Journal of Cellular Biochemistry 18:337–349 (1982)
Cellular Recognition 661–673

A Common Role for Target Cell Histocompatibility Antigens in Both Nonspecific and Specific T Lymphocyte-Mediated Cytolysis

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We have investigated the role of target cell major histocompatibility complex antigens (MHC-Ag) in nonspecific lectin-dependent lymphocyte-mediated cytolysis (LDCC). In contrast to previous reports, we provide evidence that in LDCC the lectin Concanavalin A (Con A) does not mediate lysis by simply bridging cytotoxic T lymphocytes (CTL) and targets via cell surface sugars or by activating the lytic function of CTLs attached to targets via the lectin. Lysis occurs when target cells are pretreated with lectin, but not when CTL are pretreated. Moreover, when CTL populations are used as both aggressors and targets, and only one is pretreated with lectin, lysis occurs only in the direction of the pretreated CTL target. We have observed that in LDCC, as in specific CTL-mediated killing, target recognition proceeds through interaction of CTL receptors (distinct from sugar moieties) and target cell surface determinants perhaps modified by, but distinct from, the lectin itself. We present evidence that the target determinants recognized in LDCC are MHC-Ag: 1) Cells that display reduced amounts of MHC-Ag are poor targets in LDCC; 2) removal of MHC-Ag by papain renders targets refractory to LDCC, however susceptibility is regained upon regeneration of MHC-Ag; and 3) antisera to target cell MHC-Ag block LDCC. The latter finding is also observed in oxidation-dependent CTL-mediated cytotoxicity. Involvement of MHC proteins in both specific and nonspecific CTL-mediated lysis reconciles an apparent fundamental distinction between these two processes and suggests a possible role for MHC proteins in a postrecognition step(s) leading to lysis.

Key words: lectin, concanavalin A, cytotoxic T lymphocytes, histocompatibility antigens, lymphocyte–target cell interactions, cytolysis

Abbreviations used: Con A, concanavalin A; ^{51}Cr-TC, $Na_2{}^{51}CrO_4$-labeled EL4 cells; CTL, cytotoxic T lymphocytes; EM-FCS, Dulbecco's modified Eagle's medium supplemented with fetal calf serum 10%; FDA, fluorescein-diacetate; LDCC, lectin-dependent CTL-mediated cytotoxicity; MHC-Ag, major histocompatibility complex antigens (H-2 Ag, in the mouse); PBS, phosphate-buffered saline; TC, target cells; AS, antiserum.

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Received April 17, 1981; accepted October 26, 1981.

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Cytotoxic T lymphocytes (CTL) are highly specialized immune effectors relevant to transplantation, virus, and tumor immunity. While the involvement of major histocompatibility complex antigens (MHC-Ag) as recognition structures in direct, immunologically specific, CTL-mediated cytotoxicity (CMC) is generally accepted [1-3], a role for MHC-Ag in a lytic step beyond simple recognition has been seriously questioned by the demonstration of nonspecific and even autologous CTL-mediated lysis induced by lectins or by oxidizing agents (e.g., $NaIO_4$ or neuraminidase/galactose oxidase). In lectin-dependent CTL-mediated cytotoxicity (LDCC) or oxidation-dependent cytotoxicity (ODCC), target cells (TC) of any genotype can be lysed in the presence of lectins or upon oxidation, respectively [4-7]. This apparent overriding of immunological specificity suggested that conjugation (binding) of CTL and TC [1] through specific recognition of TC MHC-Ag by indigenous CTL receptors was bypassed through intercellular crosslinking of CTL and TC via lectin bridges or oxidized moieties [6-10]. Since mere proximity of CTL and TC did not lead to lysis [11-12], it was further postulated that activation of the killing capability of the CTL by lectin in LDCC was an additional requirement for nonspecific lysis to occur [10, 13].

The studies reported here and documented fully elsewhere [14, 15] demonstrate that, in contrast to earlier views, the lectin Concanavalin A acts directly on the TC in mediating LDCC and not as an intercellular "glue" bridging CTL and TC or as a CTL activator per se. The present data suggest that a (specific) CTL surface receptor(s) other than a lectin-binding receptor is involved in lectin-dependent CTL-TC recognition. Evidence that this CTL receptor(s) is directed against TC MHC-Ag in both LDCC and ODCC is presented. The results indicate a requirement for TC MHC-Ag in at least the recognition phase of LDCC and suggest a role for MHC proteins in a postrecognition phase(s) of CTL mediated lysis.

