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Inhibition of Human Lectin-Dependent Cell-Mediated Cytotoxicity, Natural Killer-Like Cytotoxicity, and Cytotoxic T-Lymphocyte-Mediated Cytolysis by Xenoantisera Raised against Concanavalin A-Stimulated Human Lymphocytes[1]

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This report describes the *in vitro* inhibition of several classes of human lymphocyte-mediated lysis by antisera from mice and rats immunized with concanavalin A-stimulated human T-enriched lymphocytes. The inhibitory antibodies in these antisera recognized molecules on the surface of cytotoxic lymphocytes, and did not bind to B-cell lines. Experiments were performed to demonstrate that these antisera were not inhibiting cytolysis by trivial mechanisms such as (i) masking of target cell antigens by anti-target cell antibodies, (ii) agglutinating effector cells, thereby preventing effector-target cell contact, or (iii) toxicity to the effector cells. Dextran dispersion experiments revealed that these antisera inhibit the post-conjugate formation phase of cytolysis mediated by cytotoxic T lymphocytes. These antisera inhibited natural killer-like cytotoxicity and lectin-dependent cell-mediated cytotoxicity as well as lysis mediated by both mixed cell culture-generated cytotoxic T lymphocytes and cytotoxic T-lymphocyte cell lines. These findings suggest that these *in vitro* systems of cell-mediated lysis may share a common step; however the different classes of cytolysis may have been inhibited by separate antibodies in the antisera. This rapid reproducible method of generating inhibitory antisera will facilitate the study of the molecules involved in the post-conjugate formation phase of human cytotoxic T-lymphocyte-mediated lysis.

INTRODUCTION

The clinical importance of lymphocyte-mediated cytotoxicity has prompted extensive investigation of this phenomenon. *In vitro* studies have identified several classes of lymphocyte-mediated cytotoxicity. Natural killer cells, present in non-immunized individuals, selectively lyse certain tumor cells (1). Null or K lymphocytes can mediate the lysis of antibody-coated target cells (2), while lectin-coated target cells are susceptible to lysis by lymphocytes in a reaction termed lectin-dependent cell-mediated cytotoxicity (LDCC) (3). Finally, cytotoxic T lymphocytes (CTL), which have been presensitized to an antigen *in vitro* or *in vivo*, can specifically lyse target cells displaying the appropriate antigen. Cytolysis mediated by

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murine alloimmune CTL is currently the best described *in vitro* model of this process.

Several discrete phases of cytolysis have been identified in this T-cell system. Effector T lymphocytes first identify target cells, then form stable effector–target cell conjugates, and finally irreversibly compromise the integrity of the target cell membrane (4–7). These phases of cytolysis can be separated *in vitro* by altering a number of experimental parameters such as the temperature (8), the divalent cation concentration (9), or the viscosity of the medium (10). While the data are not as complete in the human system, the various classes of human lymphocyte-mediated lysis also seem to proceed through separable target cell recognition and target cell-damaging events (11–13).

A complete molecular understanding of the events occurring in the individual phases of cytolysis will require the isolation and identification of the effector cell molecule(s) operating in each phase of cytolysis. One approach to the identification of molecule(s) potentially involved in cytolysis is the production of antibodies that react with molecules expressed on the plasma membrane of the effector cells. Antibodies directed against many common lymphocyte cell-surface molecules do not inhibit cytolysis in the absence of complement (4, 14, 15), suggesting that antibodies that do inhibit cytolysis are reacting with cell-surface molecules associated with the lytic process. Furthermore the determination that an antiserum inhibits a particular phase of cytolysis provides a good indication that a cell-surface molecule recognized by that antiserum is involved in that phase of lysis. A number of investigators have recently reported the production of antiserum and monoclonal antibodies that inhibit both human (15–17) and murine (14, 18, 19) lymphocyte-mediated cytolysis *in vitro*. However, only two antisera have been shown to inhibit cytolysis after the recognition phase. In the murine system, antisera to lectin-activated murine lymphocytes were found to inhibit T-cell-mediated lysis after conjugate formation (20, 21). In the human system, antiserum to the >200,000-dalton components of supernatants from phytohemagglutinin (PHA)-activated human lymphocytes was shown to inhibit a post-Ca^{2+}-dependent step of CTL-mediated lysis (13).

