UC Irvine
UC Irvine Previously Published Works

Title

The role of lymphotoxin in target cell destruction by mitogen-activated human lymphocytes I. The correlation of target cell sensitivity to lymphotoxin and the intact lymphocyte

Permalink

https://escholarship.org/uc/item/49b6n9hz

Journal

Cellular Immunology, 15(1)

ISSN

0008-8749

Authors

Kramer, Sandra L
Granger, Gale A

Publication Date

1975

DOI

10.1016/0008-8749(75)90164-1

The Role of Lymphotoxin in Target Cell Destruction by Mitogen-Activated Human Lymphocytes

I. The Correlation of Target Cell Sensitivity to Lymphotoxin and the Intact Lymphocyte

SANDRA L. KRAMER AND GALE A. GRANGER

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92664

Four distinct sublines of mouse L 929 cells (termed alpha, beta, gamma, and delta) were derived and shown to differ markedly in their *in vitro* sensitivity to human lymphotoxin (LT). The alpha L cell is most sensitive and is rapidly destroyed by very low dilutions of LT. This cell is 100 times more sensitive to LT than the most resistant (delta) L cell. The highly lymphotoxin-sensitive alpha cell makes it possible to reproducibly detect LT activity in as little as 0.0005 ml of supernatant medium. Additional studies revealed a direct correlation between the sensitivities of the four L cell sublines to LT and to direct cytolysis mediated by mitogen-stimulated human lymphocytes. The alpha, beta, gamma, and delta L cells were shown to be equally sensitive to antibody-mediated complement-dependent lysis, indicating that the sequence of sensitivities of these L cell sublines to the direct lymphocyte and to LT does not merely reflect a general susceptibility to cell destruction. These results lend further support to the view that lymphotoxin is an important mediator of *in vitro* target cell destruction by human effector lymphocytes.

INTRODUCTION

Lymphocytes have long been recognized as important effector cells in a host of tissue-destructive cell-mediated immune reactions which encompass transplantation and tumor immunity, delayed hypersensitivity, and certain autoimmune diseases. Target cell destruction mediated by lymphocytes *in vitro* is generally thought to be a correlate of cellular immunity *in vivo*.

Studies of lymphocyte-mediated target cell destruction *in vitro* reveal a number of situations in which these reactions occur. While the mechanism(s) are complex, each of these reactions involve, in one form or another, the following general steps: (a) recognition, (b) lymphocyte activation, and finally (c) the actual effector or cytolytic step(s). The physical interaction of an immune lymphocyte with the target cell results in recognition, which is highly specific and mediated presumably by a surface-bound cell-associated receptor (1–3). Membrane recognition induces triggering or activation of the lymphoid cell which converts the lymphocyte to a functional effector cell able to cause target cell cytolysis (4–6). Cell destruction can be specific, with cellular antigens (1, 7, 8), or nonspecific, when lymphocytes are activated by soluble antigens (9–11). Nonimmune cells can also

be converted to nonspecific cytotoxic effector cells by treatment *in vitro* with one of a family of glycoproteins called mitogens (12–14).

Whether immune cytolysis and mitogen-induced cytolysis are related or different phenomena remains unclear, but it is presumed that they may be related, that certain steps are common to both systems, and that both are probably mediated by thymus-derived lymphocytes (T cells) (15, 16). There is, however, controversy centered about the final destructive step(s) that cause the actual cytolysis of the target cell by the lymphocyte. The effector mechanism(s) involved in the destructive phase may be divided into two types; the first, which will be referred to as "direct lymphocyte cytolysis," is promoted by physical contact of the lymphocyte and target cell membranes, and the second, which will be termed "indirect cytotoxicity," is produced when activated lymphocytes secrete a soluble mediator, lymphotoxin (LT), which binds to the surface membrane of the target cell and causes the death of that cell. The present study develops data which implicate LT as a major effector of target cell destruction mediated by lymphocytes both in direct and indirect cytotoxicity.

MATERIALS AND METHODS

Target cells and culture medium. Mouse L 929 cells were obtained from Grand Island Biological Company (GIBCO), Berkeley, CA, and Flow Laboratories, Los Angeles, CA, and the delta subline was generously supplied by Dr. John Holland. The culture medium used in all studies was Eagle's minimum essential medium supplemented with nonessential amino acids, 1 mM sodium pyruvate (GIBCO), 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 3% heat-inactivated (56° C for 30 min) newborn calf serum (Microbiological Associates, Inc., Bethesda, MD).

