG_{2a}) were developed in the Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston, MA. J5 (anti-CALLA, IgG_{2a}) was a gift of Dr. J. Ritz, Dana-Farber Cancer Institute. 5E9 (antitransferrin receptor, IgG_1) was obtained from the American Type Culture Collection, Rockville, MD (ATCC HB21). All antibodies were grown as ascites in pristane-primed Balb/c mice. Preparation and purification of all these antibodies, except 1HT4 has been described (22–25).

1HT4 was purified by diluting the ascites fluid fourfold with potassium phosphate buffer, pH 7.2, containing 0.58 M NaCl, and then applying the solution to a column of protein A-Sepharose CL-48 equilibrated in the same buffer. The column was washed with this buffer until the protein concentration in the eluate was close to zero, and then the MAb was eluted from the column with 0.1 M acetic acid containing 0.15 M NaCl. The 1HT4 antibody was then further purified by ion-exchange chromatography on a column of CM-cellulose (CM-52; Whatman Inc., Clifton, NJ) using the procedures described previously for J5 (26), except that the buffer was 20 mM sodium phosphate, pH 6.1, containing 50 mM glycine and 0.4 mM sodium azide, and the antibody was eluted with a gradient 0–350 mM NaCl.

Synthesis and purification of dimeric conjugates. The synthesis and purification of the J5-1 OLD 24Cl (J5-anti-$T11_2$), J5-1 MONO 2Ab (J5-anti-$T11_3$), and J5-RW24B6 (J5-anti-CD3) conjugates have been described in detail (22). The preparation of the J5-5E9 and J5-1HT4 heterodimers was done in a similar fashion. In brief, the 5 mg 5E9 and 1HT4 antibodies, in 100 mM sodium phosphate buffer, pH 7.0 (20 ml), containing 1 mM EDTA were treated with a 10-fold molar excess of succinimidyl 4-*N*-maleimidomethyl 1-cyclohexanecarboxylate at 30°C for 30 min. Excess reagent was then removed by gel filtration through a column of Sephadex G-100 equilibrated in the above buffer. The solution of the modified antibodies was then added to solutions containing 5 mg J5 antibody, which had been modified with 2-iminothiolane as described for the other three conjugates. After standing overnight at 4°C, 2 mM iodoacetamide was added to the reaction mixture, which then was subjected to gel filtration through a column of Bio-gel A 0.5 (Bio-Rad Laboratories, Richmond, CA) for the isolation of pure heterodimer conjugate using conditions described previously

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1. *Abbreviations used in this paper:* CALLA, common acute lymphoblastic leukemia antigen; CM-EBV, EBV-transformed lymphoblastoid cell line; CTL, cytolytic T lymphocytes; NK, natural killer.

(22). Samples from fractions eluting from the column were analyzed by polyacrylamide/dodecyl sulfate gel electrophoresis in gel slabs (145 mm × 90 mm × 0.75 mm) cast with acrylamide gradients (5–10% wt/vol). Gels were run under nonreducing conditions using previously described procedures (Fig. 1). Fractions containing purified antibody-antibody heterodimer were pooled, concentrated to ~ 0.5 mg/ml using a CX-30 immersible membrane (Millipore Continental Water Systems, Bedford, MA), and finally dialyzed into 10 mM potassium phosphate buffer, pH 7.2, containing 45 mM NaCl. Samples were stored frozen at −70°C.

Cell lines. The Namalwa cell line is derived from a human Burkitt lymphoma, and was obtained from ATCC (ATCC CRL1432). The K562 cell line is derived from an erythroleukemia and was a gift of Dr. James Griffin, Dana-Farber Cancer Institute. The allogeneic EBV-transformed lymphoblastoid cell line (CM-EBV) was derived from PBL that had been transformed with EBV. All cell lines were maintained in RPMI 1640 medium supplemented with 10% FCS, 100 μg/ml streptomycin, 100 U/ml penicillin, and 0.25 μg/ml fungizone.