We report here the *in vitro* inhibition of human natural killer-like cytotoxicity, LDCC, and CTL-mediated lysis by antisera raised against human concanavalin A (Con A)-activated T-enriched lymphocytes. These antisera were found to inhibit the post-conjugate formation phase of CTL-mediated lysis. The simple, rapid, and reproducible production of these inhibitory antisera promises to be a successful method for the development of reagents to study this phase of lysis.

MATERIALS AND METHODS

Media, Cell Lines, and Reagents

Continuous cells were passed biweekly in RPMI 1640 (GIBCO, Grand Island, N.Y.) and 10% fetal calf serum (GIBCO) supplemented with glutamine (Sigma, St. Louis, Mo.) to 2 m*M* (RPMI-10%). Target cell lines included the 3158, a marmoset B-lymphoblastoid cell line; the 3163, a human B-lymphoblastoid cell line (both gifts of Dr. S. Ferrone, Columbia College of Physicians and Surgeons, New York); the WI-L2, a human B-lymphoblastoid cell line; the D98, a substrain of HeLa; and the K562, a human erythroid cell line. The rabbit anti-rat IgG (IgG

fraction) was obtained from Miles Laboratories, Elkhart, Indiana. The ethylene glycol bis(β-aminoethyl ether)-*N,N'*,-tetraacetic acid (EGTA) was obtained from Sigma.

Isolation of Peripheral Blood Lymphocytes

Peripheral blood lymphocytes (PBL) were obtained by centrifugation of defibrinated human blood on Ficoll–Hypaque as described by Boyum (22).

Production of Antiserum

PBL from normal human donors were enriched for T lymphocytes by passage over nylon wool columns (Fenwal Leukopack LP-1, Fenwal Laboratories, Deerfield, Ill.) as described by Werner *et al.* (23). Either PBL or T-enriched lymphocytes were then suspended at 2×10^6 cells/ml in RPMI-10%, and stimulated with 20 μg/ml of Con A (Sigma) for 24 to 48 hr. These cells were then washed three times with 10 ml serum-free RPMI 1640 (RPMI-0%). At 2-week intervals, 20×10^6 or 10×10^6 Con A-stimulated lymphocytes were injected intraperitoneally into 250-g female Sprague–Dawley rats (Simonsen, Gilroy, Calif.) or 12- to 14-week-old Balb/c mice (Simonsen), respectively. Animals were bled from the tail vein 1 week after the last injection. The serum was diluted, heat-inactivated at 56°C for 45 min, and filter sterilized. Normal rat serum and normal mouse serum were prepared in an identical manner from naive animals.

Preadsorption of Antiserum

Cells used for preadsorption were washed three times at 4°C in RPMI-0%. Varied numbers of cells were then suspended in diluted antiserum and incubated at 4°C for 30 min with occasional agitation.

Sources of Effector Lymphocytes

CTL were generated by stimulating PBL with mitomycin C-treated 3158 or WI-L2 cells in mixed cell culture for 5 days. Stimulating cells were suspended in 1 ml of RPMI-0% containing 50 μg of mitomycin C (Sigma); after 1 hr at 37°C the cells were washed four times with RPMI-0%. Cells, at a 20:1 PBL-to-stimulator cell ratio, were cultured at 3×10^6 cells/ml in RPMI-10% supplemented to 50 μM with 2-mercaptoethanol (J. T. Baker, Phillipsburg, N.J.).

Natural killer-like cytotoxicity, the lysis of the natural killer cell-sensitive K562 cell line by mixed cell culture stimulated lymphocytes (16), was determined using effector cells from the cultures described above.