Lymphoid cells. Human lymphocytes were isolated from adenoid tissue after surgical removal from healthy children as described previously (17). The cell suspension was adjusted to 2×10^6 cells/ml and incubated 16–18 hr at 37° C in 250-ml Falcon plastic flasks to remove adherent cells. Suspensions of nonadherent cells were then washed with fresh culture medium and total viable cell counts were performed in 0.1% eosin Y using a Neubauer chamber. This method routinely yielded a cell suspension consisting of 95%–100% small to medium lymphocytes which were 90%–95% viable.

Mitogen. Phytohemagglutinin P (PHA-P, Control 573353) was obtained from Difco Laboratories, Detroit, MI, and purified phytohemagglutinin (PHA-W, Lots K4944 and K5764) was obtained from Wellcome Reagents, Ltd., Beckenham, England. The optimal dose for human LT production was determined to be 10 μg/ml PHA-P. The optimal dose for coating target cell monolayers was determined to be 10 μg/ml PHA-W.

Production and assay of human lymphotoxin. Lymphocytes were incubated in the presence of 10 μg/ml PHA-P for 4 days at 37° C in 250-ml Falcon plastic flasks at a cell density of 2×10^6 viable lymphocytes/ml. Incubation of lymphocytes alone or in the presence of target cells was in a 5% CO_2–95% air atmosphere. Medium was cleared of cells and debris by centrifugation at 2000 rpm in an International PR-2 refrigerated centrifuge and frozen at −20° C in small aliquots. The medium was assayed for LT activity by mixing with fresh culture medium to give the appropriate dilutions which were then placed on target cell monolayers.

Monolayer tube cultures (16 × 125-mm screw-capped glass tubes) were established 24 or 48 hr prior to testing at a density of 5 or 10 × 10⁴ L cells per 1.0 ml culture. After incubation at 37° C for 48 hr with the test medium, target cell viability was assessed microscopically and then cells were enumerated using a Coulter Counter (Model F). Monolayers were thoroughly washed with 5 ml phosphate-buffered saline, pH 7.2 (PBS). Each tube received 0.5 ml 0.005% trypsin and was incubated at 37° C for 5 min. Tubes were agitated vigorously and 4.5 ml of filtered PBS was added to each. The Coulter Counter sensitivity control settings were: attenuation 1, aperture 32, and threshold 30. Percentage of target cell destruction was calculated from the following formula:

$$\frac{\text{Control} - \text{Experimental}}{\text{Control}} \times 100.$$

Direct lymphocyte cytotoxicity assay. Target L cells were established 48 hr prior to assay in tube cultures at 1.5×10^5 cells per tube, which resulted in a confluent monolayer. Medium was discarded and each tube was gently washed once with warm serum-free medium and then 1.0 ml serum-free medium was added which either did or did not contain 10 μg/ml PHA-W. After 1 hr at 37° C the monolayers were rinsed with 1.0 ml warm serum-free medium to remove unabsorbed PHA and then 1.0 ml of the appropriate lymphocyte cell suspension was added. Nontreated control cultures received 1.0 ml cell-free culture medium. Experimental and control cultures contained 5×10^{-5} M 2-mercaptoethanol. The tubes were then incubated for designated intervals, at which time the monolayers were thoroughly washed with PBS. Extensive tests revealed that all nonadherent cells in these cultures were nonviable. Target cell viability and intactness of the monolayer were first assessed visually, then 0.1 ml 0.1% trypsin, 1 mM EDTA was added to each tube. After 4 min at 37° C, the tubes were agitated vigorously to insure all adherent cells were detached from the glass surface. Tubes were vortexed after adding 0.05 ml serum-containing medium to stablize the cells. Finally, 0.1 ml 0.2% eosin Y was added and viable cell counts were made in a Neubauer chamber. The few lymphocytes remaining at this point were easily distinguished from target L cells by their smaller size. Percentage of destruction was calculated from the following formula:

$$\frac{\text{Nontreated control} - \text{Experimental}}{\text{Nontreated control}} \times 100.$$

Parallel control cultures containing target cells and lymphocytes or target cells alone in the absence of PHA were assayed to determine the extent of spontaneous lymphocyte cytotoxicity.

Antibody-mediated complement-dependent lysis of L cell sublines. A young female New Zealand white rabbit was injected intravenously with a suspension of 60×10^6 L cells composed of 15×10^6 cells of each subline. After 2 wk, a booster injection was administered intravenously; 1 wk later, the rabbit was bled from the ear vein. The blood was allowed to clot at 37° C for 1 hr and the clot was then pelleted at 2000 rpm for 10 min. The serum was aspirated and centrifuged at 2500 rpm for 10 min to remove red blood cells. The serum was stored at −20° C, and heat-inactivated at 56° C for 30 min, just prior to use.

