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# Phorbol Myristate Acetate Induction of Lymphotoxins from Continuous Human B Lymphoid Cell Lines *In Vitro*

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**Summary:** The phorbol ester 4β-phorbol 12-myristate 13-acetate (PMA) was studied for its ability to induce increased lymphotoxin (LT) production from a number of continuous human B lymphoblastoid cell lines that spontaneously release low levels of LT *in vitro*. A 5–20-fold increase in LT production was seen when all seven of these cell lines were cocultured with 20 ng/ml PMA for 72 h under serum-free conditions in 0.1% lactalbumin hydrolysate. One cell line, GM3104A, was cloned and repeatedly selected to obtain a high-level LT producer in the presence of PMA. This subline, termed IR 3.4, spontaneously released the α-LT molecular weight (MW) class; however, upon PMA stimulation it released high levels of α and an unrelated β-LT MW class form. The increased amounts of LT released in the presence of PMA by IR 3.4 lymphoid cells provides a new method for the production of large amounts of α-LT for purification from a single cell source for biochemical and functional studies, and indicates that the type of induction signal may influence the type of LT released. **Key Words:** Cell lines—IR 3.4 cells—Lymphotoxin—Phytohemagglutinin—PMA.

The tumor promoter 4β-phorbol 12-myristate 13-acetate (PMA) has been shown to exert pleiotropic effects on growth and differentiation processes of cells in a variety of culture systems *in vitro* (1–4). It has been demonstrated that PMA can stimulate and potentiate the production and release of lymphokines *in vitro* (3,5). One group of lymphokines with growth inhibitory and lytic effects on nucleated cells *in vitro* is termed lymphotoxins (LT) (6–8). LT are inducible and can be released specifically when immune lymphoid cells are cocultured with the immunizing antigen or nonspecifically when nonimmune lymphoid cells are cocultured with lectins (9,10). An exception to the induced release of these effector

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molecules is that certain continuous human lymphoblastoid cell lines constitutively release low levels of LT (11,12).

LT found in supernatants from stimulated human lymphocytes are heterogeneous and can be grouped into different molecular weight (MW) classes and charge subclasses (13). Moreover, these classes also have different cell-lytic ability *in vitro* and may be derived from different populations of effector cells (14,15). Immunological and biochemical studies indicate that certain of the MW classes may be interrelated forms (16), and two LT classes, $\alpha_H$ and $\alpha$, from tonsil and adenoid cultures, have been purified to electrophoretic homogeneity (17,18). There is a need to develop adequate sources of these molecules from a single cell-type for defined biochemical and functional studies. Several investigators have previously attempted to use continuous human B lymphoblastoid cell lines as a source of $\alpha$-class LT (70–90,000 MW), but these cell lines normally release very low levels of these molecules under serum culture conditions, making biochemical purification very difficult (11,12).

The studies in this article report on the development of a new technique using PMA to obtain greatly increased quantities of $\alpha$-LT from a single, cloned cell source under serum-free conditions.

## MATERIALS AND METHODS

### Target Cells and Culture Media

The target cells used in these experiments were $\alpha$-L-929 (mouse fibroblasts). They were maintained as monolayer culture in 32-oz. prescription bottles in an atmosphere of 95% air–5% $CO_2$, and passed biweekly. Culture medium used to maintain $\alpha$-L-929 (L cells) was RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 3% heat-inactivated (1 h, 56°C) fetal calf serum (FCS) (GIBCO) (RPMI-3%).

### Continuous Human B Lymphoblastoid Cell Lines

The human B lymphoblastoid cell lines PGLC-33h, WIL-2 (supplied by Dr. Phillip Glade, University of Miami, FL), RPMI 1788, RPMI 8866 (Associated Biomedic Systems, Rockville, MD), GM3163, GM3104A (Human Gene Mutant Cell Repository, Camden, NJ), and Namalva (supplied by Dr. Michael Flickinger, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD) were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS (RPMI-10%). These suspension cells were cultured in Corning T-25-$cm^2$ flasks (Corning, NY) in an atmosphere of 95% air–5% $CO_2$, and subcultured every 3rd day at an initial density of $2 \times 10^5$ cells/ml.