The human CTL clone TB1-6 was generated from a bulk culture in which PBL were incubated for 7 d with CM-EBV, in RPMI 1640 medium supplemented with 10% human AB serum. The cells were then cloned by limiting dilution (1 cell/well) in V-bottom microtiter trays in conditioned medium. 5×10^4 cells/well irradiated (5,000 R) autologous PBL and 5×10^4 cells/well CM-EBV were added as feeders. Conditioned medium was prepared from phytohemagglutinin and phorbolester-treated PBL. The final medium consisted of RPMI 1640 with 10% conditioned medium, 20% FCS, 4 mM glutamine, and 0.05 mg/ml gentamycin. Positive wells were expanded in IL-2 containing medium with feeder cells. Fresh medium was added every 3 d; 5,000 R irradiated feeder cells (5×10^5/ml PBL + 5×10^5/ml CM-EBV) were added every 2 wk. TBI-6 is CD2+CD3+CD4−CD8+CD25+transferrin receptor+, and is cytotoxic for CM-EBV but not for other EBV-transformed lymphoblastoid lines, K562 or Namalwa, in a 4 h ^{51}Cr release assay.

Assay for cytotoxicity. The details of this assay have been published (5, 26). Briefly, 1×10^5 or 5×10^4 cloned human T cells were incubated with 5×10^3 ^{51}Cr-labeled targets for either 4 or 18 h, as indicated in the text, in a volume of 200 μl in V-bottom plastic microtiter plates in RPMI 1640 medium containing 10% FCS, antibiotics, and various reagents, as indicated in the text. After incubation, 100 μl of supernatants were harvested and assayed for released ^{51}Cr on a gamma-counter (1274 Riagamma; LKB Instruments, Gaithersburg, MD). Percent specific lysis was calculated as follows: $\%SL$ = cpm − $SR/TRC - SR$ where $\%SL$, percent specific lysis; cpm, cpm in sample; SR, spontaneous release of cpm from ^{51}Cr-labeled targets in the absence of T cells; and TRC, total number of cpm contained in 5×10^3 ^{51}Cr-labeled targets.

Cytofluorographic analysis. The details of this assay have been published (24). Briefly, 1×10^6 cells were incubated on ice for 30 min in a volume of 50 μl of HBSS containing 1% BSA and antibodies at 25 μg/ml or dimer conjugates at 50 μg/ml. After washing the cells twice in the cold, they were stained in a volume of 25 μl with goat anti-mouse Ig-FITC (Tago Inc., Burlingame, CA) on ice for 30 min. After again washing twice, cells were assayed for fluorescence intensity on an Epics C cytofluorograph (Coulter Electronics, Inc., Hialeah, FL).

Conjugate formation assay. This was done essentially as described by Schmidt et al. (27), with minor modifications. 5×10^4 TB1-6 cells and 5×10^4 Namalwa cells were admixed in the absence or presence of various heterodimers in a V-bottom plate (Falcon Labware, Becton, Dickinson & Co., Oxnard, CA) and were centrifuged (1,000 rpm for 5 min). After a 15-min incubation at 37°C, the cells were placed on ice, the pellet resuspended with a constant shear force (six cycles of a P-200 Pipetman (Gilson Co., Inc., Middleton, WI), and both the number of TB1-6 cells associated with Namalwa cells and the number of TB1-6 cells not associated with Namalwa cells were counted, and the percentage of TB1-6 forming conjugates with Namalwa was determined.

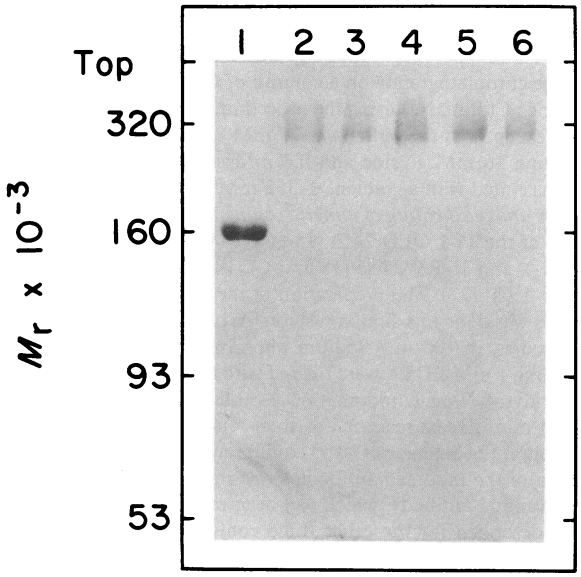

Figure 1. Analysis of the purified heterodimer antibody conjugates on a 5 to 10% (wt/vol) polyacrylamide/dodecyl sulfate gel run under nonreducing conditions. Lane *1*, antibody 5E9; lanes *2* to *6*, heterodimeric conjugates J5-anti-T11$_3$, J5-anti-T11$_2$, J5-RW24B6, J5-5E9, and J5-1HT4. The calibration of M_r was from the mobility of IgG (160,000), phosphorylase b (93,000), and glutamate dehydrogenase (53,000).

