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Inhibition of Human NK-Induced Cell Lysis and Soluble Cell-Lytic Molecules with Anti-Human LT Antisera and Various Saccharides[1]

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The present study examines and compares the cytolysis of K-562 and MOLT-4 cells mediated by human natural killer (NK) cells from fresh peripheral blood and lymphotoxins (LT) derived from human lymphoid cell populations after lectin stimulation *in vitro*. Lymphotoxins were obtained from 5-hr concanavalin A (Con A)-restimulated human peripheral blood lymphocytes (PBL) which were precultured for 5 days in medium and fetal calf serum or with allogeneic human B-lymphoid cell lines. Two classes of probes were employed in both direct (cell) and indirect (supernatant) induced target-cell lysis: (a) various saccharides and (b) antibodies reactive with human LT forms. Two sugars, *N*-acetylglucosamine and α-methylmannoside, were able to inhibit direct cell lysis of both MOLT-4 and K-562 target cells. However, saccharide inhibition was distinct for each type of target even when effector cells were obtained from the same donor. These same saccharides were also able to inhibit 20–30% of the total LT activity in a supernatant for L-929 cells and 50–90% of the lytic activity on MOLT-4 cells. Anti-human $F(ab')_2$ (IgG) and rabbit anti-α_2 LT sera blocked direct cell lysis of MOLT-4 and K-562 targets in 50% of the experiments. The anti-α_2 LT serum only recognizes a portion of the LT forms in these supernatants. These results reveal that, while both direct and indirect cell lysis are complex phenomena, they may both occur in some cases by a common mechanism(s).

INTRODUCTION

Several reports indicate preactivated human peripheral blood lymphocytes (PBL) rapidly release cell-lytic lymphotoxins *in vitro* upon receiving a secondary 5-hr lectin stimulus (1–3). Cell-lytic forms detected in supernatants from lectin-stimulated lymphocytes preactivated by coculture with fetal calf serum or allogeneic B-cell lines were lytic for L-929, K-562, and MOLT-4 targets in an 18-hr ^{51}Cr-release assay (3). Additional studies demonstrated these cell-lytic molecules are heterogeneous and can be separated by their cell-binding and lytic activities into different groups: (a) those lytic for L cells which can bind selectively to the K-562 but do not lyse the latter targets, and (b) those which can bind nonselectively to MOLT-4, K-562, and Raji cells and which lyse the NK-sensitive MOLT-4 and K-562 targets.

The present studies describe the effects of two classes of probes on both the cytotoxic activity of soluble mediators and *in vitro* cell lysis mediated by human natural killer (NK) effector cells from fresh peripheral blood. The probes utilized in these studies are (a) antisera reactive with the α component of the human LT system (4–7) and

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(b) various saccharides previously found to be inhibitory in human natural cytotoxicity (NC) systems *in vitro* (8, 9).

MATERIALS AND METHODS

Tumor cell lines and culture media. The tumor cell lines and culture media employed in these studies have been described previously (3).

Production of supernatants. Methods for production of supernatants have been previously described in detail (3).

Lymphotoxin (LT) assay. The LT assay using nondividing L-929 target cells has been described previously (3).

Sugars. Stock solutions of sugars were made in RPMI 1640 (Grand Island Biological Co. (GIBCO), Grand Island, N.Y.) culture media (RPMI-S) or RPMI 1640 plus 10% fetal calf serum (FCS, GIBCO) (RPMI-10%) at 0.2 *M* concentrations and sterilized by filtration prior to use. The following sugars were used: *N*-acetyl-D-glucosamine, α-L-fucose, and α-methyl-D-mannoside (Sigma Chemical Co., St. Louis, Mo.); D-galactose and D-mannose (Calbiochem-Behring, La Jolla, Calif.); and lactose (Mallinckrodt, St. Louis, Mo.).

Rabbit antisera. Rabbit anti-α_2 LT antiserum was obtained as previously described (4, 5). Rabbits were injected with purified $F(ab')_2$ fragments of human IgG prepared by techniques described previously (7). Each animal was immunized with 1 μg of $F(ab')_2$ in Freund's complete adjuvant by the method of Yamamoto *et al.* (5). Animals received injections every 3 weeks and were bled 7 days after each injection. Serum was collected and pooled after six bleedings. Control serum was collected from each animal before the first exposure to antigen.