METHODS

Mice, Tumor Cells, and Target Cells

Six- to twelve-week-old C57BL/6 and DBA/2 mice of both sexes were used. Leukemia EL4 of C57BL/6 was kept in syngeneic hosts as an ascites tumor by weekly transfer of 5-25 $\times$ 10^6 washed tumor cells. In addition to the EL4 leukemia of C57BL/6 mice, we used human, mouse, and chicken red blood cells, and the H-2 positive R1.1 and negative R1.E variants of a C58 lymphoma cell line obtained from the Salk Institute and maintained in culture in EM-FCS.

Cytotoxic T Lymphocytes (CTL)

The DBA/2 spleen cell suspensions were obtained by mincing the organs through stainless steel screens into phosphate-buffered saline (PBS) followed by vigorous pipetting. Nucleated cells were counted in Turk's solution. Concanavalin A (Con A)-induced lymphoblasts were generated [8] by culturing the splenocytes (2.5 $\times$ 10^6/ml) with Con A grade IV, Sigma, St. Louis, MO (2 μg/ml) in Dulbecco's modified Eagle's medium supplemented with fetal calf serum (10%; EM-FCS) in 50-ml plastic tissue culture flasks at 37°C in a CO_2 incubator for 3 days. EM and FCS were obtained from GIBCO, Grand Island, New York. Lymphoblasts were collected from cultures, washed, and counted. Cell viability was determined by trypan blue dye exclusion.

Lectin-Dependent Cellular Cytotoxicity (LDCC)

Leukemia EL4 target cells (TC) were harvested from tumor-bearing donors 3–7 days after intraperitoneal inoculation of the tumor cells. Usually, 30×10^6 TC were pelleted in a 50-ml conical plastic centrifuge tube, resuspended in 0.5 ml PBS-FCS plus 0.1–0.2 mCi $Na_2{}^{51}CrO_4$ (^{51}Cr), 1 mCi/ml, 200–500 Ci/g (New England Nuclear, Boston, MA). Target cells were labeled for 30–60 min at 37°C with occasional shaking; labeled cells were washed twice, adjusted to 2×10^6/ml EM-FCS, and kept on ice until used.

CTL were mixed with ^{51}Cr-labeled TC (^{51}Cr-TC) in a final volume of 0.2 ml EM-FCS in 12 × 75 mm plastic tubes (Falcon Plastics, Oxnard, CA), in the presence or absence of Con A, usually 10 μg/ml. The tubes were shaken, centrifuged 10 min at room temperature at 150–200g, and incubated at 37°C in a CO_2 incubator. Control tubes containing ^{51}Cr-TC were incubated without the addition of Con A-stimulated lymphoblasts. At the end of the incubation period, 1 ml ice-cold PBS-FCS was added to the assay tubes, which were then centrifuged ~1,000g 10 min at 4°C and the radioactivity in the supernate determined in a gamma counter. Results are expressed as percent ^{51}Cr released, calculated as follows (see Ref. 1):

$$\text{percent } {}^{51}\text{Cr release} = \frac{\text{cpm with CTL minus cpm without CTL}}{(0.75 \times \text{total cpm}) \text{ minus cpm without CTL}} \times 100.$$

Control release values never exceeded 10–15% of total radioactivity incorporated. With rare exceptions, 5–10% was the maximum variation between duplicate or triplicate assay tubes in all experiments.

Binding (Conjugation) of CTL and TC (12, 16)

Con A-induced effector cells, 5×10^6/ml, were labeled with fluorescein-diacetate (FDA), 0.01 mM, 10 min at room temperature, and washed 3 times. The 1×10^6 FDA-labeled effector cells were mixed with 1×10^6 TC in 1 ml EM-FCS in 12 × 75 mm plastic test tubes. After 10 min incubation at room temperature, the tubes were centrifuged 10 min at room temperature to promote CTL-TC conjugation, and the supernate was discarded. Pellets were placed on ice and resuspended in 1 ml of fresh medium with 15 strokes through a Pasteur pipette. Conjugates containing fluorescent effectors attached to non fluorescent targets were scored under a fluorescent microscope.

Antisera

The following antisera (As) were used: anti-EL4, BALB/c anti-EL4; anti-Ia. 9,20+D33, (A×B10.D2)F1 anti-B10.A(5R); anti-H-2^b; C3H anti-C.SW; anti/Thy 1.2, (PL×B6.PL-Thy 1^a) anti-C57BL/6; anti-H-2^d, BALB.B anti-BALB/c; anti-H-$2D^d$, (B10×LP. R111)F1 anti-B10.A(2R); and anti-gp70, rabbit anti-gp70 (purifed glycoprotein).

Papain Treatment of EL4 Cells

Papain (2× recrystallized, Worthington 3126) was used at a final concentration of 60 units/ml. EL4 cells, in some cases prelabeled with ^{51}Cr, were incubated at 5×10^6/ml in medium (no serum) containing papain and 5 mM cysteine. At the end of the treatment period the cells were washed twice with PBS-FCS and pipetted

to break up cell clumps caused by the papain treatment. Cell recovery and viability after 75 min of treatment were essentially indistinguishable from controls incubated under identical conditions but without papain.

