Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3375–3379, May 1987
Immunology

Resistance of cytotoxic T lymphocytes to lysis by a clone of cytotoxic T lymphocytes

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Contributed by Herman N. Eisen, January 5, 1987

ABSTRACT **To investigate how cytotoxic T lymphocytes (CTL) avoid killing themselves when they destroy target cells, we compared 20 different cell lines as target cells, including several CTL cell lines, for their susceptibility to lysis by CTL. Variations in recognition of this diverse set of target cells was circumvented by attaching to all of them a monoclonal antibody to the antigen-specific receptor of a cloned CTL cell line (clone 2C) and using the 2C cell line as the standard aggressor or effector cell. All of the nine tumor cell lines and the four noncytolytic T-helper cell lines tested as targets were highly susceptible to lysis by the aggressor CTL, but seven cytotoxic T-cell lines (six CTL and one T-helper cell line with cytotoxic activity) were largely resistant. These results, and the use of the lectin Con A as an alternative means for triggering CTL activity, point clearly to a level of resistance that could enable CTL to avoid their own destruction when they lyse target cells. The resistance of the cytolytic T cells did not appear to be accompanied by a similar resistance to complement-mediated lysis, indicating that mechanisms of CTL-mediated and complement-mediated lysis are not identical.**

Cells that have evolved mechanisms for killing other cells can reasonably be expected to have also developed mechanisms to avoid killing themselves. This generalization seems to apply to the cytotoxic T lymphocytes (CTL) that play a major role in immune defenses of vertebrates against viral and perhaps other infectious agents (e.g., see ref. 1). When a CTL adheres to a target cell whose surface antigen it recognizes, the CTL is stimulated to release cytolytic components that cause the adherent target cell to undergo lysis (reviewed in refs. 2 and 3). However, the CTL itself is evidently undamaged, since it can carry out the process repetitively, migrating from one target cell to another, lysing each in turn (4, 27). The destruction of only the target cell could mean that the plasma membrane of a CTL is resistant to the cytolytic components it releases. However, some evidence suggests that, under certain circumstances, a CTL can be destroyed if it is recognized by another CTL (4–7) as though a CTL, when recognized, is no more resistant than other target cells. The latter evidence for one-way killing, resulting from one-way recognition, could mean that the cytolytic components released by an aggressor CTL (activated by antigen recognition) are so directed that they impinge only on the membrane of the recognized target cell (CTL or other) and not on the membrane of the aggressor cell itself. However, it is also possible that cytolytic components impinge on both the aggressor and the target cell but that the aggressor CTL is largely resistant to these components, perhaps only transiently while it attacks a target cell or perhaps only locally at the site of CTL–target cell contact.

To evaluate these possibilities, we have here compared the susceptibility of diverse cell lines, including CTL, to cytolytic attack by CTL. Since cytolytic activity is normally triggered by a CTL's recognition of antigen on a target cell, and most of the 20 cell lines tested as targets had different surface antigens, we sought to standardize their recognition through the use of a monoclonal antibody (mAb) to the antigen-specific receptor of a cloned CTL cell line (clone 2C). As we showed previously, this antibody (mAb 1B2) can simulate the antigen that is normally recognized by clone 2C; thus, when the 1B2 antibody was attached to the surface of several different tumor cell lines that lacked the natural antigen of the 2C cell (L^d, a class I glycoprotein encoded by the major histocompatibility complex) the 1B2-modified cells were all lysed specifically by 2C cells (8).