Isolation of effectors. Human lymphocytes (PBL) were isolated from defibrinated blood after density gradient centrifugation on Ficoll–Hypaque (10). The interface cells were washed, resuspended in RPMI-10% at 2×10^6/ml, and incubated in 60 ml RPMI-10% in a T 75-cm^2 tissue culture flask for 60 min at 37°C. The nonadherent lymphocytes were collected, sedimented, and resuspended at 10^7 cells/ml in RPMI 1640.

Cytotoxic assay. All cytotoxic reactions were carried out in round-bottom microcytotoxicity plates (Flow Laboratories, Inglewood, Calif.) containing a final volume of 0.2 ml. To each well was added appropriate volumes of RPMI-10% and the stock sugar solution, antiserum, or control normal rabbit serum (NRS), followed by 25 or 50 μl of effector lymphocytes. The ^{51}Cr-labeled target cells were then added in 50-μl amounts. The effector-to-target (E:T) ratios were 25:1 or 50:1. After incubation at 37°C for 4 hr, the release of ^{51}Cr label was measured by determining radioactivity present in cell-free supernatant with the Titer-Tek supernatant collection system (Flow Laboratories) and quantitated in an automated gamma counter (Beckman Instruments, Fullerton, Calif.). Supernatant cytotoxicity has been previously described (3). Briefly, 10^4 ^{51}Cr-labeled MOLT-4 target cells in 0.02 ml was added to microtiter wells containing 0.05 ml of sample or control medium plus 0.05 ml of RPMI-S or RPMI-10% and then incubated at 37°C for 18 hr. The release of ^{51}Cr was measured as described above. Spontaneous ^{51}Cr release was 1–2%/hr from target cells. The total ^{51}Cr releasable was determined by the addition of 0.2 ml of 3% (w/v) sodium dodecyl sulfate solution to the target cells. The spontaneous release was determined by adding RPMI-10% in place of effector lymphocytes. Target cell destruction was calculated by the formula

$$\% \ ^{51}\text{Cr release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100.$$

The data are represented as the means of triplicate samples ± SD. Sugars or antisera alone were not toxic for the target cells at the concentrations we employed.

The percentage inhibition of target cell lysis by the sugars was calculated as

$$\% \text{ Inhibition of lysis} = 1 - \frac{\% \ ^{51}\text{Cr release with sugar}}{\% \ ^{51}\text{Cr release without sugar}} \times 100.$$

The percentage inhibition of target cell lysis by antiserum was calculated as

$$\% \text{ Inhibition of lysis} = 1 - \frac{\% \ ^{51}\text{Cr release with antiserum}}{\% \ ^{51}\text{Cr release with NRS}} \times 100.$$

The percentage inhibition, in each case, was calculated with results obtained for identical concentrations of test and control serum.

Inhibition of LT on L-929 by saccharides and antisera inhibition of LT on L-929. The appropriate concentration of each sugar in RPMI-10% was added to each L-929 test monolayer and was followed by the addition of 0.10 ml of a cell-lytic supernatant. After 18–24 hr, the average number of viable cells remaining in these cultures was compared to the average number present in tubes containing only the supernatant (+LT) or RPMI-S (control). The percentage lysis and percentage inhibition of lysis were determined as

$$\% \text{ Lysis} = \frac{(\text{control}) - (+\text{LT})}{(\text{control})} \times 100,$$

$$\% \text{ Inhibition of lysis} = \frac{(\text{LT} + \text{sugar}) - (+\text{LT})}{(\text{control}) - (+\text{LT})} \times 100.$$

The sugars alone did not affect L-929 cell viability at the concentrations used. To determine the effect of antisera on LT activity, equal volumes of supernatant and antiserum or NRS were mixed and allowed to stand at room temperature for 30 min before testing on L-929 cells. From 75 to 100 μl was then added in duplicate to 1.0-ml tube monolayer cultures. After 18–24 hr, the average number of viable cells remaining in cultures containing supernatant and antiserum (LT + Ab) was compared to the average number in cultures containing supernatant and NRS (LT + NRS) or RPMI-10% alone (control). The percentage neutralization of LT activity was determined using the formula

$$\% \text{ Neutralization} = \frac{(\text{LT} + \text{Ab}) - (\text{LT} + \text{NRS})}{(\text{control}) - (\text{LT} + \text{NRS})} \times 100.$$

The antisera or NRS alone do not affect L-929 cell viability.