Results

We constructed a series of antibody heterodimers consisting of an anti-CD10 antibody (J5; anti-CALLA) and antibodies recognizing surface structures on activated human T cells. These were an anti-CD3 antibody (RW24B6), two anti-CD2 antibodies (1 OLD 24Cl, anti-T11$_2$ and 1 MONO 2Ab anti-T11$_3$), an anti-CD25 antibody (anti-IL-2 receptor, 1HT4), and an antibody to the transferrin receptor (5E9) (Methods, Fig. 1). We have demonstrated elsewhere (22) that the functional activity (i.e., binding) of each antibody moiety in a heterodimer could be assessed by cytofluorographic analysis; thus, we showed that the J5-anti-CD3 and the two J5-anti-CD2 heterodimers bound both the CD10+CD3−CD2−Namalwa line and the CD3+CD2+Rex line (22). We performed a similar analysis on the J5-1HT4 and J5-5E9 heterodimers; the former construct bound to both the CD10+CD25−Namalwa line and to the CD10−CD25+TB1−6 clone (Fig. 2, *e, g, k, m, n,* and *o*). The latter construct (J5-5E9) bound to both the CD10−transferrin receptor+K562 line (Fig. 2, *p, q, r*), and the CD10+transferrin receptor+Namalwa line (not shown). Additionally, the TB1-6 clone expressed CD2, CD3, CD25, and transferrin receptor (but not CD10), and bound all heterodimers to the same extent as the relevant monomeric antibodies (Fig. 2, compare *b* and *h, c* and *i, d* and *j, e* and *k, f* and *l*). Thus, all five heterodimers bound the clone through the activation structure (i.e., CD2, CD3, CD25, or transferrin receptor) and the Namalwa tumor through the anti-CD10 moiety. The J5-5E9 dimer probably also bound Namalwa through the 5E9 moiety.

We and others have demonstrated that human CTL can be directed to lyse Fc receptor-expressing murine tumors such as P388D1, in the presence of antibodies to CD3 (4, 22), and we