Three T-cell growth factor-dependent CTL cell lines, (HK5, HK7, and HK9) were stimulated with B-lymphoblastoid cell lines and maintained in 50% RPMI-10%, 50% T-cell growth factor, and passaged as described by Yamamoto *et al.*[3]

Lymphocytes from the HK9 cell line served as a source of effector cells for LDCC assays.

[3] Yamamoto, R. S., Ferronc, S., Masunaka, I., and Granger, G. A., Generation of specific human cytotoxic T cells against continuous cell lines *in vitro*. Submitted for publication.

^{51}Cr-Release Assay

The lysis of ^{51}Cr-labeled target cells was determined in 200-μl volumes, using 96-well microtiter plates as previously described (24). The percentage lysis of target cells was calculated with the formula

$$\% \text{ lysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100. \quad [1]$$

Percentage inhibition of lysis was calculated with the formula

$$\% \text{ inhibition of lysis} = 1 - \frac{\% \text{ lysis in antiserum}}{\% \text{ lysis in normal serum}} \times 100. \quad [2]$$

Dextran Dispersion Assay

Dextran dispersion assays, originally described by Martz (10), were performed with the following modifications. Effector–target cell conjugates were formed by centrifuging 2×10^5 CTL and 10^4 ^{51}Cr-labeled target cells at 150*g* for 5 min in 100 μl of RPMI-10%, followed by a 7-min incubation at 25°C. The conjugates were then dispersed by gently pipetting them up and down once with a Pasteur pipet in 1 ml of dextran-containing medium. The final concentration of dextran (243,000 average molecular weight, Sigma) was 10% in all assays. After incubation for 3 hr at 37°C, the cells were pelleted by centrifugation and 0.75 ml of supernatant was removed and counted in a gamma counter. Spontaneous release was determined by incubating labeled target cells in RPMI-10% containing 10% dextran. Total release was determined by incubating labeled target cells in RPMI-10% containing 1% sodium dodecyl sulfate (Sigma) and 10% dextran. All tests were performed in triplicate and the percentage lysis was calculated using Eq [1].

LDCC Assay

Lysis of PHA (Difco, Detroit, Mich.)-coated D98 cells was performed as described by Yamamoto *et al.*[3] Briefly, we established monolayers of 10^5 D98 cells in 1-ml slant tube cultures, washed the monolayer with RPMI-0%, and added 100 μg of PHA in 1 ml of RPMI-0%. After 1 hr the PHA-coated D98 cells were washed with RPMI-0% to remove excess PHA and 1 ml of RPMI-10% containing 10^6 cytotoxic lymphocytes was added to the tubes. At the end of the assay 5 ml of cold RPMI-0% was added, and the tubes were vortexed; the medium was discarded, and the tubes were again rinsed with cold RPMI-0%. At this point the tubes were examined microscopically to ensure that all lymphocytes had been removed. The remaining viable D98 cells were then removed from the glass with trypsin (Difco), and counted in a Coulter counter as previously described (25).

RESULTS

Production of Antisera

Five mice were immunized with Con A-stimulated human PBL; 5 additional mice were immunized with Con A-stimulated human T-enriched lymphocytes. After two immunizations each mouse produced antiserum capable of inhibiting lysis mediated by human CTL in the absence of complement. In these preliminary

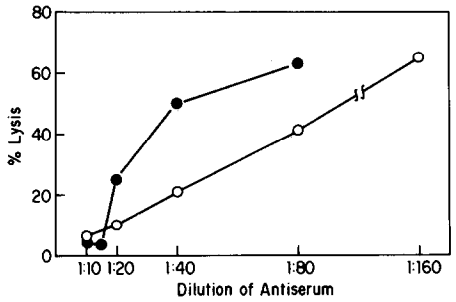

FIG. 1. Inhibition of CTL-mediated lysis by murine and rat antisera to Con A-stimulated human T-enriched cells. The percentage of Wi-L2 target cells lysed in the presence of varied dilutions of rat (O), of murine (●) antiserum is shown. Lysis of Wi-L2 target cells in this 4-hr assay was 65% in the presence of normal mouse or rat serum. A 25:1 effector-to-target cell ratio was used. Spontaneous release was 19%; the standard deviations of the points were less than 4%.