### Supernatant Production

Human B lymphoblastoid cells were grown to a density of $1.5 \times 10^6$ cells/ml in RPMI-10% Corning T-150-$cm^2$ flasks. The cultures were centrifuged at 450 *g* for 5 min and the cell pellet was resuspended in 10 ml serum-free RPMI 1640 (RPMI-0%). After repeating this procedure three times, cells were resuspended

at various cell densities in RPMI-0% supplemented with 0.1% lactalbumin hydrolysate (LAH) (GIBCO) and different concentrations of PMA (Sigma, St. Louis, MO). Each suspension was cultured at 37°C for 72–96 h in 95% air–5% $CO_2$. After the culture period, the supernatants were cleared of cells by centrifuging the cultures at 550 *g* for 10 min and stored at −70°C or subjected to concentration.

### Lymphotoxin Assays

Two types of assays were employed. One determined quantitatively the amount of LT activity present in a given supernatant, and the other indicated qualitatively its presence or absence. The details of these methods have been reported previously (13). Briefly, $10^5$ target cells in 1.0 ml were established in 16 × 125-mm screw-capped culture tubes in RPMI-3% with 0.5 μg/ml mitomycin-C (Sigma), and incubated for 24 h at 37°C in 95% air–5% $CO_2$. To determine the amount of LT present in a sample, serial dilutions of LT-containing or control medium were assayed in duplicate tubes. To determine the presence of LT in a sample, an aliquot of the sample was only added to duplicate tubes. After an additional 24 h of incubation at 37°C, the medium was discarded, the monolayers were washed with phosphate-buffered saline, and the remaining cells were trypsinized and enumerated in a model F Coulter counter (Coulter Electronics, Inc., Hialeah, FL). One unit of LT activity is defined as that amount of material that will destroy 50% of the target cells.

### Antiserum Neutralization Assays

Production and characterization of rabbit anti-whole supernatant (WS) and rabbit anti-$\alpha_2$-LT sera have been previously reported (16). All antisera were heat inactivated (1 h, 56°C) and centrifuged at 20,000 *g* for 30 min before use. Neutralization of LT was performed by adding 25–50 μl antiserum or normal rabbit serum (NRS) to 10 units of LT activity from a supernatant or fraction, preincubating for 30 min at 25°C, and then testing the remaining activity on L cells (16). The percent neutralization of LT activity was determined by the following formula:

$$\% \text{ neutralization} = \frac{(\text{cell no.} + \text{LT} + \text{Ab}) - (\text{cell no.} + \text{LT} + \text{NRS})}{(\text{cell no.} + \text{NRS}) - (\text{cell no.} + \text{LT} + \text{NRS})} \times 100$$

### Molecular Sieving

Degassed Ultrogel AcA 44 (AcA 44) (LKB, Rockville, MD) was poured to a bed height of 95–100 cm in a 2.5 × 120-cm silicladed column, equilibrated in 10 m*M* potassium phosphate and $10^{-4}$ *M* EDTA, pH 7.2. The column was calibrated numerous times over the period of these studies with blue dextran ($2 \times 10^6$ daltons), human IgG (150,000 daltons), hemoglobin (64,000 daltons), α-chymotrypsin (23,000 daltons), and phenol red (376 daltons), applied in a total volume of 1.5–2.0 ml. Fractions of 6.5 ml were collected at a flow rate of 26 ml/h, and monitored for their absorbance at 280 nm on a Beckman model 25 spectrophotometer (Beckman Instruments, Fullerton, CA). These supernatants were rou-

tinely concentrated 25× by ultrafiltration in an Amicon 8200 stirred cell (Amicon Corporation, Danvers, MA) using a 62-mm YM-30 Diaflo ultrafiltration membrane (Amicon), and stored at −70°C until used. One and one-half to two milliliters of 25× supernatants containing from 15,000 to 25,000 units of LT activity was chromatographed over the AcA 44 column, and 10–25-μl samples from the eluted fractions were assayed for LT toxic activity.