RESULTS

Inhibition of human NK activity against MOLT-4 target cells in vitro by different saccharides. Six different saccharides were tested for their effects on lysis of ^{51}Cr-labeled MOLT-4 target cells mediated by freshly isolated PBL. The results of these studies are presented in Table 1. The three sugars which inhibit lysis most frequently at 50 m*M* are *N*-acetylglucosamine, α-methylmannoside, and lactose. In eight out of

TABLE 1

The Effects of Various Saccharides on Human NK-Induced Lysis of MOLT-4 Target Cells *in vitro*[a]

Expt.[d]	E:T ratio	Percentage ^{51}Cr release[b]	Percentage inhibition of lysis in the presence of 50 m*M* concentrations[c]					
		No sugar	*N*-Acetyl-glucosamine	α-Methyl-mannoside	Lactose	L-Fucose	D-Mannose	D-Galactose
1	50:1	41 ± 5	51	54	46	39	44	54
2	50:1	65 ± 7	20	22	9	0	18	8
3	25:1	35 ± 9	54	51	ND[e]	49	77	29
4	25:1	49 ± 3	39	20	22	18	37	49
5	25:1	52 ± 6	17	13	ND	13	0	19
6	25:1	48 ± 5	23	40	12	8	21	12
7	25:1	32 ± 3	38	31	53	0	12	19
8	25:1	58 ± 5	34	34	41	28	7	19
9	25:1	42 ± 3	33	64	57	33	17	43

[a] Freshly isolated PBL were tested for levels of NK in the presence of 50 mM concentrations of different sugars and ^{51}Cr-labeled MOLT-4 target cells as described under Materials and Methods. The means ± SD are given for triplicate wells after 4 hr of incubation. Toxicity of sugars alone was less than 5%.

[b] Percentage lysis as calculated by the formula under Materials and Methods.

[c] Percentage inhibition of lysis as calculated by the formula under Materials and Methods.

[d] Seven different donors were used in these studies. Experiments 4–6 utilized freshly isolated lymphocytes obtained from a single donor on three separate occasions. Experiments 6–9 were conducted on the same day with target cells from a single culture and PBL obtained from different donors.

[e] ND, not done.

nine experiments, the presence of *N-acetylglucosamine* results in 20–54% inhibition of lysis. Also *α-methylmannoside* inhibits eight out of nine NK reactions with the percentage inhibition ranging from 20 to 64%. Lactose was effective in five out of seven assays with 20 to 57% inhibition of lysis. The remaining three sugars, *L-fucose, D-mannose,* and *D-galactose,* inhibit in four out of nine experiments. The levels of inhibition by these sugars range from 20 to 77%. Additional studies employing different levels of the various saccharides were conducted, and it was demonstrated that little or no inhibition of lysis was detectable at 10 m*M* concentrations of these sugars, and 25 m*M* concentrations were less inhibitory than 50 m*M* concentrations (data not shown). Higher levels of these sugars were not tested. However, experiments were conducted using two effector-to-target cell ratios (E:T), 50:1 and 25:1. These studies revealed that there is a higher level of inhibition of lysis by sugars at the lower E:T ratios than at the higher E:T ratios.

There are multiple explanations for the different patterns of sugar inhibition in different experiments. Several possibilities are (a) effector cells obtained from different donors are heterogeneous and exhibit different patterns of sugar inhibition, (b) the differences are due to heterogeneity within the target-cell population, and (c) alteration in the type or degree of expression of membrane components on either target or effector cell(s). To examine the first of two possibilities, the sugar inhibition patterns for lymphocytes obtained from a single donor on three separate occasions were compared (Expts. 4–6, Table 1). Clearly, although the levels of cytotoxicity in the absence of additional sugars are similar, each experiment results in a different pattern of sugar inhibition. Five sugars inhibit in Experiment 4 (*N*-acetylglucosamine, D-galactose, lactose, α-methylmannoside, and D-mannose), none of the five sugars tested in Experiment 5 is inhibitory, and three sugars inhibit in Experiment 6 (*N*-acetylglucosamine, α-methylmannoside, and D-mannose). Thus, lymphocytes obtained from the

same donor give different patterns of sugar inhibition of MOLT-4 lysis. The results of four experiments utilizing a single target-cell population and effector cells from four different donors were also compared (Expts. 6–9, Table 1). While three sugars have similar effects in each experiment (*N*-acetylglucosamine, α-methylmannoside, and D-mannose), the remaining sugars (lactose, L-fucose, and D-galactose) give variable results.