In the present study, we accordingly attached mAb 1B2 to a panel of cloned CTL and various other cell lines and compared their susceptibility to lysis by CTL clone 2C. We found that, although all of the nine tumor cell lines and four noncytolytic T-cell lines examined in this way were effectively lysed by the CTL clone, seven cytolytic T cell lines (six CTL and one T-helper cell with cytolytic activity) were all clearly resistant to lysis. Nevertheless, the resistant CTL were as effective as the susceptible cells in inducing 2C cells to discharge their cytolytic granules, as indicated by the secretion of a serine esterase that is associated with these granules (19). The findings suggest that at least one mechanism that enables CTL to avoid their own destruction when they kill other cells is their resistance to the cytolytic components they release.*

MATERIALS AND METHODS

Cell Lines. Mouse tumor cell lines, P815 ($H\text{-}2^d$), EL4 ($H\text{-}2^b$), BW5147 ($H\text{-}2^k$), S49 ($H\text{-}2^d$), R1.1 ($H\text{-}2^k$), and mouse L cells transfected with D^d (cell line T4.83) were maintained in culture with RPMI 1640 medium as described (9).

Cloned CTL lines 2C (anti-L^d) (9), 2.1.1 (anti-L^d) (8), G4 (anti-D^d) (10), 3C11 (anti-D^d) (11), 4K3 (anti-L^d) (11), and 3 (anti-D^d) (12) and cloned T-helper cell lines TH-1 (anti-trinitrophenyl/$H\text{-}2^d$) (G. Sigal, personal communication), 18.1 (anti-$H\text{-}2^b$) (13), 5-5 (anti-ovalbumin/$H\text{-}2^d$) (14), and D10 (anti-conalbumin/$H\text{-}2^k$) (15) were all maintained in the same medium containing irradiated stimulator cells (BALB/c, BALB.K, or BALB.B spleen cells) plus either recombinant interleukin 2 (generously provided by Biogen, Boston) or supernatants from rat spleen cells that were cultured for 48 hr in the presence of Con A. Ovalbumin, conalbumin, or trinitrophenyl-coupled spleen cells were included for the T-helper lines 5-5, D10, and TH-1, respectively. The noncytotoxic line CTLL (16) was maintained with Con A supernatants only.

mAbs. The mAb (1B2) that recognizes the $\alpha\beta$ heterodimeric

Abbreviations: CTL, cytotoxic T lymphocyte(s); mAb, monoclonal antibody; MLC, mixed lymphocyte culture(s); SPDP, *N*-succinimidyl-3-(2-pyridyldithio)propionate.

*A preliminary account of this work was presented at a minisymposium at the 70th annual meeting of the American Association of Immunologists, St. Louis, MO, April 17, 1986 (28).

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T-cell receptor of clone 2C and its purification have been described (8). For attachment to cells, the heterobifunctional crosslinking reagent *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) was coupled to mAb 1B2. Fifty microliters of 20 mM SPDP (in ethanol) was added to 1 mg of 1B2 in 1 ml of phosphate-buffered saline (PBS) (pH 7.2). After 30 min at room temperature, the modified mAb (SPDP-1B2) was dialyzed against PBS (pH 7.2). Anti-Thy-1 mAb (13-4; see ref. 17) was purified from ascites by ammonium sulfate precipitation for complement-mediated lysis (see below).

Mixed Lymphocyte Cultures. BALB.K (H-2^k) spleen cells (5×10^6 cells per ml) were cultured with irradiated (2000 rad; 1 rad = 0.01 Gy) BALB.B spleen cells (5×10^6 cells per ml) for 5 days and then layered over Ficoll to remove dead cells prior to use in cytotoxicity assays.

Cytotoxic Assays. CTL-mediated target cell lysis was measured by a standard ^{51}Cr release assay (18) using two different methods to trigger the cytolytic process. In one, mAb 1B2 was coupled to ^{51}Cr-labeled target cells (at room temperature). ^{51}Cr-labeled target cells (10^7 cells) were washed twice with PBS, reduced with 500 μM dithiothreitol for 30 min, again washed with PBS, and resuspended in 200 μl of SPDP-1B2 at 0.5 mg/ml. After 30 min, the cells were washed three times with RPMI 1640 medium containing 10% fetal calf serum and adjusted to 2×10^5 cells per ml. In the second procedure (lectin-mediated cytotoxicity), CTL and ^{51}Cr-labeled target cells were incubated with Con A at 10 μg/ml.