## RESULTS

### Release of LT *In Vitro* by Established Cell Lines in the Presence of PMA

The continuous human B lymphoblastoid cell lines, RPMI 1788, RPMI 8866, PGLC-33h, WIL-2, Namalva, GM3163, and GM3104A, and normal lymphocytes were cultured in the presence or absence of PMA or phytohemagglutinin-P (PHA). Cell lines were first grown to a cell density of $1.5 \times 10^6$ cells/ml in RPMI-10% and then transferred into fresh RPMI-10% at an initial density of $5 \times 10^5$ cells/ml in the presence of either 20 ng/ml PMA or 20 μg/ml PHA at 37°C. Normal lymphocytes were obtained from tonsils and and adenoids and cultured at two cell densities, $5 \times 10^5$ and $4 \times 10^6$ cells/ml, in the presence of either 20 ng/ml PMA or 20 μg/ml PHA at 37°C in RPMI-10%. After 72 h the test and control culture supernatants were cleared of cellular debris by centrifugation and assayed for LT activity on L cells. The results of several experiments are presented in Table 1.

Each cell line produced different levels of LT activity. However, it is evident there was a 10–20-fold increase in the levels of LT released by the various B cell lines in the presence of PMA when compared with non-PMA–treated controls. It is also evident that PMA does not induce LT activity from normal lymphocytes, but PHA, as previously described (13), does induce normal lymphocytes to produce LT—however, at a cell density that is eight times that of the continuous B cell cultures employed in these studies. The levels of LT produced by PHA-stimulated normal lymphocytes were three to seven times lower than those from the PMA-activated B cell lines, RPMI 1788, PGLC-33h, GM3104A, and RPMI 8866. Additional studies revealed that PMA did not increase the sensitivity of the L cells to LT-induced lysis, for the addition of PMA to a standard LT preparation gave the same number of cell-lytic units as the standard tested without PMA (Table 2). Further studies also demonstrated that the lytic activity from PMA-stimulated B cell lines is not due to PMA itself or due to the PMA that has been modified by cells in culture (Table 3).

### Selection and Cloning of an LT-Producing Cell Subline

The GM3104A cell line was chosen for these studies because it is a natural high-level LT producer and is also homozygous at the HLA and Dr loci (Human Genetic Mutant Cell Respository). This cell line was cloned by the limiting dilution method in the presence of a feeder layer of $2 \times 10^4$ BALB/c peritoneal macrophages per microtiter well in a 96 flat-bottomed well Linbro plate (19). A high-LT–producing clone was identified and then recloned two additional times

by the limiting dilution technique. Four high-LT–producing clones were identified and termed IR 3.1, IR 3.3, IR 3.4, and IR 3.9. Subsequently, each cloned line was grown to $2 \times 10^8$ cells, and cryopreserved. The IR 3.4 cell subline was used in all these studies.

### Kinetics of LT Production by PMA-Induced Cells

IR 3.4 cells were cultured at an initial density of $5 \times 10^5$ cells/ml, with varying concentrations of PMA, for 24, 48, and 72 h in RPMI-10% at 37°C. At the end of each culture period supernatants were cleared of cellular debris and the number of units of cell-lytic activity was determined on L cells. The results shown in Table 4 indicate that PMA, at very low doses, rapidly induces the release of increased levels of LT *in vitro*. Additional studies were conducted to attempt to transfer cultures maintained in serum-containing medium into protein-free medium (RPMI-0%) or serum-free RPMI 1640 supplemented with 0.1% LAH (RPMI-LAH). The results of these studies, presented in Table 5, indicate that IR 3.4 cells produce as much LT in serum-free conditions as in the presence of serum.