Normal rabbit serum was used as a source of complement; it was obtained from a normal rabbit in the same way the antiserum was collected from the immunized rabbit. The complement serum was stored in small aliquots at $-70°$ C. It was determined to be nontoxic to the L cell sublines and active at a 1:20 dilution.

The sensitivity of the L cell sublines to antibody-mediated complement-dependent lysis was determined in the following assay. Target cell monolayers of each of the four sublines were established in tube cultures at 5×10^4 cells per tube. Forty-eight hour later the medium was discarded and monolayers were washed once with serum-free medium. One milliliter of fresh medium containing the appropriate dilution of antiserum and 0.05 ml complement serum was added to duplicate tubes. Control cultures received fresh medium only. Tubes were then incubated for 1 hr at 37° C at which time viable cell counts were made in a Neu-bauer chamber and percentage of destruction was calculated as described above.

Radiolabeling. PHA-W was radiolabeled with [^{125}I] Na (ICN Corporation, Irvine, CA) by a modification of the chloramine T method used by Klinman and Taylor (18). Briefly, the reaction mixture (0.25 ml volume) contained 800 μg PHA-W, 1.5 mCi carrier-free ^{125}I, and 25 μg chloramine T. After 5-min incubation at room temperature, 120 μg sodium metabisulfite in 0.05 ml volume was added to halt the reaction. The mixture was diluted to 2.0 ml with PBS, and dialyzed against four changes of PBS over 24 hr at 4° C to remove inorganic iodide. The iodinated mitogen was used immediately.

Binding of radiolabeled mitogen. Monolayer tube cultures of each of the four L cell sublines were established 48 hr prior to the binding assay at 1.5×10^5 cells per tube. Medium was discarded and each tube was gently washed once with warm serum-free medium and then 1.0 ml serum-free medium containing 10 μg [^{125}I]-PHA-W was added to each of 10 replicate tubes for each L cell subline. After 1 hr at 37° C the monolayers were rinsed with 1.0 ml warm serum-free medium to remove unabsorbed PHA and these tube cultures were then monitored for ^{125}I in a Baird Atomic gamma counter. Controls consisted of blank tubes containing no cells, labeled for 1 hr with 10 μg [^{125}I]-PHA-W. These tubes were washed twice with PBS to remove unbound mitogen. At the same time replicate monolayer cultures were incubated in an identical fashion with 10 μg/ml unlabeled PHA-W for 1 hr and the cells in these tube cultures were enumerated using the Coulter Counter.

RESULTS

Four distinct sublines originally derived from mouse L 929 cell have been intro-duced into our tissue culture regime at various times during the past 2 yr. They were obtained from commerical suppliers and private laboratories. The alpha L sub-line has been carried in our laboratory for approximately 2 yr, the beta L sub-line for 1 yr, the gamma L subline for 9 mo, and the delta L subline for 6 mo. During routine experiments, it became apparent that there are great differences in the sensitivities of the cells from the four L cell sublines to human LT. The sub-lines are treated identically and routinely passaged just prior to reaching confluency, twice weekly, at a cell density of 5×10^5 cells per 8-oz prescription bottle. Each of the four sublines appears healthy morphologically and the alpha, beta, and gamma L cells are visibly indistinguishable. The delta L cell is slightly different in morphological appearance and seems to have more points of attachment to the

glass surface than cells of the other three sublines. Extensive growth studies revealed no significant difference in their dividing times (22–24 hr).

The four L cell sublines were repeatedly assayed for their sensitivity to a standard preparation of human LT over the entire time course that these studies were in progress (8 mo), which demonstrated that their sensitivity did not change with time. The sublines were tested over an LT dilution range of three log factors, i.e., from 1:2 through 1:2048. The assays were performed a total of eight times with duplicate tubes at each point, and only negligible variation was observed. The results of a typical experiment are shown in Fig. 1. Similar results were obtained with LT medium generated by cells obtained from several different lymphocyte donors and also by lymphocytes cultured with 2 μg/ml PHA-W. It is apparent that the sublines longest in our possession are considerably more sensitive to LT that those more recently obtained. The alpha L cell is six times more sensitive than the beta cell and 110 times more sensitive than the resistant delta cell. The difference in sensitivities of the four sublines is also expressed in the kinetics of target cell cytolysis. Complete destruction of alpha L cell monolayers is routinely observed as soon as 6 hr with potent preparations of LT and in 24 hr with less active supernatant mediums. Monolayers of delta cells, to the contrary, as minimally destroyed by strongly active supernatant mediums and longer incubation periods are required to demonstrate a cytotoxic effect.