Various numbers of CTL (in 100 μl) were added to 2×10^4 ^{51}Cr-labeled target cells (in 100 μl). After 4 hr at 37°C, cells were pelleted by centrifugation, supernatants were assayed for radioactivity, and percentage specific ^{51}Cr release was calculated from $100 \times (a-b)/(t-b)$, where a is ^{51}Cr release in the presence of CTL, b is the spontaneous ^{51}Cr release in the absence of CTL, and t is the total ^{51}Cr released from target cells with 0.5% Nonidet P-40.

Serine Esterase Assay. Serine esterase released by CTL (10^6 cells per well) after incubation with various target cells (10^6 cells per well) for 3 hr at 37°C was measured in 10 μl of culture supernatant by using the substrate *N*-benzyloxycarbonyl-L-lysine thiobenzyl ester (BLT) in a colorimetric assay as described (19); enzyme activity was expressed as absorbance (A_{412}) measured after 30 min at room temperature.

Complement-Mediated Lysis. ^{51}Cr-labeled cells (2×10^4) were incubated with a saturating level (5 μg/ml) of anti-Thy-1 mAb (13-4) (17) and various concentrations of guinea pig serum as a source of complement. After 3 hr at 37°C, the cells were pelleted by centrifugation, and supernatants were assayed for radioactivity and percentage specific ^{51}Cr release was calculated as described above.

In some experiments, anti-H-2^b alloantisera were used to activate complement and, in others, serum from 3- to 4-week- old rabbits (Pel-Freez) was used as the source of complement. In both cases, the results were the same as with anti-Thy-1 and guinea pig serum.

RESULTS

Susceptibility to Cytolytic Attack by CTL Clone 2C. The mAb 1B2 was attached under uniform conditions (see *Materials and Methods*) to ^{51}Cr-labeled cells of the various cell lines tested as targets. The mouse T-cell lymphoma EL4 was included, as it is widely used as a standard target in studies of CTL activity. As illustrated in Fig. 1 and summarized in Table 1, at the highest effector cell (2C) to target cell ratio (20:1) there was substantial lysis (70%) of the EL4 cells, as expected. Three T-helper cell lines (TDH-1, D10, 18.1) and the noncytolytic T-cell line CTLL were also effectively lysed (65–75%). However, there was little or no lysis of most of the CTL cell lines, especially clones 2.1.1, G4, and 4K3. Intermediate results (≈37% lysis) were observed with one CTL line (clone 3C11) and with one T-helper cell line (clone 5-5) that is also cytolytic. Since 3C11 (anti-L^d) is poorly cytolytic and subcloning has shown it to have a high proportion of cells with little cytolytic activity (E. B. Reilly, personal communication), it is possible that noncytolytic (e.g., CTLL) or poorly cytolytic CTL variants are less resistant to lysis than highly cytolytic CTL.

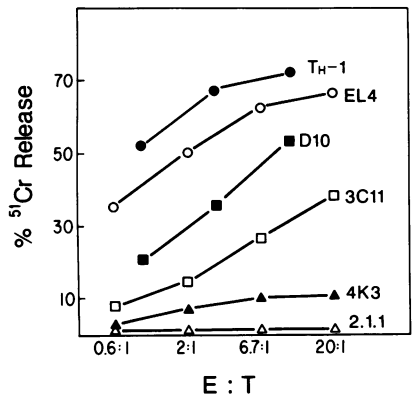

FIG. 1. Susceptibility of 1B2-modified target cells to lysis by CTL clone 2C. Anti-T-cell-receptor antibody 1B2 was coupled to ^{51}Cr-labeled target cells (T-lymphoma EL4; T-helper cell clones TH-1 and D10; CTL clones 3C11, 4K3, and 2.1.1), which were incubated with CTL clone 2C at various effector (2C)/target ratios (E:T). After 4 hr, supernatants were assayed for radioactivity. Spontaneous release values were EL4, 12%; TH-1, 22%; D10, 20%; 3C11, 10%; 4K3, 17%; 2.1.1, 23%.