Treatment of TC With Sodium Periodate (7)

Target cells, 50 × 10^6 in 1 ml Ca^{2+}-free PBS, were treated with 4 ml of 2.5 mM sodium periodate in Ca^{2+}-free PBS pH 7.2 for 10 min at room temperature unless otherwise stated. The treated cells were then washed twice with PBS/5% FCS and the cell pellet resuspended in EM-FCS.

RESULTS

The DBA/2 spleen cells were cultured as described in Methods in the presence of Con A, 2 μg/ml. After 3 days of culture the cells were harvested and assayed for cytotoxicity against ^{51}Cr-labeled EL4 TC in the presence of increasing concentrations of Con A (Fig. 1A) or Con A at 10μg/ml plus increasing concentrations of glucose or α-methyl-mannopyranoside (Fig. 1B). Complete and specific lectin-dependence of cytotoxicity was observed. Con A pretreatment of the TC prior to the assay resulted in equal lysis (Fig. 1A). Despite the paradoxical observation that pretreatment of either CTL or TC with lectin does not give rise to equivalent lysis of the TC (Table I and Refs. 9, 10), it has been postulated that Con A serves as an intercellular bridge ("glue") and antigen substitute that nonspecifically triggers the cytolytic function of CTL in LDCC [9, 10,13]. The arguments that lectin (e.g., Con A) at certain concentrations may inhibit CTL [17] or lead to autologous lysis of CTL [18] are overcome by the demonstration of the functional activity of Con

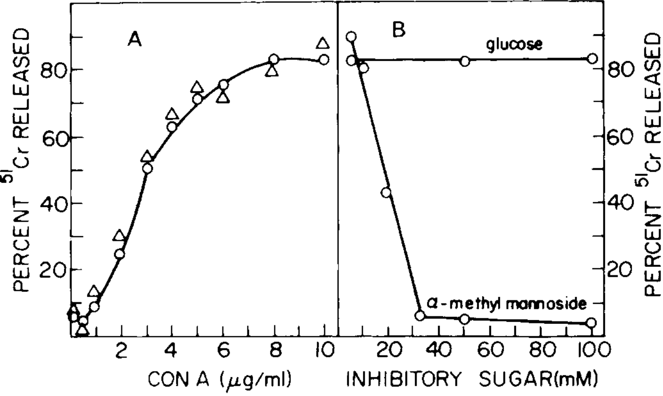

Fig. 1. Lectin (Con A)-dependent CTL-mediated cytotoxicity. (A) Dependence of cytotoxicity on the concentration of Con A in the assay or during TC pretreatment. Effector cells were generated from DBA/2 spleen cells in the presence of 2 μg/ml Con A, as described in the Methods section, and assayed for cytotoxicity against ^{51}Cr-labeled EL4 target cells pretreated with (△) or in the presence (○) of the indicated concentrations of Con A. CTL:TC ratio was 5:1, assay time was 90 min. (B) Effect of glucose and α-methylmannopyranoside on cytolysis in LDCC. Effector cells were generated as in A and assayed for lysis against EL4 cells for 2 h in the presence of 10 μg/ml Con A at an EC:TC ratio of 5:1.

A-pretreated CTL (Con A-CTL) against Con A-pretreated TC (Con A-TC) (Table I), which is in some cases a substantial fraction of the killing activity of nontreated CTL. The possibility that the low killing activity by Con A-CTL against untreated TC was owing to low Con A binding was investigated and excluded [14]. The ability of Con A-CTL to conjugate (bind) to TC [16] was investigated and found to be drastically impaired relative to untreated CTL with Con A-TC (Table II). In part this reduction was also due to Con A-CTL autologous conjugates that were not scored, but which reduced the availability of Con A-CTL for conjugation. However the conjugation of Con A-CTL with Con A-TC (61 per 0.5 μl) was significantly higher than with TC (10 per 0.5 μl), which correlated with the lysis observed. The inability of Con A-CTL to conjugate with TC could by itself account for the decrease in their killing activity. To circumvent the problem of defective conjugation of Con A-CTL and TC, a "killer-antikiller" experiment [11, 12] was designed, using the same CTL population as both aggressors and targets. This experiment exploited the fact that conjugation between Con A-CTL and an identical target (also a CTL) was assured, since it is known that untreated CTL will bind to and lyse any Con A-treated TC (including syngeneic TC [8]). Thus, while it was expected that CTL would lyse Con A-CTL, would Con A-CTL, now conjugated to a CTL, lyse