experiments, immunization with Con A-stimulated T-enriched lymphocytes generated antisera with higher inhibitory titers than did immunization with Con A-stimulated PBL. Therefore, only Con A-stimulated T-enriched lymphocytes were used in subsequent immunizations. We immunized 4 rats and more than 20 mice with Con A-stimulated T-enriched lymphocytes. After two immunizations the serum of each of these animals caused greater than 50% inhibition of CTL-mediated lysis at an antiserum dilution of 1:10.

Typical titers of murine and rat antisera after three immunizations are shown in Fig. 1. The titer of these antisera increased with continued immunizations. In Fig. 1, antiserum from the rat caused 50% inhibition of lysis at a 1:60 dilution; antiserum from the same rat caused 50% inhibition of lysis at a 1:120 dilution after five immunizations. Treatment of these antisera with rabbit anti-rat IgG abrogated the capacity of these antisera to inhibit cytolysis, indicating that the observed inhibition of cytolysis was antibody mediated (data not shown).

Inhibitory Antibodies Recognize Effector Cells but Not Target Cells

The following experiments were performed to demonstrate that the inhibition of cytolysis was mediated by antibodies reacting with the effector cells. Anti-target cell antibodies have been shown to inhibit cell-mediated lysis *in vitro* (26). To prevent inhibition of cytolysis by this means we used target cells from the 3158 marmoset cell line, and preadsorbed the antisera on target cells. The data in Table 1 demonstrate that preadsorption on 3158 and WI-L2 target cells did not remove the capacity of these antisera to inhibit CTL-mediated cytolysis. WI-L2 target cells were used in Experiment I and 3158 target cells were used in Experiments II–IV. Experiments V and VI demonstrated that preadsorption on K562 target cells did not remove the capacity of these antisera to inhibit the natural killer-like cytotoxicity. Murine antisera was used in Experiments III and IV, and rat antisera was used in the remainder of the experiments in Table 1. Three bleeds from rat 3 were pooled and termed P3 antiserum. This serum pool, used in Experiments II and V, is further characterized in subsequent experiments described in this paper.

The data in Table 1 also demonstrate that molecules recognized by the inhibitory antibodies are expressed on human lymphocyte cell surfaces. Lymphocytes acti-

TABLE 1

Inhibition of Natural Killer-Like Cytotoxicity and CTL-Mediated Lysis by Antisera to Con A-Stimulated Human T-Enriched Lymphocytes

Experiment	Unadsorbed antiserum	Preadsorbed antiserum[a]						Normal serum
		Target cells		PBL		Effector cells		
		5	40	5	40	5	40	
		Percentage lysis of ^{51}Cr-labeled target cells[b]						
I	16	8	16	—[c]	—	—	—	73
II	8	10	9	14	45	43	57	63
III	2	4	2	3	29	45	64	62
IV	23	24	—	—	—	—	—	58
V	5	9	4	—	—	—	—	38
VI	8	6	15	—	—	—	—	53

Note. The final dilution of antiserum or normal serum was 1:20 in all cases.

[a] The number of cells $\times 10^{-7}$ used to preadsorb 1 ml of antiserum is given below.

[b] A 20:1 effector-to-target cell ratio was used in these 4-hr assays; standard deviations were less than 5%; spontaneous release was less than 22%.

[c] Not done.

vated in mixed cell culture for 5 days were most effective on a per cell basis in the adsorption of inhibitory antibodies. Fresh PBL were less effective in adsorbing the inhibitory antibodies, nevertheless three sequential adsorptions with 4×10^8 fresh PBL/ml of antiserum abrogated the inhibitory capacity of these antisera (data not shown).