Table 1. Susceptibility of cell lines to lysis by CTL clone 2C and activated complement

Cell line	Type	Maximum % ^{51}Cr release* +1B2	Maximum % ^{51}Cr release* +Con A	Complement†
Human				
JY	B-cell lymphoma	65		
HPB-ALL	T-cell lymphoma	75		
K-562	Myelogenous leukemia	57		
Mouse				
P815	Mastocytoma	85		
EL4	T-cell lymphoma	72	54	1.6
S49	T-cell lymphoma	73		0.7
BW5147	T-cell lymphoma	89		
R1.1	T-cell lymphoma	60		
BW5147X2.1.1	T-cell hybridoma	73–86‡		
TH-1	T helper	72	40	2.5
18.1	T helper	75		3.0
D10	T helper	53		
CTLL	Noncytolytic CTL	61	31	
5-5	T-helper/killer	37	9	
3C11	CTL	38	8	
2C	CTL	11	17	2.7
3	CTL	11		3.1
4K3	CTL	8	0	2.6
G4	CTL	1	5	9.1
2.1.1	CTL	0	1	6.7

*Values represent % ^{51}Cr released from ^{51}Cr-labeled target cells at effector (2C)/target ratios of 10:1 or 20:1. Target cells were coupled with 1B2 (see *Materials and Methods*) or were incubated with Con A at a final concentration of 10 μg/ml. Spontaneous ^{51}Cr release values were 9–26% (except T-helper/killer 5-5 were 38%).

†Values represent the volume (μl) of complement (guinea pig serum) required to yield 33% specific ^{51}Cr release from target cells preincubated with anti-Thy-1 mAb.

‡Range of values for five different hybridomas.

Fusion of the susceptible tumor cell line BW5147 with the resistant line 2.1.1 yielded noncytolytic hybridomas that were susceptible to lysis by CTL clone 2C (Table 1). Thus, the resistant phenotype of CTL clone 2.1.1 could not be transferred by cell fusion.

Resistant CTL Can Be Recognized by and Can Activate Aggressor CTL. To verify that the apparently resistant 1B2-modified CTL were still recognized by the CTL clone 2C, we tested one of the resistant clones (2.1.1) as an unlabeled inhibitor ("cold target") in a cytolytic assay involving 2C cells as aggressors and ^{51}Cr-labeled 1B2-modified EL4 (i.e., 1B2-EL4) as target cells. As shown in Fig. 2, the resistant 1B2-modified 2.1.1 cells (i.e., 1B2-2.1.1) and the susceptible 1B2-EL4 cells were equally effective as specific inhibitors and were thus equally well recognized by the cytolytic 2C cells.

To determine whether the resistant CTL targets actually stimulated the aggressor CTL's release of cytolytic components, we took advantage of a previous finding that when a CTL recognizes and attacks a target cell it releases cytotoxic granules that contain a cytolytic protein, termed perforin (20, 21), and also a serine esterase (19); the latter is readily measured in the culture medium by a sensitive chromogeneic assay (see *Materials and Methods*). Table 2 shows the amount of serine esterase released from 2C cells when incubated in the presence of four different target cells: resistant 1B2-modified CTL (clone 2.1.1), susceptible 1B2-modified EL4 cells, P815 cells (which are susceptible because they express L^d, the natural antigen recognized by clone 2C), and unmodified EL4 cells (which do not express the L^d antigen and are therefore not recognized by clone 2C). The resistant target (1B2-2.1.1) and the susceptible targets (P815 and 1B2-EL4), but not the control EL4 cells, induced the release of similar amounts of serine esterase from the CTL clone 2C. Thus, three targets that were recognized by 2C cells were equally effective in stimulating the release of cytotoxic granules from the 2C cells, whether the targets were resistant CTL (1B2-2.1.1) or susceptible cell lines (P815, 1B2-EL4).