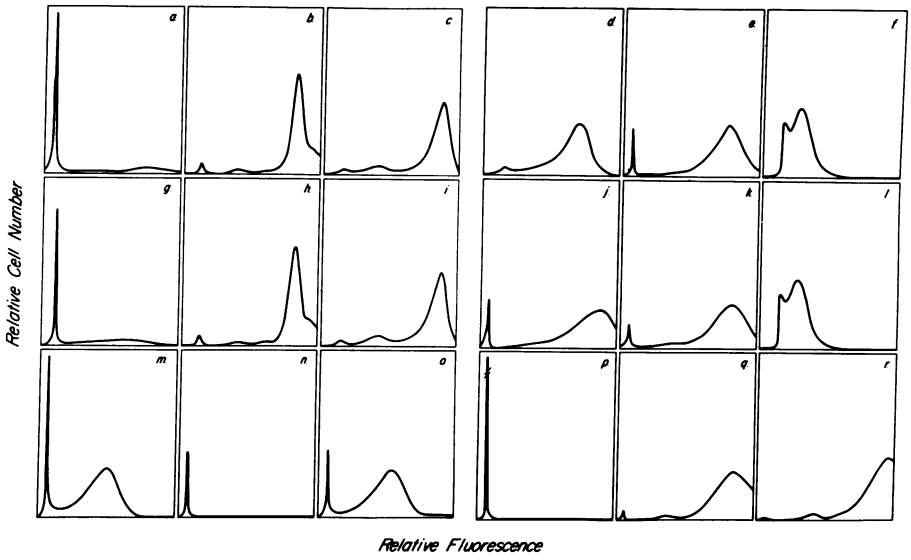

Figure 2. Cytofluorographic analysis of the binding of antibodies and heterodimers; 1 × 10^6 TB1-6 (*a–l*), Namalwa (*m–o*), or K562 cells (*p–r*) were incubated with the following reagents and then stained with GAMG-FITC, as described in Methods: (*a*) no antibody, (*b*) RW24B6, (*c*) anti-T11$_2$, (*d*) anti-T11$_3$, (*e*) 1HT4, (*f*) 5E9, (*g*) J5, (*h*) J5-RW24B6, (*i*) J5-anti-T11$_2$, (*j*) J5-anti-T11$_3$, (*k*) J5-1HT4, (*l*) J5-5E9, (*m*) J5, (*n*) 1HT4, (*o*) J5-1HT4, (*p*) J5, (*q*) 5E9, (*r*) J5-5E9.

extended these observations by demonstrating that human CTL could also lyse P388D1 in the presence of certain anti-CD2 antibodies (anti-T11$_2$ and anti-T11$_3$), but not in the presence of antibodies to such antigens as CD25, CD8, or transferrin receptor (22). We therefore wished to determine what pattern of lysis of a CD10+ tumor target would be observed when the human CTL clone TB1-6 was incubated with this tumor target in the presence of the various heterodimers described above (Table I). It was evident that while both the J5-anti-CD3 and J5-anti-CD2 heterodimers directed the clone to efficiently lyse the Namalwa target (which was not lysed in their absence), the J5-5E9 and J5-1HT4 heterodimers had no effect at the same concentration. The J5-anti-CD2 heterodimers required the presence of the reciprocal monomeric anti-CD2 antibody for optimal lysis, that is, both anti-T11$_2$ and anti-T11$_3$ are required for optimal lysis and either one can be conjugated (22). A dose response assay demonstrated that the anti-CD3 heterodimer promoted more efficient lysis of the target than the more efficient of the two anti-CD2 heterodimers, (J5–anti-T11$_3$) in a 4-h assay (Fig. 3).

A trivial explanation for the inefficiency of the J5-1HT4 and the J5-5E9 heterodimers was that the 1HT4 and 5E9 moieties in the dimers inhibited the cytolytic function of the clone by interfering with the function of the relevant receptor on the clone. If this were the case, then the lysis by TB1-6 of its appropriate target (CM-EBV) would be inhibited in their presence, or in the presence of the heterodimers with J5. This was not the case (Table II), although certain anti-CD3 antibodies could inhibit this type of lysis by the clone (Kalish, R., unpublished observations).

It was then of interest to determine if any of the heterodimers enhanced conjugate formation between the CTL and the target, and if there were any differences between them that correlated with the previous data. TB1-6 was incubated with

Table I. Lysis of CD10 + Namalwa Cells in the Presence of Anti-CD10 Heterodimers

		Exp. 1		Exp. 2		Exp. 3	
Target	Heterodimer	10:1	5:1	7.5:1	2.5:1	10:1	5:1
				% Specific release (E/T ratio)			
a. CM-EBV	—	72	66	67	58	35	33
b. Namalwa	—	0	0	15	8	3	1
c. Namalwa	J5-anti-CD3	100	94	100	92	ND	ND
d. Namalwa	J5 + anti-CD3	1	0	13	6	ND	ND
e. Namalwa	J5-anti-T11$_3$/T11$_2$	31	22	68	54	49	37
f. Namalwa	J5-anti-T11$_2$/T11$_3$	ND	ND	47	36	30	26
g. Namalwa	J5 + anti-T11$_2$ + anti-T11$_3$	21	19	29	21	28	21
h. Namalwa	J5-5E9	0	0	21	8	1	1
i. Namalwa	J5-1HT4	0	0	16	7	7	5

The human CTL clone TB1-6 was incubated at the indicated E/T ratios with 5 × 10^3 ^{51}Cr-labeled targets in the presence of the indicated antibodies or the heterodimers. The anti-T11$_3$, anti-T11$_2$, 5E9, and 1HT4 heterodimers were used at a final concentration of 10 μg/ml, with 5 μg/ml of anti-T11$_2$ or anti-T11$_3$ added to the anti-T11$_3$ and anti-T11$_2$ heterodimers, respectively. The final concentration of the anti-CD3 heterodimer was 5 μg/ml in experiment 1, and 1 μg/ml in experiment 2 and experiment 3. Antibody admixture controls were at the same final concentration as the relevant heterodimer.