Experiments were then designed to determine if the sensitivity of the four target cell sublines to human LT parallels their susceptibility to direct lymphocyte-mediated cytolysis. It seemed clear that the probability of a positive correlation with four distinct, yet very closely related cell lines would be highly improbable unless the destruction in the direct lymphocyte system were mediated by LT. Experiments were, therefore, devised in which the triggering or activation of the lymphocyte would be maximal and held constant for all four sublines in order that we would be measuring only the destructive phase. We selected, therefore, the nonspecific
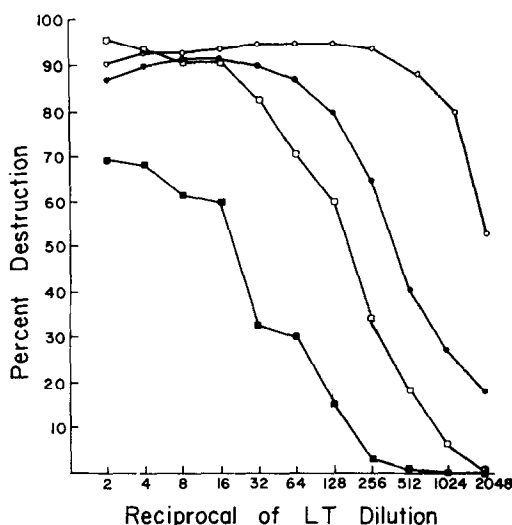

FIG. 1. Target cell destruction mediated by human "lymphotoxin." Various dilutions of human LT were placed on monolayers of alpha (O——O), beta (●——●), gamma (□——□), and delta (■——■) L cells. After 48 hr, the target cells were assayed for viability.
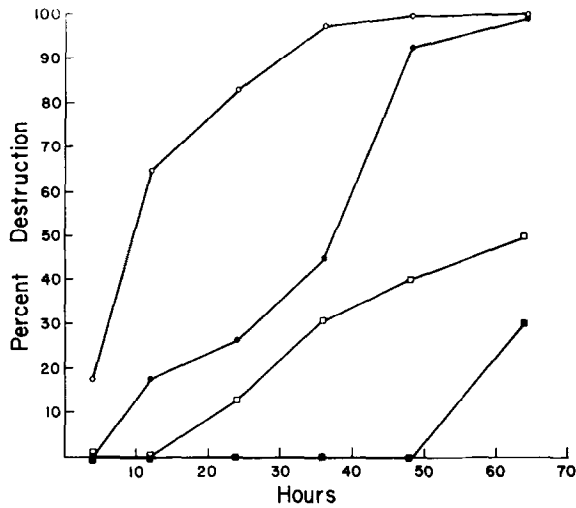
FIG. 2. Kinetics of target cell destruction by PHA-stimulated human lymphocytes. Target cell monolayers, precoated with PHA-W, were exposed to two human lymphocytes per target cell. Viability of the target cells was assayed at various times: alpha L cells (O——O), beta L cells (●——●), gamma L cells (□——□), and delta L cells (■——■).

mitogen system in which target cells are precoated with mitogen and then exposed to nonimmune small lymphocytes.

The sensitivity of each of the four L cell sublines to cytolysis mediated by the intact lymphocyte was determined by two criteria: (1) the kinetics of destruction, and (2) the lymphocyte-to-target cell ratio required for destruction. Intactness of the monolayer was assessed microscopically in each case prior to assaying cell viability and visual destruction always mirrored the actual viable cell counts.
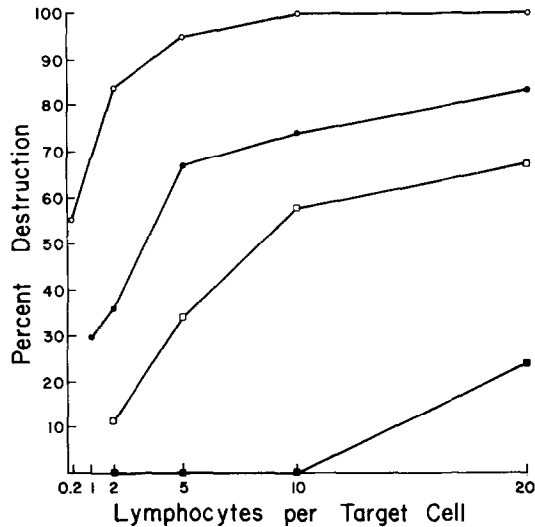