CTL that Resist Lysis Retain Their Own Cytolytic Activity. Previous studies have demonstrated that the cytolytic activity of CTL in a heterogeneous spleen cell population can be almost totally destroyed (>90%) by another spleen cell population whose CTL recognize cell-surface antigens of the first population (5, 6). It seemed possible, therefore, that

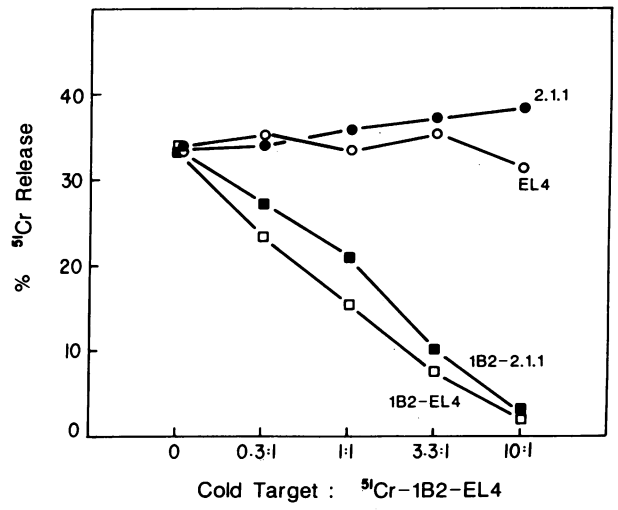

FIG. 2. Resistant CTL are recognized by CTL clone 2C. Cold target inhibition was performed by incubating various numbers of unlabeled target cells (2.1.1, EL4, 1B2-2.1.1, and 1B2-EL4) with ^{51}Cr-labeled 1B2-modified EL4 target cells (^{51}Cr-1B2-EL4) in the presence of CTL 2C cells (at a 2C/^{51}Cr-1B2-EL4 ratio of 1:1). After 4 hr, supernatants were assayed for radioactivity.

Table 2. Resistant CTL target cells induce the release of serine esterase from CTL clone 2C

Target	Serine esterase released on addition of: Medium alone	CTL 2C
Medium	0.0	1.0
P815	1.2	*10.8*
EL4	1.1	2.0
1B2-EL4	0.9	*10.9*
2.1.1	2.4	3.8
1B2-2.1.1	2.9	*13.6*

Values represent the units of serine esterase activity (A_{412} per 30 min) in supernatants after incubation for 3 hr. Supernatants were examined in the BLT assay (see *Materials and Methods*). Elevated values are italicized. CTL to target cell ratio was 1:1.

although the 2C cells did not cause the ^{51}Cr-labeled 1B2-targeted CTL to release ^{51}Cr, they might have damaged the targeted CTL to the point where their cytolytic activity was substantially lost. To test this possibility, we examined a 1B2-modified CTL line (G4) whose specificity (anti-D^d) differed from that of the aggressor CTL (2C cells, which are anti-L^d). After incubating 1B2-G4 cells together with 2C cells for 4 hr, ^{51}Cr-labeled target cells for clone G4 (L cells transfected with D^d) were added to assess the remaining cytotoxic activity of the 1B2-G4 cells. As shown in Table 3, there was absolutely no decrease in the cytotoxic activity of the 1B2-modified G4 cells as compared to unmodified G4 cells or to 1B2-modified G4 cells that had not been exposed to the aggressor 2C cells. Thus, in addition to not lysing G4 cells, CTL clone 2C had no detectable effect on the cytolytic activity of the G4 cell line.