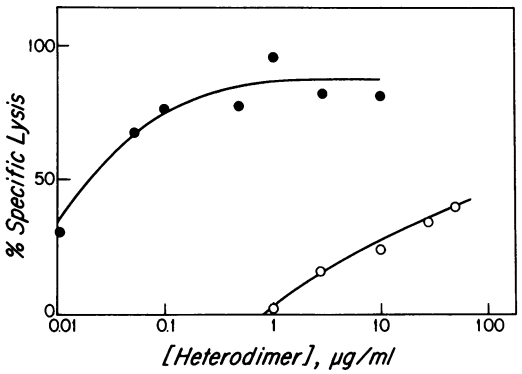

Figure 3. Comparison of efficacy of heterodimer-mediated lysis. TB1-6 was incubated for 4 h with 5×10^3 ^{51}Cr-labeled Namalwa an E/T ratio of 5:1 in the presence of the indicated concentrations of either J5-RW24B6 (●) or J5-anti-T11$_3$ (○). Anti-T11$_2$ was added at a final concentration of one-half the concentration of J5-anti-T11$_3$ to the wells containing J5-anti-T11$_3$. Percent specific lysis was calculated as described in Methods.

Namalwa alone or in the presence of the five different heterodimers; the percentage of TB1-6 cells forming conjugates with Namalwa was enumerated by light microscopic examination. Approximately 35% of these T cells formed conjugates with Namalwa under these conditions in the absence of heterodimers, and this percentage was not significantly raised by the presence of any heterodimers (not shown).

Thus, it appeared that heterodimers containing anti-CD25 or anti-transferrin receptor antibodies could not direct a human T cell clone to kill a tumor target, perhaps because the antibodies, while recognizing a marker of activation on the clone, could not trigger its cytolytic program. We therefore asked whether these heterodimers could be induced to direct lysis of the target by the clone if the clone were additionally triggered through CD3 or CD2, which are structures known to be involved in the activation of the cytolytic program of CTL clones (2, 5, and Table I). We therefore incubated the clone with anti-CD3 antibodies, and then assayed for its ability to lyse the CD10+ target in the presence of the J5-5E9, J5-1HT4,

Table II. 5E9 and 1HT4 Do Not Inhibit the Lysis of CM-EBV by TB1-6

Target	In vitro addition	5:1	2.5:1
		% *Specific release (E/T ratio)*	
CM-EBV	—	43	35
	1HT4	46	29
	5E9	43	31
	J5	46	30
	J5-1HT4	38	32
	J5-5E9	35	26
	J5 + 1HT4	35	28
	J5 + 5E9	37	29

TB1-6 was incubated at the indicated E/T ratios with 5×10^3 ^{51}Cr-labeled CM-EBV in the presence of the indicated monomeric antibodies, heterodimers, or admixtures of antibodies, all at a final concentration of 10 μg/ml.

and the two J5-anti-CD2 heterodimers (Table III). It is clear that this concentration of anti-CD3 is activating because it caused the clone to lyse the Fc receptor bearing target P388D1, (Table III, *j* and *k*), and because an equivalent concentration of anti-CD3 caused an increase in the concentration of free Ca^{2+} in the clone (not shown), although it had no effect by itself on the lysis of the Namalwa target (Table III, *b* and *c*, *m* and *n*). The results show that while the anti-CD3 antibody enhanced J5-anti-CD2 heterodimer-mediated killing (Table II, *d* and *e*, *o* and *p*), it had no effect on the lack of J5-5E9 and J5-1HT4 mediated lysis (Table III, *f* and *g*, *h* and *i*, *q* and *r*, *s* and *t*). Additionally, anti-CD2 antibodies, which by themselves activated the clone and enhanced its lysis of Namalwa (Table I, *b* and *g*; Table III, *m* and *u*) as has been reported for other human CTL clones (5), also failed to increase the lysis of Namalwa in the presence of the J5-5E9 and J5-1HT4 heterodimers (Table III, *u*, *v*, *w*).