FIG. 3. Ratio of lymphocytes to PHA-coated target cells. Monolayers of target cells, precoated with PHA-W, were exposed to various ratios of lymphocytes to target cells. Viability of the target cells was assayed at 24 hr; alpha L cells (O——O), beta L cells (●——●), gamma L cells (□——□), and delta L cells (■——■).
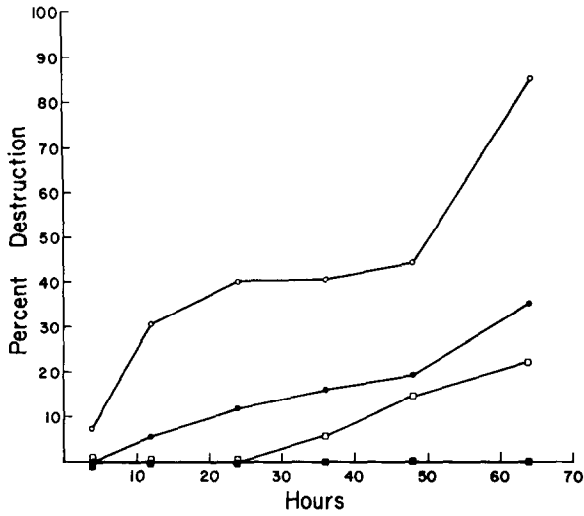
FIG. 4. Kinetics of target cell destruction by unstimulated human lymphocytes. Target cell monolayers were exposed to two unstimulated human lymphocytes per target cell. Viability of the target cells was assayed at various times; alpha L cells (O——O), beta L cells (●——●), gamma L cells (□——□), and delta L cells (■——■).

Each experiment was repeated a total of five to eight times employing lymphocytes from different donors, and with duplicate tubes for each dilution. Less than 10% variation was observed between experiments. The experimental values shown are from representative experiments. Microscopic observation verified that uniform attachment of lymphocytes to the target cells occurred regardless of the target subline used. Results of the kinetics experiments are illustrated in Fig. 2. Monolayer cultures of the four L cell sublines were exposed to two lymphocytes per target
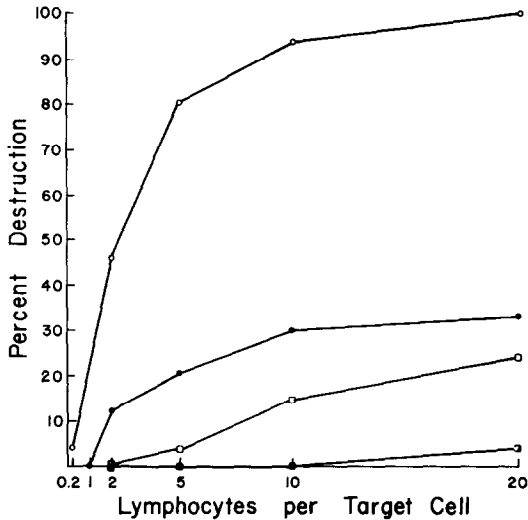

FIG. 5. Ratio of unstimulated lymphocytes to target cells. Monolayers of target cells were exposed to various ratios of unstimulated lymphocytes to target cells. Viability of the target cells was assayed at 24 hr: alpha L cells (O——O), beta L cells (●——●), gamma L cells (□——□), and delta L cells (■——■).

cell and viability of the target cells was assayed at various times, from 4 to 64 hr. The ratio of 2:1 was selected as optimal for these studies, because it was determined in preliminary experiments that a higher ratio would destroy the sensitive alpha target cells too rapidly and a lesser ratio would not result in measurable destruction of the resistant delta cells even at later assay times. Interestingly, dramatic destruction of alpha L cells was observed in 12 hr, a time known to precede appreciable lymphocyte morphologic transformation and DNA synthesis. In contrast, the delta target cell monolayers were only 30% destroyed at 64 hr.

The effect of exposing monolayers of the four distinct target cells to various numbers of lymphocytes for 24 hr is shown in Fig. 3. Marked destruction of alpha target cell monolayers was visibly evident after 4 to 6 hr at the higher lympocyte-to-target cell ratios, while delta target cells completely resisted destruction at ratios less than 20 lymphocytes per target cell. One lymphocyte was fully capable of destroying more than one target cell as evidenced in cultures where 1 lymphocyte per 5 alpha L cells caused greater than 50% destruction in 24 hr.