Recent studies have suggested that effector cells display new molecules, or new determinants on molecules already present, during interaction with target cells (19, 27). Therefore we performed the following experiment to determine whether P3 antiserum recognizes molecules or determinants preferentially expressed during target–effector cell interaction. Antisera were preadsorbed on either 5×10^6 viable

TABLE 2

Adsorption of Inhibitory Antibodies by CTL and Conjugates

	Unadsorbed antiserum[a]	Antisera preadsorbed on				Normal serum
		10^6 3158	5×10^6 3158	5×10^6 CTL	Conjugates	
		Percentage lysis of 3158 target cells[b]				
P3 antiserum	16	17	16	62	60	75
Murine antiserum	23	—[c]	23	62	62	78

[a] The final dilution of antiserum or normal serum was 1:15 in all cases.

[b] A 25:1 target-to-effector cell ratio was used in these 4-hr assays; standard deviations were less than 5%; spontaneous release was less than 20%.

[c] Not done.

TABLE 3

The Capacity of CTL Pretreated with Antiserum to Mediate Lysis

Antiserum source	Pretreatment of CTL		
	2 hr, 37°C	2 hr, 22°C	10 min, 4°C
	Percentage inhibition of lysis[a]		
P3	87	49	45
Mouse 1	75	84	50
Mouse 2	—[b]	48	—
Mouse 3	73	64	34
Mouse 4	8	5	4

[a] A 25:1 effector-to-target cell ratio was used in these 4-hr assays; standard deviations were less than 5%; spontaneous release was less than 18%.

[b] Not done.

CTL (equivalent to 17×10^7 cells/ml of serum) or conjugates formed by pelleting 5×10^6 CTL and 10^6 3158 target cells and incubating them at 37°C for 30 min. We found that effector–target cell conjugates adsorbed no more inhibitory antibodies than effector cells alone (Table 2).

Pretreatment of CTL with Antiserum

The data in Table 3 reveal that 45 to 87% inhibition of 3158 target-cell lysis resulted when CTL were pretreated with either P3 antiserum or antiserum from three of the four mice tested. In these experiments the CTL were pretreated with a 1:20 dilution of antiserum, and then washed two times with RPMI-10%. Effector cells were suspended at the same density during both pretreatment and the cell-killing assay. Pretreatment of CTL with normal serum had no affect on target-cell lysis, which was greater than 38% in all cases. When CTL were pretreated with antiserum from mouse 4 no more than 8% inhibition of lysis was observed. The data in Table 3 represent the maximum amount of inhibition caused by antiserum from mouse 4 in three separate pretreatment experiments. However antiserum from mouse 4 was just as effective as antiserum from other mice at inhibiting cytolysis when the antiserum was mixed directly with the targets and CTL (Table 1, Experiment IV). Pretreatment of CTL with these antisera for 2 hr at 37°C had no effect on their viability as judged by eosin Y dye exclusion. Pretreatment of the CTL with these antisera for 2 hr at 37° caused only slight aggregation (>60% of the CTL were not attached to other cells and no aggregates of >10 cells were seen). Aggregation is not a plausible mechanism for the inhibition of lysis observed since antiserum from mouse 4 caused the same amount of aggregation as P3 antiserum caused, yet lymphocytes pretreated with antiserum from mouse 4 were still able to lyse target cells.

P3 Antiserum Inhibits CTL-Mediated Lysis after Conjugate Formation

The following experiments were performed to determine whether the P3 antiserum was able to inhibit the phase of cytolysis occurring after the CTL had formed conjugates with the target cells. Target–effector cell conjugates were formed at

TABLE 4

Inhibition of CTL-Mediated Lysis after Conjugate Formation

	Conjugates dispersed in 10% dextran and			
Experiment[a]	P3 antiserum[b]	Normal serum	5 m*M* EGTA	—
	Percentage lysis of 3158 cells[c]			
I	32	61	18	67
II	23	28	1	32
III	10	81	0	77

[a] A 50:1, 100:1, and 20:1 CTL-to-target cell ratio was used in Experiments I, II, and III, respectively.