Discussion

In this report we have investigated the phenomenon of the antibody-heterodimer–mediated lysis of the human targets by activated human T cells with specificity for nominal or alloantigen. This has been the subject of recent interest by several groups because it is hoped that such antibody heterodimers may represent novel and clinically useful reagents for the treatment of neoplasia (15–21). These reagents have the theoretical ability to recruit the host's own immune system into a tumor-specific response. Several groups have constructed antibody heterodimers with anti-CD3 antibodies and demonstrated that activated human T cells can be directed to lyse, in vitro, tumor targets that they would not normally lyse in the presence of these reagents. It was therefore of interest to determine how general this phenomenon of heterodimer-directed lysis was; that is, whether antibody heterodimers composed of antibodies recognizing other surface structures present on activated T cells could also direct the lysis of tumor targets by these activated cells. In this report we compare heterodimers recognizing four such structures: CD3 (T cell receptor-associated T3 glycoproteins), CD2 (T11-E rosette receptor), CD25 (IL-2 receptor), and the transferrin receptor, all of which are expressed on activated T cells, the latter three in a preferential fashion compared with nonactivated cells (6, 11–14). We constructed antibody heterodimers with antibodies to these structures and with an antibody to the CD10 structure (J5; anti-CALLA). We demonstrated the purity of these preparations (Fig. 1), and that these preparations bound both the effector CTL through the antibody to the activation structure and the target through the J5 moiety (Fig. 2) (22).

We found that only heterodimers containing anti-CD3 or anti-CD2 antibodies, but not heterodimers containing certain antibodies to other markers of activation, such as the IL-2 receptor (1HT4) or transferrin receptor (5E9) could mediate lysis of a tumor target by an activated T cell clone. Furthermore, it was unlikely that the 1HT4 or 5E9 containing heterodimers actually inhibited the clone by interfering with the function of these receptors, because neither antibody had any effect on the lysis by TB1-6 of its appropriate target. Moreover, 5E9 does not inhibit the binding of transferrin to its receptor and hence has no toxic effect in vitro (25). Thus, while all the heterodimers bound target and effector cells, the mere formation of an antibody bridge between the activated cytolytic cell

Table III. Effect of anti-CD3 and anti-CD2 Antibodies on the Heterodimer-mediated Lysis of CD10 + Namalwa Cells

Target	Activating antibody	Heterodimer	10:1	5:1
			% *Specific release*	
Experiment 1				
a. CM-EBV	—	—	72	66
b. Namalwa	—	—	0	0
c. Namalwa	Anti-CD3	—	0	1
d. Namalwa	—	J5 anti-$T11_3/T11_2$	31	22
e. Namalwa	Anti-CD3	J5 anti-$T11_3/T11_2$	58	60
f. Namalwa	—	J5-1HT4	0	0
g. Namalwa	Anti-CD3	J5-1HT4	3	2
h. Namalwa	—	J5-5E9	0	0
i. Namalwa	Anti-CD3	J5-5E9	0	0
j. $P338D_1$	—	—	0	1
k. $P338D_1$	Anti-CD3	—	99	86
Experiment 2			7.5:1	2.5:1
l. CM-EBV	—	—	67	58
m. Namalwa	—	—	15	8
n. Namalwa	Anti-CD3	—	13	3
o. Namalwa	—	J5-anti-$T11_2/T11_3$	47	36
p. Namalwa	Anti-CD3	J5-anti-$T11_2/T11_3$	76	63
q. Namalwa	—	J5-1HT4	16	7
r. Namalwa	Anti-CD3	J5-1HT4	10	2
s. Namalwa	—	J5-5E9	21	8
t. Namalwa	Anti-CD3	J5-5E9	17	9
u. Namalwa	Anti-$T11_2/T11_3$	—	29	26
v. Namalwa	Anti-$T11_2/T11_3$	J5-1HT4	30	26
w. Namalwa	Anti-$T11_2/T11_3$	J5-5E9	31	30

TB1-6 was incubated with 5×10^3 ^{51}Cr-labeled targets in the presence of the indicated heterodimers that were at 10 μg/ml (+5 μg/ml of anti-$T11_2$ or anti-$T11_3$, with the J5-anti-$T11_3$ and J5-anti-$T11_2$ heterodimers, respectively). In some wells, anti-CD3 (RW24B6) at 1 μg/ml or anti-$T11_2$ + anti-$T11_3$ at 5 μg/ml each were added to additionally activate the clone.

and the target was not sufficient for lysis to occur, even when the clone's cytolytic program was additionally triggered through CD2 or CD3 (Table III); the antibody bridge itself had to contain a moiety capable of additionally activating the clone in order to effect target lysis. Our data with the 5E9- and 1HT4 containing heterodimers also confirmed data with a heterodimer made with an anti–class I antibody (W6/32) that was ineffective in targeting human T cells to lyse tumor targets (19).