TABLE I. Effect of Con A Pretreatment of Effector and Target Cells on LDCC

Group[a]	^{51}Cr released[b] (percent)
CTL + (Con A-TC)	54.4
(Con A-CTL) + TC	3.5
(Con A-CTL) + (Con A-TC)	36.0
CTL + TC + Con A (10 μg/ml)	32.5

[a]CTL = Con A stimulated (2 μg/ml, 3 days) lymphoblasts, washed prior to assay. Con A-CTL = CTL preincubated with Con A (10 μg/ml) for 30 min at 37°C, washed three times prior to assay. TC = ^{51}Cr-labeled target cells. Con A-TC = Target cells preincubated with Con A (10 μg/ml) for 30 min at 37°C, washed three times prior to assay.
[b]Average of two experiments, duplicate samples, corrected for spontaneous release.

TABLE II. Effect of Con A Pretreatment of Effectors and Targets on Conjugation and Cytotoxicity in LDCC

Group[a]	Conjugates (Number per 0.5μl)	^{51}Cr released (percent)
CTL + (Con A-TC)	203	31.5 ± 0.8
(Con A-CTL) + TC	10	0.4 ± 0.1
(Con A-CTL) + (Con A-TC)	61	18.6 ± 0.6
CTL + TC	25	0

[a]Con A pretreatments of CTL and TC were performed as described in Table I. In addition, the ^{51}Cr-labeled, Con A-treated or untreated targets were labeled with fluoresceindiacetate, followed by three washes in PBS/FCS. For the cytotoxicity assay, 5 × 10^5 CTL were incubated with 10^5 ^{51}Cr-TC for 2 h at 37°C. For the conjugation assay, 10^6 CTL were copelleted at 150g for 10 min at room temperature with 2 × 10^6 TC, resuspended in 1 ml EM-FCS, and kept on ice until conjugates were counted in a hemacytometer. See [16] for quantification, type of conjugate, and correlation between number of conjugates formed and TC lysis.

TABLE III. Lack of Reciprocal Lysis of Con A-Treated CTL and Untreated CTL

Group[a]	Ratio of CTL:Con A-CTL	^{51}Cr released (percent)
(Con A-^{51}Cr-CTL) + CTL	1	47.4 ± 2.0
(Con A-^{51}Cr-CTL) + CTL	5	61.1 ± 2.5
(Con A-^{51}Cr-CTL) + CTL	10	66.6 ± 2.0
(Con A-CTL) + (^{51}Cr-CTL)	1	4.2 ± 0.5
(Con A-CTL) + (^{51}Cr-CTL)	5	2.7 ± 0.5
(Con A-CTL) + (^{51}Cr-CTL)	10	0.9 ± 0.3
(Con A-^{51}Cr-CTL)		19.7 ± 1.2

[a]Unlabeled and ^{51}Cr-labeled CTL were pretreated with Con A at 10 μg/ml, 37°C, 30 min, and diluted 10-fold with PBS/FCS. Untreated CTL were added to the Con A-treated CTL, spun, and resuspended in EM/FCS. Following conjugation the cells were incubated for 2 h at 37°C, then assayed for released radioactivity. The number of Con A-treated effectors in each assay was 5×10^5.

TABLE IV. Susceptibility of Different Cell Types to LDCC*

Cell type	^{125}I-Con A bound[a] (cpm ± SE)	^{51}Cr released (percent ± SE)
E14 (H-2$^+$)	8,095 ± 1162	89.1 ± 0.4
R1.1 (H-2$^+$)	11,593 ± 1532	60.1 ± 1.9
R1.E (H-2$^-$)	10,807 ± 572	22.9 ± 2.4
CRBC	–	0.3 ± 0.06
HRBC	–	-0.5 ± 0.3

*Nucleated mouse cells and erythrocytes (human and chicken) were pretreated with Con A at 10 μg/ml for 30 min at 37°C, washed, and resuspended in EM/FCS. Effector cells were mixed with the Con A-treated TC, additional Con A (10 μg/ml) was added, and the assay tubes were centrifuged and incubated for 85 min at 37°C, after which radioactivity in the supernate was measured.
[a]For the Con A binding studies, 2×10^6 cells were incubated with ^{125}I-Con A (0.4 μCi/μg, 4μg Con A/ml) in 200 μl PBS for 30 min at 37 °C. The cells were washed twice, resuspended, and triplicate aliquots counted.

the bound CTL? The data presented in Table III indicate that lysis is not symmetrical between Con A-CTL and CTL. That is, lysis occurs only in the direction of the Con A-coated cell, confirming indications already derived from data in Table I that Con A acts primarily as a target cell modifier in mediating LDCC. As will be discussed more extensively below, these CTL anti-CTL data unequivocally show that Con A per se does not act as a CTL activator, since if it did conjugation of two CTL (Con A-CTL and CTL) via a Con A linkage would lead to activation of both CTL and result in bidirectional lysis, which is not observed (see [12] for lack of simultaneous mutual lysis during killer/killer interaction).