Figures 4 and 5 represent the spontaneous background destruction manifested in tube cultures where mitogren was omitted, showing the kinetics of destruction and the lymphocyte-to-target cell ratio, respectively. The nature of the destruction manifest in these cultures was visibily indistinguishable from the destruction
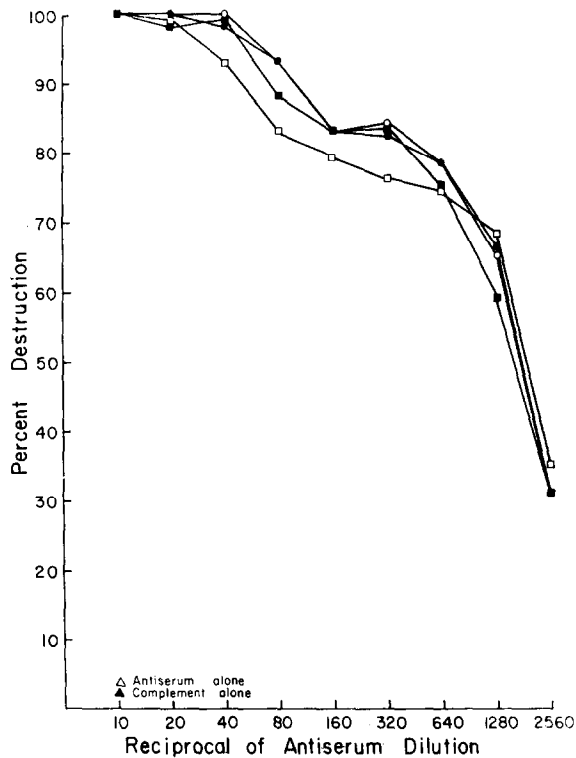

FIG. 6. Sensitivity of target cells to antibody-mediated complement-dependent lysis. Monolayers of target cells were exposed to serial dilutions of rabbit antiserum prepared against the target cells and a 1:20 dilution of rabbit complement for 1 hr at 37° C. Viability of the four target cell sublines was then determined; alpha L cell (O——O), beta L cell (●——●), gamma L cell (□——□), and delta L cell (■——■).

TABLE 1

Binding of ^{125}I-labeled PHA-W to Target L Cells[a]

L cell subline	[^{125}I]PHA-W (cpm bound/10^5 target cells)
Alpha	51,024 ± 2,163
Beta	52,559 ± 1,574
Gamma	59,395 ± 3,751
Delta	80,027 ± 2,727

[a] Monolayer cultures of alpha, beta, gamma, and delta L cells were labeled at 37°C for 1 hr in the presence of 10 μg/ml [^{125}I]PHA-W. Target cells in replicate tubes were enumerated using a Coulter Counter. Results are given as mean ± standard deviation. Blank control tubes bound 9.0% of the total [^{125}I]PHA-W bound in tubes containing a confluent monolayer of alpha L cells.

observed in mitogen-treated cultures. The direct lymphocyte cytotoxicity experiments gave identical results when 50 μg/ml PHA-P replaced the 10 μg/ml PHA-W, or when 3% human AB-positive serum replaced the newborn calf serum, or both.

The possibility remained that the more sensitive target L cells are inherently more fragile to lysis regardless of the means used to destroy them. Experiments were, therefore, conducted to determine the susceptibility of the four subline cells to another means of target cell destruction, namely, antibody-mediated complement-dependent lysis. The results are illustrated in Fig. 6 and demonstrate no significant difference in the sensitivities of the sublines to antibody-mediated lysis.

In order to eliminate the possibility that the four sublines might be binding different amounts of mitogen and thereby stimulating the lymphocytes to different degrees, radioiodinated PHA-W was prepared and the target cells were assayed for their ability to bind the radiolabeled mitogen. Parallel cultures were assayed for total cell number and the results computed to cpm bound per 10^5 target cells. The results are shown in Table 1. There was no significant difference in the amount of mitogen bound by the four target cell sublines.

DISCUSSION

Our previous *in vitro* studies have indicated that continuous cell lines derived from various tissues and different animal species differ greatly in their *in vitro* sensitivity to human LT (19–21). The present study demonstrates the marked differences in *in vitro* sensitivity to human LT which exist between distinct, yet closely related L cells originally derived from a single parent cell strain. Comparing LD_{50} dilutions, the alpha L cell is six times more sensitive than the beta cell, and 110 times more sensitive than the resistant delta cell. The alpha L cell is by far the most sensitive target cell to the *in vitro* action of LT tested to date. The basis for these differences is not clearly understood, but there are several possible explanations. Human LT-induced target cell destruction is at least a two-step phenomenon; the first involves binding of LT to trypsin-sensitive cell surface receptors, and the second, subsequent to binding and temperature-sensitive, causes cytolysis (22). Preliminary experiments indicate they all bind human LT to the same degree which indicates the differences may lie in the final or actual cytolytic step(s).