There is evidence that once appropriately triggered, CTL effectuate target cell lysis through the exocytosis of perforin-containing granules (28–30). If this is indeed the case, our data may suggest that exocytosis of these granules can only be directed by antibody bridges CD2 or CD3. The finding that target lysis mediated by anti-CD2 heterodimers can indeed be enhanced by monomeric anti-CD3 antibody suggests a functional linkage between the CD3 and CD2 surface structures on T cells, an observation that has been demonstrated in a variety of systems (6, 31). Finally, while we did not observe any significant enhancement in conjugate formation between the CTL and the CD10-expressing target in the presence of any of the anti-CD10 heterodimers, it has, in fact, been shown that conjugate function between CTL and targets is a process mediated by nonspecific adhesion molecules, such as LFA-1, CD8, and LFA-3, with the specificity of the CTL recognition and activation demonstrable only by measuring actual cytotoxicity, either by ^{51}Cr-release or trypan-blue exclusion (32, 33). Thus, it is likely that conjugate formation between TB1-6 and Namalwa is also mediated by LFA-1, with the J5-anti-CD2 and J5-anti-CD3 heterodimers serving primarily to trigger the clone's cytolytic program. In fact, preliminary data suggest that anti–LFA-1 antibodies can abolish heterodimer-mediated lysis (not shown).

It is noteworthy that the anti-CD3 heterodimer triggered target lysis by this clone more efficiently than did either anti-CD2 heterodimer. It may be that the triggering of CD3/TCR is a more potent activator of the cytolytic program than triggering of CD2, although both these structures are clearly involved in the activation of CTL (1–6). It is also likely that the activating properties of the anti-T113 antibody are reduced by its modification and conjugation to J5, whereas no such inactivation is observed with the RW24B6 antibody (Lambert, J. M., unpublished observations). We are currently studying improved methods for the modification of anti-T113 in order to eliminate this reduction in activity. Furthermore, the use of anti-CD2 heterodimers may present several important advantages in vivo that are not apparent from the in vitro data. First, $T11_3$ is a unique epitope, expressed on the CD2 antigen after cellular activation (6), while there is no such "neo-epitope" known for the CD3 antigen (3). In vivo, the number of acti-

vated, $T11_3$-expressing cells is low, whereas CD3 is expressed on all circulating cells of T lineage (1, 6). Since it is known that antibodies or immunoconjugates that recognize tissue determinants in vivo have much shorter $t_{1/2}$ than those that do not (34), anti-tumor-anti-$T11_3$ heterodimers may have more favorable circulatory pharmacokinetics than anti-tumor-anti-CD3 heterodimers; additionally, delivery to the tumor mass may be better for similar reasons, namely that normal CD3-expressing tissues such as spleen and lymph nodes will bind an anti-CD3 heterodimer, but not an anti-$T11_3$ heterodimer. In addition, anti-CD2 heterodimers have the potential advantage of being able to recruit natural killer cells as well as T cells, since both NK and T cells express CD2, whereas CD3 is expressed primarily by only T cells (1, 5, 35). In fact, preliminary experiments with activated populations of PBL suggest that anti-CD2 heterodimers can recruit both T cells and NK cells, whereas anti-CD3 heterodimers recruit only the latter in vitro (Scott, C. F. Jr., unpublished observations).

Our findings may have implications in the design of clinically useful reagents; it may not be possible to focus an infusion of activated or lymphokine-activated killer cells onto tumor targets with heterodimers recognizing any activation antigens, only heterodimers containing anti-CD3 or anti-CD2 antibodies may be useful. However, it may be that other structures that have been demonstrated not simply to represent markers for activation, such as CD25 and the transferrin receptor, but structures through which T cells can actually be activated, could be useful in the construction of heterodimers. These include T44 (8, 9), Tp103 (7), and 2H1 (10). Studies to address this question with the last antibody are in progress.

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