CTL Are Not Uniformly Resistant to All Other CTL. In most previous studies in which one set of CTL appeared to have been inactivated by other CTL, the aggressor CTL were contained in heterogeneous spleen cell populations [mixed lymphocyte cultures (MLC)] that had been stimulated by an allogeneic class I major histocompatibility complex-encoded antigen (4–6). Fig. 3*A* shows the effect of a MLC [from spleen cells of BALB.K (*H-2^k*) mice that had been stimulated by spleen cells from BALB.B (*H-2^b*) mice] on various ^{51}Cr-labeled CTL with the *H-2^b* haplotype, including 2C and two other CTL (G4 and 2.1.1) that were resistant to lysis by clone 2C. All three of the ^{51}Cr-labeled *H-2^b* CTL were clearly lysed, although it required 3–10 times more MLC cells to lyse them to the same extent as the standard EL4 lymphoma cells.

Table 3. Lytic activity of 1B2-modified and unmodified G4 cells after incubation with CTL 2C

First incubation*	Second incubation: % ^{51}Cr release at G4/L cell (D^d) ratios of 5:1	1:1
G4 alone	60.2	38.5
1B2-G4 alone	57.5	37.0
2C + G4 (5:1)	45.9	30.0
2C + G4 (1:1)	53.7	32.0
2C + 1B2-G4 (5:1)	49.2	31.6
2C + 1B2-G4 (1:1)	56.0	34.6

*CTL G4 cells or 1B2-G4 cells were incubated with or without CTL 2C for 4 hr at 37°C. ^{51}Cr-labeled L cells transfected with D^d (T4.83) were then added at G4 or 1B2-G4 to T4.83 ratios of 5:1 or 1:1. After 4 hr at 37°C, supernatants were assayed for ^{51}Cr released. Release of ^{51}Cr from T4.83 cells in the presence of 2C (at 25:1) was negligible (2%).

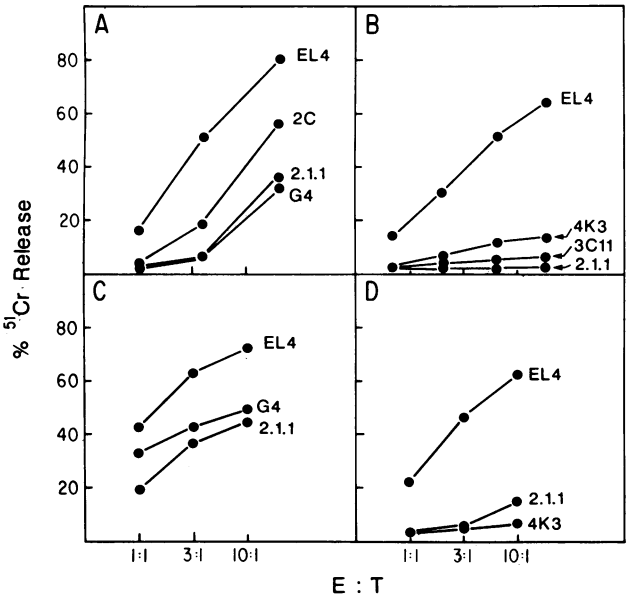

FIG. 3. Susceptibility of CTL to lysis by mixed lymphocyte cultures and other CTL clones. (*A*) ^{51}Cr-labeled cells (EL4, 2C, 2.1.1, G4; all H-2^b) were incubated with a BALB.K anti-BALB.B (H-2^k anti-H-2^b) mixed lymphocyte culture at various effector/target ratios (E:T). (*B*) ^{51}Cr-labeled cells (EL4, 4K3, 3C11, 2.1.1) were incubated with Con A (10 μg/ml) and CTL 2C at various effector (2C)/target ratios. (*C*) ^{51}Cr-labeled cells (EL4, G4, 2.1.1) were incubated with Con A (10 μg/ml) and CTL 4K3 at various effector (4K3)/target ratios. (*D*) ^{51}Cr-labeled cells (EL4, 2.1.1, 4K3) were incubated with Con A (10 μg/ml) and CTL G4 at various effector (G4)/target ratios.