The precise mechanism which led to the *in vitro* divergence in LT sensitivity of the different L cell sublines remains obscure for the moment. The cells are remarkably similar with respect to cellular morphology, growth rate, and with the exception of the delta L cell, to plating efficiency. The importance of the relative plating efficiencies of target cells in the interpretation of certain *in vitro* direct lymphocyte cytotoxicity results has recently been emphasized by Fidler, who suggested that the actual lymphocyte-to-target cell ratio employed for maximum cell destruction is directly related to the specific plating efficiency of the target cell used in the studies (23). We found the plating efficiencies of alpha, beta, and gamma cells to be 95%–100%, while the delta cell has a plating efficiency of 60%. The effective dosage of LT and activated lymphocytes in these studies was, therefore, 40% higher for the delta cell than for the other sublines, further emphasizing the high resistance of the delta cell.

Employing the highly sensitive alpha L cell has resulted in greatly increasing the sensitivity of the *in vitro* LT assay system. Previously undetectable amounts of LT can now be easily assayed, thus eliminating medium concentration step(s). The alpha L cell has proved invaluable in physicochemical studies of human LT, for fractions can be tested directly, without dialysis or reconstitution, by adding small aliquots to complete tissue culture medium. In addition, the time required for the assay has been shortened from 48 to 16–24 hr.

The present studies were undertaken primarily to determine if the direct and indirect destructive mechanisms are related. We sought to hold constant the initial phases of the reaction, namely, recognition and activation, and to measure the effective capacity of the activated lymphocyte to cause direct and indirect *in vitro* cytolysis of related L cell sublines. In order to reduce aggregation of lymphocytes in free suspension: (1) PHA-W was selected, and (2) the target cells were precoated with PHA-W, thus eliminating the need for mitogen in the suspension. Target cell monolayers were confluent at the time of the addition of lymphocytes to insure that the mitogen was not adhering to the glass surfaces between cells. As near as could be determined, all four L cell sublines bound PHA to the same degree. The more resistant target L cells did, in fact, bind slightly more mitogen, eliminating the possibility that the differences in sensitivity might be due to differences in the degree of activation of the lymphocyte. Employing these conditions, we compared the behavior of these closely related target cells in response to direct lymphocyte-mediated cytotoxicity.

There is a postive correlation between target L cell sensitivity to direct and indirect lymphocyte-mediated *in vitro* cytotoxicity. We were able to demonstrate a direct relationship with respect to both the kinetics of destruction and the lymphocyte-to-target cell ratio required for destruction. The kinetics of cytolysis reveal that the more sensitive the target cell to LT, the more rapidly it is destroyed by effector lymphocytes. The rapid destruction of alpha cells correlates with the observation that LT is exported within a few hours after the lymphocytes have been activated (24). That the direct destruction mediated by lymphocytes requires an active cellular response has been reported previously (25, 26), and is substantiated by our finding that heat-killed lymphocytes (56° C for 30 min) are unable to destroy mitogen-coated alpha cells.

The same direct correlation was observed when we compared the effectiveness of various numbers of lymphocytes to cause direct cytolysis of the various L cell

sublines. It is clear that the more sensitive the target cell to LT, the fewer the number of lymphocytes required to induce cytolysis. The alpha L cell monolayer is 55% destroyed in 24 hr in the presence of 1 lymphocyte per 5 target cells, indicating that the alpha cell is exquisitely sensitive to direct lymphocyte-induced cytotoxicity. In contrast, the delta cell monalyers are only 24% destroyed by a ratio of 20 lymphocytes to 1 target cell. These data indicate that the alpha L cell is 50–100 times more sensitive than the delta L cell, a figure remarkably similar to the 110× difference in sensitivity of the alpha and delta L cells to lymphotoxin.

We have observed significant spontaneous destruction mediated by lymphocytes in the absence of mitogen. Interestingly, the sensitivity of the four L cell sublines to the spontaneous cytotoxicity parallels their sensitivity to LT and to the mitogen-activated lymphocyte. Mononuclear and polymorphonuclear cells have been reported to be capable of causing nonspecific *in vitro* target cell destruction but the aggressor cells employed in this study were purified lymphocyte preparations containing none of these cells. In any case, these activated effector cells are easily detected on highly LT-sensitive target cell monolayers and not detectable on LT-resistant cells strongly suggesting that LT may be operative in these reactions. This is further supported by our previous observations in murine systems that antibodies to mouse LT block both PHA-induced and spontaneous background cytotoxicity (27).

The observation that target cells are not uniformly sensitive to the cytotoxic lymphocyte *in vivo* or *in vitro* is not new. Earlier these differences were ascribed to be differential densities or strengths of the target cell surface antigens. The differences in target cell sensitivity to the lymphocyte *in vitro* were then thought to reflect differences in the degree of triggering at the stage of lymphocyte activation. Further studies have indicated, however, that this may not be the case, and have suggested that the difference in target cell sensitivities may reside in the actual effector or cytolytic phase. The present experiments strongly implicate LT as an important effector in the terminal destructive phase of the mitogen-induced direct lymphocyte cytotoxic reaction. The nonuniformity of target cell sensitivities to the cytotoxic lymphocyte may simply be reflective of the difference in sensitivties to LT. The evidence that each of the four L cell sublines is equally susceptible to antibody-mediated complement-dependent lysis clearly demonstrates that the difference in sensitivities of the target cells to the direct lymphocyte and to LT is not merely the manifestation of an inherent fragility of the target cells to destruction.