[b] The final dilution of antiserum or normal serum was 1:10 in Experiment I and 1:20 in Experiments II and III.

[c] The spontaneous release in these 3-hr assays was less than 25%; standard deviations were less than 4%.

25°C. The data in Table 4 reveal that P3 antiserum inhibited the lysis of target cells in these preformed conjugates. The failure of the target cells in these conjugates to lyse after dispersion and incubation at 37°C in medium containing 5 m*M* EGTA and 10% dextran demonstrated that the effector cells did not damage the target cells during conjugate formation (Table 4). When CTL and target cells were dispersed in medium containing 10% dextran without prior conjugate formation lysis was never higher than 3%, indicating that the viscous dextran-containing media prevented effector cells from recycling and forming new conjugates with additional target cells. Thus P3 antiserum inhibited not the recycling of the effector cells, but the post-conjugate formation phase of lysis.

Inhibition of LDCC and Lysis Mediated by T-Cell Growth Factor-Dependent CTL Lines

We found that P3 antiserum inhibited lysis mediated by the three T-cell growth factor-dependent CTL lines tested. The HK7 CTL line was generated by stimulation with the human B-lymphoblastoid cell line 3163. The other two CTL lines, HK5 and HK9, were stimulated with the 3158 marmoset cell line. The ability of P3 antiserum to inhibit cytolysis mediated by HK5 effector cells is shown in Table 5.

We also found that P3 antiserum inhibited LDCC. In these experiments lymphocytes from the HK9 cell line were placed on monolayers of PHA-coated D98 cells. These effector cells lysed 50–80% of the PHA-coated D98 cells in 2 hr. However, D98 cells not coated with PHA were not susceptible to lysis during this time. Figure 2 reveals that the addition of P3 antiserum to the target cells at the same time as the effector cells resulted in 90% inhibition of lysis; even 30 min after the effector cells had been placed on the target cells, the addition of P3 antiserum caused 44% inhibition of lysis.

DISCUSSION

In this manuscript we have described the development of antisera in rats and mice that can inhibit different classes of human lymphocyte-mediated lysis *in vitro*.

TABLE 5

Inhibition of Lysis Mediated by the CTL Cell Line, HK-5

Preadsorption on 3158 cells[a]	Final serum dilution				
	P3 Antiserum				Normal serum
	1:10	1:20	1:40	1:80	1:10
	Percentage lysis of 3158 target cells[b]				
—[c]	−1	0	2	22	58
5	0	1	4	26	—[d]
40	−1	2	3	27	—

[a] The number of 3158 cells × 10^{-7} used to preabsorb the antiserum is given below.

[b] A 20:1 effector-to-target cell ratio was used in this 4-hr assay; spontaneous release was 9%; standard deviations were less than 3%.

[c] Unadsorbed serum.

[d] Not done.

These antisera do not inhibit lysis by trivial means such as killing effector cells, masking target cell antigens, or preventing target–effector cell contact by agglutinating the effector cells. These findings support the concept that these antisera are reacting with effector cell molecules involved in the cytolytic process. The exclusion of eosin Y by effector cells incubated in these antisera, and the ability of effector cells pretreated with antiserum from mouse 4 to lyse target cells demonstrate that these antisera are not toxic to effector cells. Additionally, the use of a xenogeneic marmoset target cell, and the failure of target cells to adsorb inhibitory antibodies eliminated the possibility that anti-target cell antibodies were responsible for the inhibition of lysis. Finally one of the conclusions that can be drawn from the dextran dispersion experiments is that agglutination of effector cells by the antisera is an unlikely explanation for the observed inhibition of lysis. The viscous dextran-containing media prevented the agglutination of effector cells, yet the antiserum was still able to inhibit lysis. It is possible that these antibodies are binding