An investigation into the nature of the target cell determinants affected by lectin was then initiated. It was first noted in the literature [19, 20], and confirmed here for one cell type (Table IV), that nucleated cells deficient in MHC-Ag were less susceptible to LDCC than MHC-positive cells, although they could bind ^{125}I-Con A equally well. This pattern of low or no susceptibility in LDCC was also observed with chicken and human erythrocytes known to be deficient in MHC-Ag. Interestingly, not only were erythrocytes not lysed in LDCC, but Con A-RBC failed even to bind to CTL.* However, refractoriness to LDCC of erythrocytes or

*The mode of action of other lectins may be found elsewhere (Berke G, Rosen D, Moscovitch M, submitted for publication).

H-2-deficient TC may be due to the absence of MHC-Ag. Therefore, the susceptibility of EL4 TC, known to express MHC-Ag, to specific CTL-mediated lysis and LDCC was tested following treatment with papain or exposure to anti-MHC-Ag sera, which digest or block MHC-Ag, respectively. Papain treatment of the TC prior to LDCC resulted in the removal of serologically detectable MHC-Ag, as well as other cell surface components, which correlated well with a decline in susceptibility to specific CTL, as well as to LDCC (Fig. 2A). All three parameters, that is, MHC-Ag expression, lysis by specific CTL and by LDCC regenerated with similar kinetics (Fig. 2B).

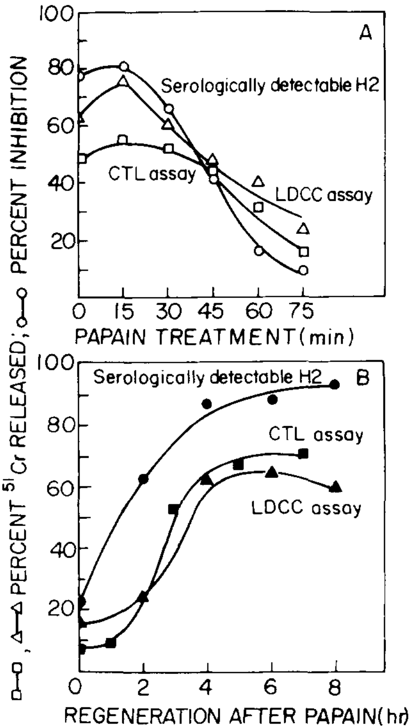

Fig. 2. (A) Effect of papain treatment of EL4 target cells on their antibody-binding ability and susceptibility to specific CTL-mediated lysis and LDCC. EL4 cells were treated with papain for varying lengths of time. The 0.5×10^6 papain-treated cells were used to absorb 100 μl of C3H anti-C3H.SW antiserum (titer 1:80) for 30 min at 5°C. After centrifugation, an aliquot of the cell-free supernate was tested in a complement-mediated cytotoxicity assay against ^{51}Cr-labeled EL4 cells. The results represent the percent inhibition of the cytolytic capacity of the antiserum at each time point (○ – ○). The ^{51}Cr labeled EL4 cells were treated with papain, washed, and used as targets in a direct CTL assay with specific BALB/c anti-EL4 CTL. These were obtained from spleens of BALB/c mice immunized i.p. 4 to 6 wk earlier with 2.5×10^7 EL4 cells and restimulated in vitro with irradiated C57BL/6 spleen cells. Responding to stimulating ratio was 2:1, and cultures were carried out for 3 days in EM-FCS. Effector to target ratio was 5:1, assay time was 3 h (□ – □). A portion of the cells used as targets in the CTL assay were incubated for 30 min at room temperature with 10 μg/ml Con A, and then incubated with DBA/2 polyclonally activated effector cells at an effector-to-target ratio of 4:1 for 90 min (△ – △). (B) Recovery of EL4 target cells from papain treatment. Papain-treated cells were washed and incubated in nutrient medium plus serum at 37°C for varying lengths of time. At each time point, the following assays were performed exactly as described in Figure 1A. Absorption of H-2^b antiserum (● – ●), direct CTL assay (■ – ■), and LDCC assay (▲ – ▲).

The C57BL/6 leukemia EL4 used as TC in our experiments express MHC-Ag of the H-2^b type. Therefore, it was decided to test whether antisera against H-2^b type surface antigens (anti-EL4, anti-H-2^b, and anti-D33) would block LDCC, while antisera against non-MHC cell-surface determinants (anti-GP70, anti-Thy 1.2) would not. It was found that antisera directed against TC MHC-Ag specifically inhibited LDCC, while antisera directed against non-MHC components (e.g., Thy 1.2 and GP70) or irrelevant MHC-Ag did not (Fig. 3). Whether target MHC-Ag are also involved in ODCC was checked. Papain treatment of targets, which removes MHC-Ag (Fig. 2) and incubation with antisera specific for TC MHC-Ag-inhibited ODCC (Table V and Fig. 4), similar to the findings with LDCC.