The PHA-activated direct lymphocyte cytolytic system provides a model which may have broader relevance and encompass the mechanisms operative at the terminal phase of the cellular immune system. Recent experiments have demonstrated that target cell cytolysis by immune lymphocytes requires an active secretory system (28). Walker and Lucas have recently demonstrated that a rabbit antiserum prepared against human LT inhibits target cell destruction by PHA-activated nonimmune and PPD-activated immune lymphocytes and by lymphocytes sensitized *in vivo* to renal transplants and tumor tissue (29). The precise degree of correlation between target cell sensitivity to direct and indirect cytotoxicity in these experiments further attests to LT as a potent effector molecule in direct lymphocyte-mediated cytotoxicity reactions *in vitro*.

ACKNOWLEDGMENTS

REFERENCES

1. Rosenau, W., and Moon, H. D., *J. Nat. Cancer Inst.* **27**, 471, 1961.
2. Canty, T. S., and Wunderlich, J. R., *J. Nat. Cancer Inst.* **45**, 761, 1970.
3. Golstein, P., Svedmyr, E. A. J., and Blomgren, H., *Eur. J. Immunol.* **2**, 380, 1972.
4. Brunner, K. T., Mauel, J., Cerottini, J. C., and Chapuis, B., *Immunology* **14**, 181, 1968.
5. Brondz, B. D., *Transplant. Proc.* **1**, 416, 1969.
6. Henney, C. S., and Bubbers, J. E., *J. Immunol.* **110**, 63, 1973.
7. Govaerts, A., *J. Immunol.* **85**, 516, 1960.
8. Wilson, D. B., *J. Cell. Comp. Physiol.* **62**, 273, 1963.
9. Ruddle, N. H., and Waksman, B. H., *J. Exp. Med.* **128**, 1267, 1968.
10. Granger, G. A., Shacks, S. J., Williams, T. W., and Kolb, W. P., *Nature (London)* **221**, 1155, 1969.
11. Dumonde, D. C., Wolstencroft, R. A., Panayi, G. S., Matthew, M., Morley, J., and Howson, W. T., *Nature (London)* **224**, 38, 1969.
12. Holm, G., Perlman, P., and Werner, B., *Nature (London)* **203**, 841, 1964.
13. Moller, E., *Science* **147**, 873, 1965.
14. Granger, G. A., and Kolb, W. P., *J. Immunol.* **101**, 111, 1968.
15. Cerottini, J. C., Nordin, A. A., and Brunner, K. T., *Nature (London)* **228**, 1308, 1970.
16. Janossy, G., and Greaves, M. F., *Clin. Exp. Immunol.* **9**, 483, 1971.
17. Kolb, W. P., Williams, T. W., and Grander, G. A., *In* "In Vitro Methods in Cell-Mediated Immunity" (B. R. Bloom and P. R. Glade, Eds.), pp. 333–342. Academic Press, New York, 1971.
18. Klinman, N. R., and Taylor, R. B., *Clin. Exp. Immunol.* **4**, 473, 1969.
19. Williams, T. W., and Granger, G. A., *Nature (London)* **219**, 1076, 1968.
20. Granger, G. A., and Williams, T. W., *In* "Progress in Immunology," pp. 437–445. Acadmeic Press, New York, 1971.
21. Williams, T. W., and Granger, G. A., *Cell. Immunol.* **6**, 171, 1973.
22. Hessinger, D. A., Daynes, R. A., and Granger, G. A., *Proc. Nat. Acad. Sci. USA* **70**, 3082, 1973.
23. Fidler, I. J., *J. Nat. Cancer Inst.* **50**, 1307, 1973.
24. Williams, T. W., and Granger, G. A., *J. Immunol.* **103**, 170, 1969.
25. Holm, G., and Perlmann, P., *Nature (London)* **207**, 818, 1965.
26. Lundgren, G., and Moller, G., *Clin. Exp. Immunol.* **4**, 435, 1969.
27. Granger, G. A., *Amer. J. Pathol.* **60**, 469, 1970.
28. Plant, M., Lichenstein, L. M., and Henney, C. S., *J. Immunol.* **110**, 771, 1973.
29. Walker, S. M., and Lucas, Z. J., *Transplant. Proc.* **5**, 137, 1973.