### Inhibition of IR 3.4 LT by Anti-LT Serum *In Vitro*

Antisera were developed in rabbits immunized with either serum-free WS from PHA-stimulated lymphocytes (anti-WS) or a purified subclass of α-LT (anti-$\alpha_2$-LT) (16). Both these sera were first tested to determine their effectiveness in neutralizing standard human LT preparations of WS or α-LT obtained from lectin-stimulated tonsil and adenoid cultures *in vitro* (13). For these tests, the amount of LT was held constant at 10–15 units, and various amounts of antiserum were added. Maximum neutralization of lytic activity (100%) from WS or α-LT class

**TABLE 1.** *Release of lymphotoxin* in vitro *by phorbol 12-myristate 13-acetate (PMA)- or lectin-stimulated human B lymphoblastoid cell lines and normal lymphocytes*

| Cell line tested | Units of lymphotoxin per milliliter in culture medium | | |
|---|---|---|---|
| | PMA | Control | PHA |
| RPMI 1788 | 999.95 | 68.16 | 57.32 |
| RPMI 8866 | 2,228.31 | 177.52 | 152.72 |
| PGLC-33h | 1,259.32 | 78.01 | 63.44 |
| WIL-2 | 219.76 | 28.93 | 11.29 |
| Namalva | 121.96 | 7.53 | 1.13 |
| GM3163 | 357.92 | 32.31 | 21.49 |
| GM3104A | 1,562.72 | 78.45 | 67.99 |
| Normal lymphocytes[a] | 17.41 | 20.21 | 72.57 |
| Normal lymphocytes[b] | 59.23 | 67.43 | 304.61 |

[a] Normal lymphocytes cultured at a cell density of $5 \times 10^5$ cells/ml.

[b] Normal lymphocytes cultured at a cell density of $4 \times 10^6$ cells/ml.

PHA, phytohemagglutinin-P.

**TABLE 2.** *Phorbol 12-myristate 13-acetate (PMA) effects of lymphotoxin activity on L cells* in vitro[a]

| Cell line tested | PMA | Lymphotoxin units |
|---|---|---|
| L cells | — | 382.72 |
| L cells | 20 ng/ml | 375.86 |

[a] Lymphotoxin was added to L-cell tube cultures in either the presence or absence of PMA. Lymphotoxin activity was determined as described in Materials and Methods.

was achieved with 25–50 μl anti-WS. Twenty-five to fifty microliters anti-$\alpha_2$-LT serum neutralized 100% of the α-LT class and 50–65% of the lytic activity expressed in the WS. In these experiments with IR 3.4 supernatants, 50 μl of either anti-WS or anti-$\alpha_2$-LT serum was preincubated with 10–15 units of lytic activity from the PMA- or non-PMA–stimulated IR 3.4 supernatants. The results of these studies are presented in Table 6. The anti-WS and anti-$\alpha_2$-LT significantly neutralized the LT activity of the non-PMA–stimulated IR 3.4 supernatants under all three culture conditions. These same antisera caused significant but variable levels of neutralization of LT activity from the PMA-stimulated IR 3.4 supernatants.

### Definition of the Various MW Classes of LT in PMA-Induced IR 3.4 Supernatants by Gel Filtration Chromatography

IR 3.4 supernatants were concentrated and subjected to gel filtration chromatography on AcA 44 as described in Materials and Methods. Shown in Fig. 1 is the cytotoxic elution profile of 72-h spent medium. It is clear that the cytolytic activity can be resolved into one major α-MW class (80,000–100,000 daltons),

**TABLE 3.** *Control of L-cell number in the presence of supernatants from phorbol 12-myristate 13-acetate (PMA)-treated cell lines* in vitro[a]

| | Average no. of L cells in PMA culture medium | |
|---|---|---|
| Cell line tested | No PMA | PMA |
| —[b] | 15,768 ± 273[c] | 15,649 ± 456 |
| α-L-929 (mouse fibroblast) | 15,701 ± 473 | 16,104 ± 265 |
| T-24 (human bladder carcinoma) | 15,582 ± 243 | 15,921 ± 448 |
| HeLa (human cervical carcinoma) | 15,365 ± 321 | 15,446 ± 364 |
| MOLT 3 (human T lymphoma) | 15,946 ± 407 | 15,736 ± 269 |

[a] Various cell lines were treated with or without 20 ng/ml PMA for 48 h at 37°C, and assayed for cytotoxic activity on α-L-929 cells *in vitro*.