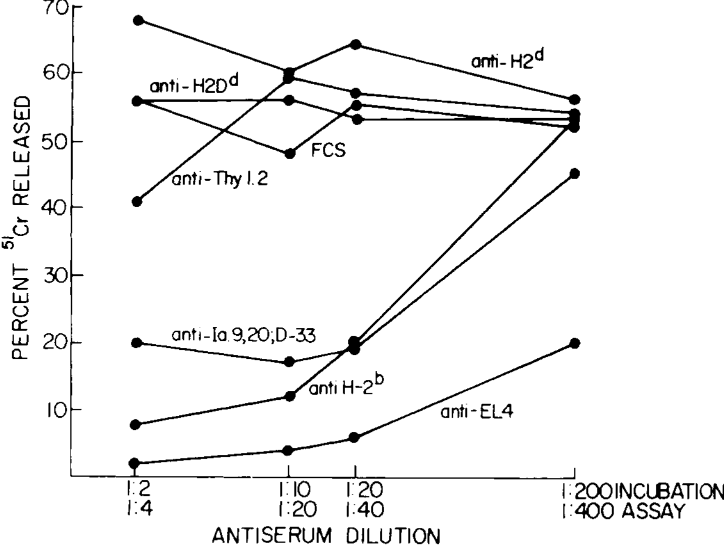

Fig. 3. Inhibition of LDCC by antisera. Two million ^{51}Cr-labeled EL4 target cells were pelleted and resuspended in 50 μl of the various antisera (As) dilutions. Following 30 min incubation at room temperature, the cells were washed 2 × with PBS to remove excess As. The As-treated targets were then mixed with Con A-generated DBA/2 spleen effector cells, CTL:TC ratio of 5:1. Con A was present in the assay at 5 μg/ml. The assay was carried out for 90 min at 37°C.

TABLE V. Influence of Papain Treatment of Target Cells on Their Susceptibility to ODCC

Papain treatment of TC[a] (min)	CTL:TC	^{51}Cr released (percent)
0	2.5	13.4 ± 5.5
	5	19.3 ± 1.8
	10	27.1 ± 1.2
60	2.5	0
	5	0
	10	0

[a]The ^{51}Cr-labeled TC (EL4) were treated with 60 units/ml papain in medium with 5 mM cysteine for 0 or 60 min at 37°C, washed twice with PBS, then oxidized with 2.0 mM $NaIO_4$ in PBS for 10 min at room temperature, and washed. The 10^5 TC were used per assay, which was run in duplicate. Values are corrected for spontaneous release.

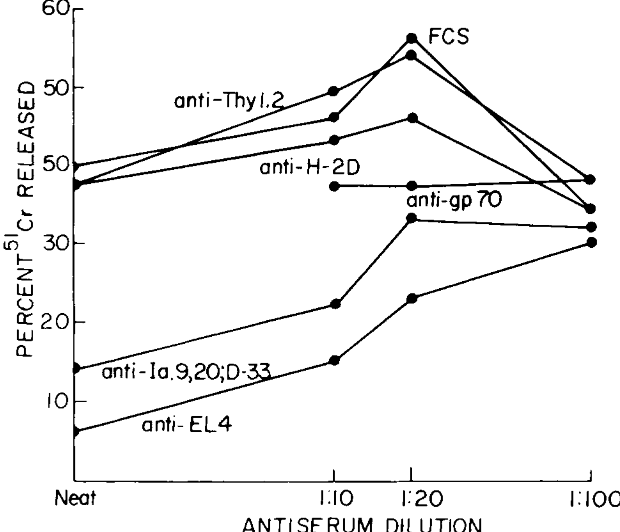

Fig. 4. Antiserum inhibition of lymphocyte-mediated cytolysis of periodate-treated EL4 cells (ODCC). The ^{51}Cr-labeled EL4 cells were incubated at room temperature for 10 min in 2 mM sodium periodate, washed twice, and then incubated with various dilutions of the indicated antisera for 30 min at room temperature. CTL were added to give a CTL:TC ratio of 5:1, and the mixture was centrifuged to promote cell-cell contact. The assay was carried out for 90 min at 37°C.