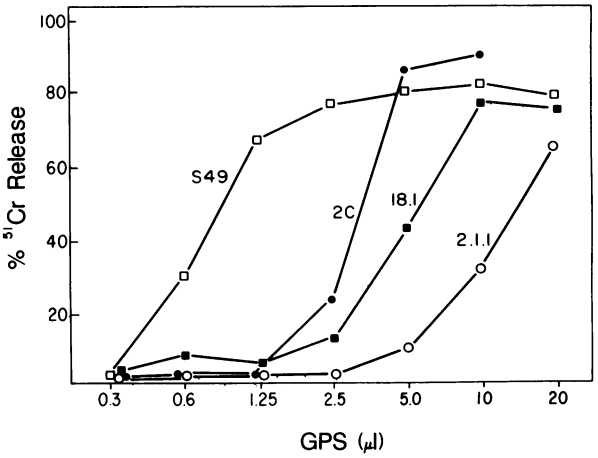

FIG. 4. Susceptibility of various cells to lysis by activated complement. ^{51}Cr-labeled target cells (T lymphoma S49, T helper 18.1, CTL 2C, CTL 2.1.1) were incubated with anti-Thy-1 mAb 13-4 and various concentrations of guinea pig serum (GPS) complement. After 3 hr at 37°C, supernatants were assayed for radioactivity. The density of Thy-1 on the surface of these cells and the other cells tested (Table 1) were shown by fluorescence-activated cell sorter analysis to be nearly identical (data not shown).

In considering these results, it is important to realize that a MLC contains diverse cytotoxic cells. Hence, the lysed target CTL (2.1.1, G4, 2C) might have been subjected to the combined attack of various aggressor cells, including CTL that were specific for diverse *H-2^b* epitopes on the target CTL and also perhaps by activated macrophages; the latter are cytotoxic not by virtue of pore-forming activity but rather by release of reactive oxygen intermediates.

To explore further the extent to which diverse CTL cell lines can serve as effective targets for other CTL, we took advantage of the well-known ability of the lectin Con A to activate CTL so that they will lyse cells to which they are adherent, regardless of the CTL's own specificity or the antigens on the prospective target cells. Three cloned CTL cell lines were tested for their ability in the presence of Con A to lyse various ^{51}Cr-labeled CTL and EL4 lymphoma cells. As shown in Fig. 3 *B* and *D*, in the presence of Con A two of the CTL tested as aggressors (G4 and 2C) had little (up to 15%) or no lytic activity against the other ^{51}Cr-labeled CTL target cells (2.1.1, 4K3, and 3C11), although they lysed the lymphoma EL4 cells very effectively (60%). The third CTL cell line tested as aggressor, 4K3, clearly lysed two CTL targets, but 10 times more 4K3 cells were required to lyse the CTL targets to the same extent as the standard EL4 lymphoma (Fig. 3*C*). Therefore, these results show that the cloned CTL cell lines tested were clearly resistant to lysis by some but not all of the CTL tested as aggressors.

Susceptibility of Cells to Lysis by Activated Complement. Considerable evidence suggests that a CTL destroys a target cell by releasing a cytolytic protein, termed perforin, that resembles complement factor nine (C9) (21, 23). Accordingly, cells that are resistant to lysis by CTL might also be resistant to lysis by activated complement.

To compare the susceptibility of various T-cell lines to lysis by complement, an excess of mAb to Thy-1 (present on all murine T cells) and various amounts of guinea pig serum (as a source of complement) were added to the cells. Representative titrations are illustrated in Fig. 4 and all results are summarized in Table 1 by listing the amount of complement required to lyse the cell lines to the same extent (33%). There was only a limited correlation between susceptibility to lysis by complement and by CTL. For example, a cloned T-helper cell line (18.1) that was highly susceptible to lysis by CTL clone 2C required as much complement as did three CTL lines (4K3, 2C, and 3) that were all resistant to lysis by CTL clone 2C. However, the two CTL clones that were most resistant to complement (2.1.1 and G4) were also the most resistant to lysis by CTL clone 2C. Hence, these two clones might share a mechanism, such as the efficient clearance of channels or pores from cell membranes, that may account for the decreased susceptibility of some nucleated cells to lysis by complement (22). However, the lack of a strict correlation between susceptibility to lysis by CTL and complement suggests that there are significant differences between these two mechanisms for destroying antigen-bearing cells (as previously proposed, see ref. 24).