[b] RPMI-3% was incubated with or without 20 ng/ml PMA for 48 h at 37°C, and assayed for cytotoxic activity on α-L-929 cells *in vitro*.

[c] Average triplicate value of L-cell number from sample counted by the Coulter counter.

**TABLE 4.** *Kinetics of lymphotoxin release by phorbol 12-myristate 13-acetate (PMA)-induced IR 3.4 cells* in vitro

| PMA concentration | Units of lymphotoxins per milliliter at incubation intervals of: 24 h | 48 h | 72 h |
|---|---|---|---|
| 0 | 84.54 | 91.54 | 93.16 |
| 5 ng/ml | 963.72 | 1,184.49 | 1,386.62 |
| 10 ng/ml | 1,076.43 | 1,107.85 | 953.67 |
| 20 ng/ml | 1,011.73 | 944.57 | 904.86 |
| 40 ng/ml | 1,103.13 | 1,370.19 | 2,039.00 |
| 80 ng/ml | 950.02 | 1,476.49 | 1,763.33 |

and three very minor MW classes—β (40,000–60,000 daltons), γ (15,000–20,000 daltons), and Cx (>200,000 daltons). Shown in Fig. 2 is the cytotoxic elution profile of a 72-h PHA-stimulated normal lymphocyte culture in which the normal lymphocytes were cultured at a cell density of $4 \times 10^6$ cells/ml. Clearly, the major peak of lytic activity resides in the α-LT region, which is the same as the α-LT region of the IR 3.4 culture. The lytic activities seen in the column profile from the PHA-stimulated normal lymphocytes are much broader. Shown in Fig. 3A and B are the cytotoxic elution profiles of supernatants from PMA-stimulated IR 3.4 cells at 24 and 72 h, respectively, under RPMI-0% cell culture conditions. Clearly, there are two major peaks of lytic activity, the α- and β-MW classes. Low levels of Cx and γ-classes are evident at 24 h, and only the γ-class is present at 72 h. Shown in Fig. 4A and B are the cytotoxic elution profiles of supernatants from PMA-stimulated IR 3.4 cells at 24 and 72 h, respectively, under RPMI-LAH cell culture conditions, where there are only two major peaks of lytic activity, α- and β-classes, at both 24 and 72 h.

**TABLE 5.** *Release of lymphotoxin by phorbol 12-myristate 13-acetate (PMA)-stimulated IR 3.4 cells* in vitro *under various culture conditions*

| Culture conditions | PMA concentration | Units of lymphotoxin per milliliter at incubation intervals of: 24 h | 48 h | 72 h |
|---|---|---|---|---|
| RPMI-10% | 0 | 62.00 | 107.09 | 49.05 |
| | 20 ng/ml | 825.03 | 744.19 | 831.52 |
| RPMI-0% | 0 | 37.65 | 66.93 | 61.23 |
| | 20 ng/ml | 797.28 | 805.55 | 945.37 |
| RPMI-LAH | 0 | 38.96 | 35.75 | 41.55 |
| | 20 ng/ml | 734.72 | 769.33 | 1,071.55 |

LAH, 0.1% lactalbumin hydrolysate.