DISCUSSION

The exquisite specificity of CTL-mediated lysis, allowing detection of minor changes in amino acid sequence of MHC proteins [21], is probably due to interaction of highly specialized CTL surface receptors with specific MHC-Ag expressed on the TC plasma membrane. The notion that MHC-Ag, while operative as specific recognition molecules, are not obligatory for CTL-mediated cytolysis in general rests heavily on the facts that 1) nonspecific lysis of TC could be obtained in the presence of lectins (LDCC) [5] or following oxidation of TC (ODCC) [7] and 2) cell lines deficient in MHC-Ag could still be lysed in LDCC, although to a lesser extent that non-MHC-Ag deficient TC [19, 20]. The abolition of immunological specificity in LDCC and ODCC was thought to be owing to crosslinking or bridging of CTL and TCL through lectin or Schiff-base bridges, respectively [6–9], which supposedly accounted for the recognitive nonspecificity, coupled with activation of the cytolytic function of the CTL by the lectin or oxidative mitogen [10, 13]. However, the work presented herein and in greater detail elsewhere [14, 15] presents a strong case for the role that TC membrane MHC-Ag plays in CTL-mediated lysis.

In LDCC our arguments against the role of lectin as simply an intercellular bridge and killer cell activator are best summarized by the data in Table III and Figure 4, which outline the expected and observed results of the interaction between two killer populations (Con A-CTL and CTL) based on two different hypotheses for the role of Con A in mediating LDCC. The observation that lysis proceeds only in the direction of the Con A-treated CTL partner and not in both directions as predicted by the bridging and activation model, suggests that the lectin facilitates

irrelevant TC lysis by affecting some structures on target cells that are involved in LDCC, but that lectin does not advantageously affect cytolysis-related structures on CTL.

Experiments have been presented which suggest that the TC structures affected by Con A during LDCC and ODCC are products of the MHC. This conclusion is supported by three types of evidence. The ability of TC to be lysed in LDCC as well as in specific CTL-mediated lysis following papain treatment parallels TC expression of serologically detectable H-2 in both disappearance and regeneration. Furthermore, the three parameters are restored with similar kinetics (Fig. 2). Moreover, H-2 deficient TC are relatively poor targets in LDCC (Table IV). Finally lysis in LDCC can be blocked efficiently by antisera directed against TC MHC-Ag, but only poorly if at all by antisera directed against other TC surface antigens (Fig. 3). Collectively, these experiments are compatible with a model for CTL-TC interaction involving recognition of TC MHC-Ag via MHC receptors on the CTL surface. We propose that these receptors are identical with those involved in immunologically specific CTL-mediated lysis of target cells.

The CTL-mediated lysis of periodate-treated TC (ODCC) is similar to LDCC in that periodate treatment of targets gives rise to nonspecific lysis by cytotoxic effectors [6, 7]. It should be noted that this nonspecific lysis of targets occurs irrespective of whether the CTL are induced by allogeneic cells, oxidized syngeneic cells, or lectin [7]. As in LDCC, the role of the TC MHC antigens in ODCC has been unclear. MHC-Ag have been implicated in the proliferative reaction induced by periodate or by neuroaminidase/galactose oxidase [22, 23]. MHC involvement has also been implicated in the $NaIO_4$-treated targets that were MHC-matched with $NaIO_4$-treated stimulating cells were preferentially lysed. Furthermore, preferential blocking of F1 effectors was obtained with cold parental cells of the same MHC as the stimulating parental cells [7]. However, both parental types were lysed equally well by F1 CTL stimulated by $NaIO_4$-treated cells of one parental type, and $NaIO_4$-treated allogeneic as well as syngeneic cells could block lysis by allogeneically stimulated effectors [7]. While the former observations have been used to suggest the participation of MHC components in at least the recognition phase in ODCC [7], the latter observations have been used to argue that mere bridging of TC and CTL through crosslinking of oxidatively generated aldehyde groups on targets, and amino groups on effectors, gives rise to the observed lysis [6, 7]. The inhibition of ODCC demonstrated in this study by papain treatment of TC and by antisera (AS) directed against TC MHC-Ag strongly implicates the involvement of MHC-Ag in this lytic process (Table V and Fig. 4).

A role for TC MHC-Ag in nonspecific lytic processes such as LDCC and ODCC is apparently incompatible with the finding that mouse embryonal carcinoma cells F9, devoid of serologically detectable H-2 components, can in the presence of Con A be lysed by CTL [19]. Embryonal carcinoma cells, however, were lysed to a substantially lesser extent than the control TC [19]. Moreover, F9 cells can be induced to differentiate in vitro to endodermal cells expressing MHC-Ag and be lysed by CTL [24]. Furthermore, monoclonal H-$2D^b$ antibody detected a low percentage of F9 embryonal carcinoma cells expressing MHC-Ag [24]. In another study, Bevan and Hyman reported that an H-2 negative cell line could undergo lysis in an LDCC type reaction; however, the susceptibility of the H-2 negative TC was considerably lower when compared with the H-2 positive TC [20].