Inhibition of human NK activity against K-562 target cells in vitro by different saccharides. The same sugars were tested for their effects on lysis of ^{51}Cr-labeled K-562 target cells by freshly isolated PBL *in vitro.* The results are presented in Table 2. In three out of five experiments, *N*-acetylglucosamine and α-methylmannoside exhibit significant levels of inhibition, ranging from 21 to 44%. Lactose inhibits two out of four experiments (24–53% inhibition of lysis). The remaining sugars inhibit lysis less frequently. The levels of inhibition by these sugars, L-fucose, D-mannose, and D-galactose, range from 21 to 36%. Lymphocytes obtained from the same donor on different occasions give different patterns of inhibition (Expts. 4 and 5, Table 2).

Inhibition of supernatant lysis of L-929 cells (LT activity) by saccharides. Experiments were designed to determine if the same sugars which inhibit NK activity also inhibit lysis of L-929 cells by soluble lymphocyte products. Supernatants were obtained from human PBL by culturing lymphocytes for 5–7 days in RPMI-10% (cPBL) and then stimulating with Con A for 5 hr. The cell-free supernatants were tested for lytic activity on L-929 cells in the presence of different sugars at 20 m*M*. The results of these studies are shown in Table 3. Two of the six sugars inhibit a portion of the supernatant activity: *N*-acetylglucosamine inhibits (21–32%) in four out of six experiments, α-methylmannoside inhibits (20–26%) in two out of six experiments, and finally, none of the other sugars inhibited lysis. Higher concentrations of *N*-acetylglucosamine, 100 and 50 m*M*, did not result in a significant increase in the level of inhibition of LT activity, and 100 m*M* concentrations of the other sugars were also not inhibitory for LT activity on L-929 cells in these supernatants (data not shown).

Inhibition of supernatant lysis of MOLT-4 target cells by N-acetylglucosamine and

TABLE 2

The Effects of Various Saccharides on Human NK-Induced Lysis of K-562 Target Cells *in Vitro*[a]

Expt.[d]	E:T ratio	Percentge ^{51}Cr release[b]	Percentage inhibition of lysis in the presence of 50 mM concentrations[a]					
		No sugar	*N*-Acetyl-glucosamine	α-Methyl-mannoside	Lactose	L-Fucose	D-Mannose	D-Galactose
1	50:1	36 ± 6	44	25	53	14	36	31
2	50:1	63 ± 3	11	6	16	16	11	2
3	25:1	62 ± 6	16	21	24	21	5	11
4	25:1	37 ± 2	35	0	3	0	0	16
5	25:1	53 ± 5	32	26	ND[e]	23	15	36

[a] Freshly isolated PBL were tested for levels of NK in the presence of 50 mM concentrations of different sugars and ^{51}Cr-labeled K-562 target cells as described under Materials and Methods. The means ± SD are given for triplicate wells after 4 hr of incubation. Toxicity of sugars alone was less than 5%.

[b] Same as footnote *b* in Table 1.

[c] Same as footnote *c* in Table 1.

[d] Four different donors were used in these studies. Experiments 4 and 5 were conducted on different days and utilized freshly isolated PBL from the same donor.

[e] ND, not done.

TABLE 3

The Effects of Different Saccharides on Lymphotoxin-Induced Lysis of L-929 Cells[a]

Sample	Percentage lysis[b]	Percentage inhibition of lysis in the presence of different sugars at 20 m*M* concentrations[c]					
	No sugar	*N*-Acetyl-glucosamine	α-Methyl-mannoside	Lactose	L-Fucose	D-Mannose	D-Galactose
1	59 ± 1	27	19	10	14	0	10
2	61 ± 4	7	26	2	ND	0	ND
3	60 ± 6	32	18	ND[d]	ND	ND	ND
4	64 ± 5	23	20	12	5	3	12
5	75 ± 1	21	12	8	1	1	3
6	75 ± 3	11	8	0	3	0	0

[a] Supernatants obtained from cPBL after 5 hr of stimulation with 10 μg/ml Con A were tested for lytic activity on L-929 cells in the presence of different sugars at 20 m*M* as described under Materials and Methods. The mean ± SD for percentage lysis was determined for duplicate or triplicate culture tubes after 18–24 hr.