**TABLE 6.** *Percent neutralization of IR 3.4 lymphotoxin (LT) by anti-WS and anti-$\alpha_2$-LT sera* in vitro

| Culture conditions | PMA concentration | IR 3.4 supernatants tested at incubation intervals of: 24 h Anti-α | 24 h Anti-WS | 72 h Anti-α | 72 h Anti-WS |
|---|---|---|---|---|---|
| RPMI-10% | 0 | 89.31 ± 3.51 | 74.19 ± 0.42 | 100.10 ± 5.32 | 79.27 ± 0.00 |
| | 20 ng/ml | 64.71 ± 12.42 | 56.13 ± 3.74 | 74.23 ± 9.00 | 56.50 ± 14.48 |
| RPMI-0% | 0 | 51.92 ± 7.58 | 55.83 ± 3.78 | 69.36 ± 2.83 | 63.99 ± 15.37 |
| | 20 ng/ml | 27.68 ± 4.19 | 32.64 ± 4.73 | 62.46 ± 5.21 | 48.34 ± 8.24 |
| RPMI-LAH | 0 | 100.28 ± 11.79 | 109.00 ± 18.82 | 100.27 ± 3.83 | 100.43 ± 9.44 |
| | 20 ng/ml | 36.78 ± 16.02 | 57.98 ± 7.14 | 66.07 ± 8.83 | 100.35 ± 2.83 |

PMA, phorbol 12-myristate 13-acetate; WS, whole supernatant; LAH, lactalbumin hydrolysate.

## Capacity of Anti-WS and Anti-$\alpha_2$-LT to Neutralize Different MW Classes of IR 3.4 LT Activity *In Vitro*

These two antisera were tested for their ability to neutralize the LT MW classes obtained from AcA-44–fractionated IR 2.4 supernatants. The α- and β-LT classes were collected, as shown by the horizontal bars in Figs. 3 and 4. Fractions containing the α- and β-LT classes were immediately pooled and quantitated for units of lytic activity on L-929 cells. Each sample was tested for lytic activity in the presence of the two antisera. As shown in Table 7, the anti-$\alpha_2$-LT serum failed to neutralize β-LT activity from serum-free cultures and was weakly reactive against the β-LT from the PMA-stimulated IR 3.4 LAH cultures. This partial neutralization may be due to the presence of contaminating α-LT in the β-MW

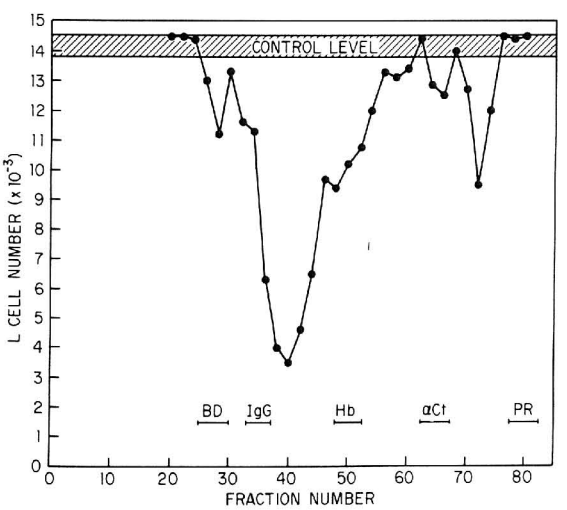



**FIG. 1.** Cytotoxic elution profile of a 3-day IR 3.4 spent medium on an AcA 44 molecular sieving column. The elution profiles of various molecular weight markers are indicated by the horizontal bars: blue dextran (BD, >200,000 daltons), human IgG (150,000 daltons), hemoglobin (Hb, 64,000 daltons), α-chymotrypsin (α-Ct, 23,000 daltons), and phenol red (PR, 376 daltons).