DISCUSSION

The destruction of a target cell by a CTL is the end result of a reaction sequence that begins with the formation of a CTL–target cell conjugate. In such a conjugate the CTL, activated via its receptor's recognition of antigen on the target cell, releases the contents of cytotoxic granules at the CTL-target cell junction (19, 20). One of the released components, perforin, resembles complement factor nine (C9) and appears, like C9, to lyse target cells by forming transmembrane channels in target cell membranes (21, 23). If perforin reacts in the same way with CTL membranes, how does a CTL avoid killing itself when it kills a target cell?

The evidence presented here suggests that the answer, at least in part, is that CTL are largely resistant to the effects of the cytolytic components released by activated CTL. Our most direct evidence derives from observations with a mAb (1B2) to the antigen-specific receptor of a cloned CTL line (clone 2C). When attached to diverse cells this antibody, acting as an antigen substitute, renders the "1B2-modified" cells susceptible to lysis by 2C cells. Altogether, we have now examined 24 1B2-modified cell lines, including 9 tumor cell

lines (of human and mouse origin), 5 noncytolytic T-cell hybridomas, 4 cloned mouse T-cell lines (D10, 5-5, TH-1, 18.1) with the helper phenotype ($CD4^+$,$CD8^-$), and 7 mouse T-cell clones (CTLL, 3C11, 4K3, G4, 2.1.1, 2C, 3) with the CTL phenotype ($CD4^-$,$CD8^+$). The susceptibility of these cell lines to lysis by CTL clone 2C correlated consistently with their own cytolytic capacity—i.e., the cytotoxic cell lines were more resistant to lysis than the noncytolytic cell lines (Table 1).

These results are supported by a recent observation that CTL specific for a peptide from the nucleoprotein of influenza virus can kill target cells, but not themselves, in the presence of added peptide (25). Other evidence that CTL are spared from self-killing was provided in another study using the lectin Con A to mediate CTL–target cell interaction (26). In the latter study, as in the results shown here with lectin-mediated killing (Fig. 3), not all CTL were resistant to the lethal effects of all other CTL. The results suggest that a targeted CTL may be highly resistant to some CTL and less resistant to others, as though effector CTL vary in their aggressiveness (e.g., the amount of cytolytic components released). This variability may account for the many reports that show CTL are susceptible to lysis by alloreactive mixed lymphocyte populations (4–6; see also Fig. 3*A*) and the occasional observation that one CTL clone can kill another CTL clone (7).

It is widely believed that CTL kill target cells by a unidirectional process, as though CTL, when activated by recognition of targets, eject cytotoxic granules toward the adherent target and away from the CTL surface. Our results do not rule out such a process. But they do establish that CTL, unlike the other cells tested, have unusual resistance to their released toxic components. This conclusion is supported by preliminary efforts using isolated cytotoxic granules to examine a wide array of eukaryotic cells for susceptibility to lysis (R. Verret, A. Firmenich, D.M.K., and H.N.E., unpublished data).

We thank the following for the indicated cell lines: Mark Pasternack (G4); Ed Reilly (3C11, 4K3); George Sigal (T-helper TH-1); John Russell (3); Charles Janeway, Jr. (D10 and 5-5); and John Seidman (D^d-transfected L cells, T4.83). Recombinant IL-2 was a generous gift of Biogen Research (Boston, MA). We also thank Laura Ayasse for technical assistance and Ann Hicks for secretarial assistance. This work was supported by Public Health Service Grants CA28900, CA15472, and CA42504, and by Cancer Center Core Grant CA14051, all awarded by the National Cancer Institute, Department of Heath and Human Services. D.M.K. was supported by a Postdoctoral Fellowship from the Charles A. King Trust (Boston, MA).

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