[b] Percentage lysis of L-929 cells as calculated by the formula under Materials and Methods.

[c] Percentage inhibition of lysis as calculated by the formula under Materials and Methods.

[d] ND, not done.

α-methylmannoside. The two sugars which inhibit NK activity against MOLT-4 target cells and supernatant lysis of L-929 cells were tested for inhibition of supernatant lysis of MOLT-4 target cells. Supernatants were generated from cPBL as described under Materials and Methods. The results shown in Table 4 indicate that 50 m*M* concentrations of each sugar inhibit (54–92%) MOLT-4 lytic activity present in 5-hr Con A-stimulated supernatants from cPBL. There were no significant levels of inhibition detected at 20 m*M* concentrations of these sugars.

Inhibition of natural killing by rabbit anti-human α_2 and rabbit anti-human F(ab')$_2$ antisera. Two antisera capable of neutralizing L-929 cell-lytic forms of LT activity from human lymphocytes were tested for inhibition of natural killing against MOLT-4 and K-562 target cells. Rabbit antiserum was raised against the α_2 subclass of human α LT and against human F(ab')$_2$ IgG fragments as described under Materials and

TABLE 4

The Effects of Sugars on MOLT-4 Lysis Mediated by Supernatants from Lectin-Stimulated cPBL[a]

Expt.	Percentage ^{51}Cr-release[b]	Percentage inhibition of lysis in the presence of sugars at 50 m*M* concentrations[c]	
	No sugar	*N*-Acetylglucosamine	α-Methylmannoside
1	48 ± 8	92	85
2	46 ± 14	65	ND[d]
3	31 ± 13	81	68
4	26 ± 6	54	81

[a] Supernatants from 5-hr Con A-stimulated cPBL were tested for lytic activity on ^{51}Cr-labeled MOLT-4 target cells in an 18-hr ^{51}Cr-release assay in either the presence or absence of 50 mM concentration of sugars as described under Materials and Methods.

[b] Same as footnote *b* in Table 1.

[c] Same as footnote *c* in Table 1.

[d] ND, not done.

Methods. The results of studies employing these antisera are presented in Table 5. Of seven separate experiments with MOLT-4 cells as targets, anti-α_2 LT inhibits in three experiments (36–72% inhibition) and anti-human F(ab')$_2$ inhibits in four experiments (21–58% inhibition). With K-562 target cells, both antisera inhibit in two of the three experiments (45–49% inhibition with anti-α_2 LT, 62% inhibition with anti-human F(ab')$_2$).

Four of the experiments presented in Table 5 were performed on the same day with target cells obtained from a single cell culture (Expts. 4–7, Table 5). The effector lymphocytes were obtained from four different donors. The results of these four experiments indicate that the more lytically active PBL populations are inhibited to a greater extent by these antisera. This does not appear to be true when experiments performed on different days are compared.

The effects of rabbit anti-human α_2 LT and rabbit anti-human F(ab')$_2$ antisera on supernatant lysis of L-929 and MOLT-4 target cells. These same antisera were tested for inhibition of LT activity on L-929 cells in supernatants obtained from cPBL or LCCL-derived PBL after stimulation with Con A for 5 hr. The results (not shown) indicate that significant levels of inhibition ($P < 0.05$) were obtained with each antiserum in two out of five experiments (30% inhibition of lysis).

These sera were also tested for inhibition of supernatant lysis of MOLT-4 target cells (data not shown). In experiments utilizing five different supernatants, no inhibition of lysis by anti-F(ab')$_2$ serum was detected. Two supernatants were inhibited when anti-α_2 LT serum was added to the assay for cytotoxicity. The percentage ^{51}Cr release in these experiments was reduced from 40 to 50% from 63 ± 6 to 37 ± 6% by the anti-α_2 LT antiserum.