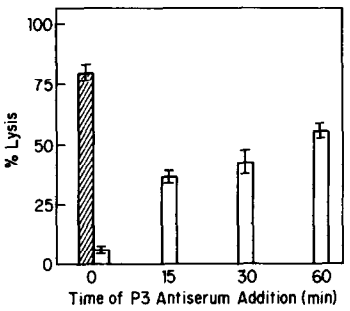

FIG. 2. Inhibition of LDCC by P3 antiserum. The open bars represent the percentage lysis of PHA-coated D98 cells when P3 antiserum was added from 0 to 60 min after the addition of the effector cells. The hatched bar represents the level of lysis that occurred in the presence of normal rat serum. The final serum dilution was 1:30 in all cases; the error bars indicate the range of the data in this 2-hr assay.

to a molecule that is not involved in the lytic process, and are inhibiting cytolysis by steric hindrance. However, the failure of most antibodies directed against cell-surface components to inhibit lysis makes this mechanism of inhibition unlikely.

The adsorption experiments have identified the cell populations expressing molecules associated with the cell lytic process. Lymphocytes activated in mixed cell culture adsorbed inhibitory antibodies at least eight times more effectively on a per cell basis than fresh PBL. The diameter of these activated lymphocytes was less than two times that of the fresh PBL, and, if these cells can be considered spheres, even a twofold increase in diameter would cause only a fourfold increase in surface area. This indicates that cell surface molecules involved in the lytic process are present at a lower density on PBL or that only a small subpopulation of PBL displays molecules expressing determinants recognized by these antisera. The inhibitory antibodies were not adsorbed by the human B lymphoblastoid cell line Wi-L2 or the human erythroblastoid cell line K562. These results combined with the enhanced capacity of mixed cell culture-activated lymphocytes to adsorb the inhibitory antibodies suggest that the molecules recognized by these antibodies are unique to effector cells.

Pretreatment of effector cells with antiserum failed to inhibit lysis only in the case of antiserum from mouse 4 (Table 2). Yet antiserum from mouse 4 was capable of inhibiting lysis when added directly to the cell-killing assay (Table 3). Thus more than one type of inhibitory antibody was generated by this immunization procedure. These different antibodies may recognize two or more different proteins associated with the cytolytic process. Alternatively one protein may express new determinants as it undergoes conformational changes during cytolysis.

At present there is no convenient assay that can be used to identify and isolate molecules involved in the post-conjugate formation phase of cytolysis. Thus antibodies that inhibit lysis after conjugate formation would be useful tools in the study of these molecules. The dextran dispersion experiments have shown that the P3 antiserum inhibits human CTL-mediated lysis after conjugate formation. This strongly suggests that this antiserum recognized molecules associated with the post-conjugate formation phase of cytolysis. The inhibition of natural killer-like cytotoxicity provides additional evidence that P3 antiserum is reacting with molecules involved in the post-conjugate formation phase of lysis. Platsoucas and Good have found that anti-OKT3, an antibody thought to inhibit the recognition phase of CTL-mediated lysis, does not inhibit natural killer-like cytotoxicity (17) while P3 antiserum is able to inhibit both CTL and natural killer-like cytotoxicity.

The inhibition of LDCC, natural killer-like cytotoxicity, and CTL-mediated lysis by these antisera suggests these classes of human lymphocyte-mediated cytotoxicity share a common molecule in their lytic mechanism. However, these different types of cell-mediated cytotoxicity may be inhibited by different antibodies in the antisera. This question will ultimately be resolved by the development of monoclonal antibodies that inhibit the post-conjugate formation phase of lysis. The current lack of such monoclonal antibodies in both the human and the murine system might be remedied by the use of immunization protocols known to elicit antibodies that inhibit this phase of cytolysis. We are currently attempting to develop such inhibitory monoclonal antibodies, and are examining the effector cell surface peptides recognized by these inhibitory antisera.

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