In fact, we feel that both of these studies [19, 20] do not contradict our conclusion that TC MHC-Ag do play a role in TC lysis, since in both cases when surface MHC-Ag were reduced lysis was also significantly reduced. The fact that some degree of lysis was still obtained may be attributed to very low levels of MHC antigens still present, or to the existence of other surface antigens structurally related to or antigenically crossreactive with MHC-Ag. An interesting analogy may be found in the almost complete refractoriness either to antigen-specific [25] CTL-mediated lysis or to LDCC, of mouse, human, and chicken red blood cells (Table IV), known to display extremely low concentrations of MHC-Ag.

Although the above experiments support the idea that MHC-Ag are involved in at least the recognition phase of nonspecific LDCC or ODCC, they do not explain how lectin or oxidative modification of TC structures result in CTL-TC recognition ultimately leading to lysis. We would like to propose that MHC determinants are affected by certain lectins or oxidizing agents in a manner causing their redistribution on the target cell surface. This redistribution results in microclustering of MHC-Ag that could stabilize interactions with otherwise nonspecific (low affinity) receptors on the CTL surface by increasing the avidity of the interaction. Such an immunologically nonspecific interaction would not ordinarily take place when the TC MHC-Ag are randomly dispersed. A key element of this hypothesis is that the lectin per se plays no direct role in the intercellular recognition process, i.e., it is not engaged by any antigen-specific receptor on the CTL. TC recognition would take place exclusively through indigenous CTL receptors other than lectin receptors (see also Fig. 5).

On the basis of the results presented thus far, we propose that the mechanism by which cytotoxic lymphocytes recognize and lyse targets in LDCC and ODCC is similar, if not identical, to that employed in specific CTL-mediated killing [1]. The analogy between specific and lectin-dependent T cell killing is supported by the demonstration that both types of killing are multiphasic, proceeding through a Mg^{2+}-dependent adhesion step, a Ca^{2+}-dependent programming for lysis stage and a killer cell-independent lysis [26]. Also, both are similarly susceptible to the same type of inhibitors. Our data furthers the analogy by suggesting that the same receptors are involved in the recognition of both specific and nonspecific targets. The demonstrated obligatory involvement of MHC-Ag in both nonspecific and direct CMC reconciles an apparent fundamental distinction between these two processes and moreover suggests a role for these transmembrane determinants in postrecognition steps of cytolysis.*

This work was supported by USPHS AI 14747 and ACS IM 48 (W.C.) and in part by CB-74183 (G.B.). Part of the work reported in this paper was undertaken during the tenure of an American Cancer Society-Eleanor Roosevelt International Cancer Fellowship awarded by the International Union Against Cancer to G.B. Additional support was provided by the Concern Foundation. G.B. is Isaac and Elsa Bourla Professor of Cancer Research. V.H. is the recipient of American Cancer Society Postdoctoral Fellowship J-415 and W.C. is the recipient of USPHS Research Career Development Award AI 00009.

*Berke G, Clark WR: In Golstein P, Clark W (eds): "Mechanism of Cell-Mediated Lysis." New York: Plenum Press, 1981.

HYPOTHESIS	KILLER CTL (Con A ◆) TARGET CTL	^{51}Cr RELEASE PREDICTED	OBSERVED
A	Con A-pretreated + untreated (^{51}Cr)	+	-
CON A ACTS AS A BRIDGE AND ACTIVATES THE KILLER	untreated + Con A-pretreated (^{51}Cr)	+	+
B	Con A-pretreated + untreated (^{51}Cr)	-	-
CON A MODIFIES THE TARGET CELL, MAKING IT RECOGNIZABLE BY THE KILLER	untreated or Con A-pretreated + Con A-pretreated (^{51}Cr)	+	+

Fig. 5. How Concanavalin A mediates LDCC. According to hypothesis A, Con A (◆) serves to bridge the CTL and the target by binding to lectin-specific cell surface carbohydrates (—<), and also to activate the CTL. Indigenous CTL receptors (—c) capable of reacting with TC MHC-surface antigens are not involved. This hypothesis would predict that Con A bound to one CTL should allow it to bind to and lyse an identical (but not Con A-treated) ^{51}Cr-labeled CTL. This was not observed experimentally (Table III). Hypothesis B states that Con A creates a TC determinant (—●) recognizable by a CTL receptor (—c), probably in addition to the binding to other cell determinants (—<). Both CTL possess —c receptors, but lysis could only occur in the direction of the CTL bearing —● determinants. Actually, when the "aggressor" is pretreated with Con A, it can also be lysed, but since it is not labeled with ^{51}Cr, its lysis is not measured.

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