TABLE 5

The Effects of Rabbit Anti-Human α_2 LT and Rabbit Anti-Human F(ab')$_2$ Antisera on NK of MOLT-4 and K-562 Target Cells *in Vitro*[a]

Expt.[d]	Target cell line	Dilution of sera	Percentage ^{51}Cr release[b]	Percentage inhibition of lysis in the presence of antisera[c]	
			NRS	Anti-α_2 LT	Anti-F(ab')$_2$
1	MOLT-4	1:10	70 ± 2	6	13
2	MOLT-4	1:20	63 ± 3	13	29
3	MOLT-4	1:10	62 ± 7	10	37
4	MOLT-4	1:20	60 ± 5	72	58
5	MOLT-4	1:20	50 ± 4	36	12
6	MOLT-4	1:20	24 ± 1	37	21
7	MOLT-4	1:20	18 ± 5	6	0
8	K-562	1:10	55 ± 5	11	15
9	K-562	1:20	47 ± 5	45	62
10	K-562	1:10	37 ± 6	49	62

[a] Rabbit anti-α_2 LT and rabbit anti-human F(ab')$_2$ antisera were tested for inhibition of NK in a 4-hr ^{51}Cr-release assay as described under Materials and Methods. The E:T ratio for all experiments was 25:1. Data represent the means ± SD for triplicate wells.

[b] Same as footnote *b* in Table 1.

[c] Percentage inhibition of lysis as calculated by the formula under Materials and Methods.

[d] PBL from different donors were used in each experiment. Experiments 4–7 were conducted on the same day with target cells obtained from a single cell culture.

DISCUSSION

Recent studies in the murine natural killer (NK) and natural cytotoxic (NC) systems have revealed that certain simple sugars, in particular, D-mannose, inhibit target-cell destruction (8). Each of these systems, murine NK and NC, employs different tumor cell lines for detection of cell-mediated cytotoxicity by nonimmune spleen cells. The patterns of inhibition by different sugars were found to be different for the NK and NC systems. These authors suggested that simple sugars, perhaps as part of complex glycoproteins or glycolipids on the target-cell surface, act as recognition sites for murine NK and NC cells. Similar experiments have been conducted in the human system using human Chang cells as targets (9). Several sugars, including α-lactose and L-mannose, were found to have an inhibitory effect in this cell-mediated lytic system. The results of these two sets of studies suggested that saccharides may provide one means of relating the NK and NC lymphocyte-mediated and supernatant-mediated cell-lytic systems.

In the present studies, six different saccharides were examined for their effects on NK-induced lysis of MOLT-4 target cells (*N*-acetylglucosamine, α-methylmannoside, lactose, L-fucose, D-mannose, and D-galactose). Of these six sugars, the three which inhibit lysis of MOLT-4 cells most frequently are *N*-acetylglucosamine (NAG), α-methylmannoside (αMM), and lactose. Inhibition is dosage dependent up to the highest concentration employed in these studies (50 m*M*). Higher levels were not tested due to the possibility that greater changes in the ionic strength of the medium could be sufficient to adversely affect the lytic reaction. A similar set of experiments employing K-562 cells as targets revealed that NAG is inhibitory in this NK system as well. However, NAG and αMM are not as inhibitory for NK lysis of K-562 cells as for lysis of MOLT-4 cells. There was also quite a bit of variation from lymphocyte donor to donor and also between the K-562 and MOLT-4 target cells in the ability of these sugars to inhibit *in vitro* lysis. Because of this variation, these sugars were not the definitive probes we had hoped for; however, additional studies were conducted to determine if they had effects in supernatant-induced lysis.

Supernatant lytic activity detectable on both L-929 and MOLT-4 cells was examined for inhibition by the same six sugars. The results of these studies reveal that NAG and αMM also inhibited some supernatant lysis of L-929 cells. At 20 m*M* concentrations, the levels of inhibition range from 20 to 32%. These data indicate that a discrete subpopulation of LT forms within the total populations lytic on the L-929 cell may be inhibited by these sugars. It should be noted that these values are calculated based on the percentage lysis rather than units/milliliter of LT activity. The percentage neutralization of LT units is actually higher in many cases. In contrast, when supernatant lytic activity for MOLT-4 target cells was tested for inhibition by NAG and αMM, both sugars were found to be quite inhibitory. While higher levels are needed for inhibition of MOLT-4 lysis than for inhibition of L-929 cell lysis, the percentage inhibition of lysis is higher, ranging from 54–92%. Thus the sugar probes were more effective in blocking MOLT-4 lysis than L-929 cell lysis. One explanation of these findings is that LT forms in these supernatants are a mixture of different forms and the L cells are sensitive to multiple LT forms, some of which are blocked by these sugars and others are not, whereas MOLT-4 is sensitive to a more discrete population of LT forms that are almost totally inhibited by these sugars and less sensitive to those that are not inhibited. These data are in basic agreement with

previous findings that L-929 cells are more sensitive to the smaller α, β, and γ LT forms than other targets (11).

Previous work by Pape *et al.* (12) with antisera directed at various portions of human IgG molecules, including the $F(ab')_2$ fragment, revealed that these antisera will inhibit natural cytotoxicity for K-562 target cells *in vitro*. The authors interpreted these results as suggesting that a significant part of NK is actually attributable to ADCC. Certain human LT components now known to be the Cx and α_H forms are partially neutralizable by anti-human $F(ab')_2$ antiserum (7, 13). The present results indicate that both the anti-$F(ab')_2$ and anti-α_2 LT antisera will inhibit NK lysis of both MOLT-4 and K-562 target cells. However, the amount of inhibition is quite variable for both reagents. It is interesting that the anti-α serum only inhibited in about half of these experiments. These data suggest these NK-induced cell-lytic reactions may occur via different types of effector cells and/or lytic mechanisms as suggested by Pape *et al.* (12).

The effect of anti-$F(ab')_2$ and anti-α_2 on supernatant lysis of MOLT-4 and L-929 targets reveals this situation is also complex. Both sera neutralized a portion of LT lytic activity on L-929 cells; however, with certain supernatants there were no measurable effects. In contrast, anti-$F(ab')_2$ antisera had no effect on lysis of MOLT-4 targets, whereas anti-α_2 blocked lysis in 2 of 5 experiments tested. There are several explanations for these findings: (a) each supernatant contains multiple LT forms and the proportion of each form may vary from supernatant to supernatant; (b) Abs bind to the LT forms but do not block lysis on the targets; and (c) the antigens involved are masked and not accessible to antibody-binding sites. We have found that α LT Ag determinants can be masked when this component is incorporated into the larger Cx and α_H forms; however, preliminary studies employing molecular sieving columns suggest the MOLT-4 lytic forms are of the smaller α and β molecular weight classes. While it cannot yet be stated with certainty, we favor the concept that these supernatants contain a mixture of different LT forms.

Previous and present results substantiate that a polyclonal lectin signal triggers release, within 5 hr, of cell-lytic molecules accumulated in the intracellular pools of preactivated human lymphocytes (1–3). Extensive studies reveal the LT forms in these supernatants are heterogeneous in their size (14, 15), cell-lytic capacity (3, 11, 16, 17), and their ability to bind to different cell types *in vitro* (3, 6, 17). One possible explanation for this effect is that the lectin signal stimulates the release of discrete LT forms from different classes of effector cells; i.e., NK, anomalous T killers, and/or specific cytotoxic lymphocytes. The heterogeneity of the supernatant forms may thus reflect the heterogeneity of the cell population from which they were derived. An important question not resolved by the present studies is the molecular nature of these binding events. Are they specific or is the binding via nonselective mechanism(s)? This will require further study with cell-lytic forms derived from highly refined populations of effectors. Previous studies with LT forms from human T cells and MLC blasts suggest that some of these molecules are associated with immunoglobulin-like materials because they are reactive with anti-$F(ab')_2$ (7, 13) and, when derived from cells immune to soluble antigens, will specifically bind to the antigens when immobilized on beads (6). More recent data obtained studying LT forms obtained from alloimmune murine cells suggest they may not have V-region specificity (16).

Lymphotoxins are not as rapid as intact effector cells in causing cell lysis *in vitro*.

We find they are not capable of inducing cell lysis sooner than 4 to 8 hr, and the longer the incubation time, the greater the effects on the targets. This is considerably slower than lysis induced by intact effector NK or CTL lymphocytes. Yet, while soluble forms are less effective than the intact cell, the present studies provide preliminary evidence that these lytic materials may be involved as effectors in NK-induced rapid cell lysis. Previous studies with antibodies made against various molecular weight LT classes from humans and guinea pigs indicate that these antisera can inhibit various types of lymphocyte-induced cell-lytic reactions *in vitro* (13, 18–22). Cell lysis induced by intact lymphoid cells is complex, and lysis and growth inhibition induced by supernatants released by these cell populations are also complex. The relationship between the two phenomena will clearly only be resolved after characterization and comparison of the components involved in each.

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