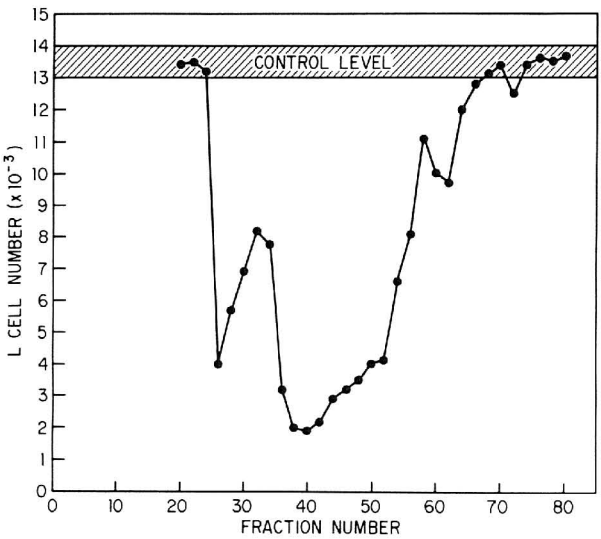



FIG. 2. Cytotoxic elution profile of a 72-h PHA-stimulated normal lymphocyte culture chromatographed on an ACA 44 molecular column.

material because increased anti-$\alpha_2$-LT fails to increase the neutralization of β-LT activity. In contrast, the anti-WS significantly neutralized from 40 to 70% of the β-LT activity from both culture conditions and time points. Both antisera almost completely neutralized the α-MW class activity.

## DISCUSSION

The LT activity released *in vitro* by mitogen-stimulated normal human lymphoid cells is heterogeneous by both biochemical and functional critera (13,14). The advantage tonsils and adenoids had over continuous cell lines was that multiliter volumes of supernatant could be routinely generated under essentially serum-free conditions from a constant supply of tissue from local hospitals. The disadvantages to these cultures were the following: (a) they contained mixed populations of lymphocytes obtained from various donors, making specific identification of the population of lymphocyte releasing LT impossible; (b) these cells had to be cultured at a high density to obtain optimal LT release; and (c) these tissues today are not as available as they have been previously. The major LT class in these supernatants is the α-MW class (13). Rabbit antibodies generated against a refined human α have been shown to block antibody-dependent cell-mediated cytotoxicity (20), a form of specific T cell–lysis (21), lectin-induced cell destruction (22), and natural killing induced by human natural killer effectors from fresh peripheral blood lymphocytes on K-562 and MOLT 4 targets (23). These studies with α-MW class indicate that this is an important form and may be involved in cell-mediated lysis.

It was the objective of the present study to identify a well-defined lymphoid cell line releasing large amounts of the α form. These studies have been previously attempted, and continuous human B cell lines releasing α-LT were identified;

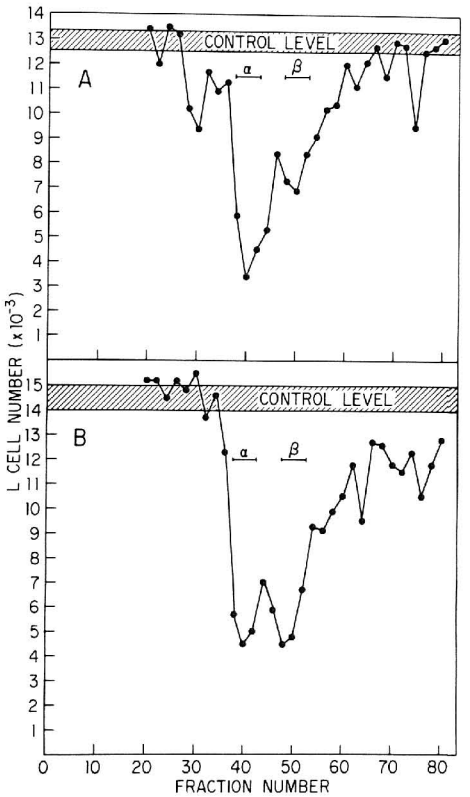



FIG. 3. (Left). Cytotoxic elution profiles of 24-h (A) and 72-h (B) RPMI-0% PMA-stimulated IR 3.4 supernatant chromatographed on an AcA 44 molecular sieving column.

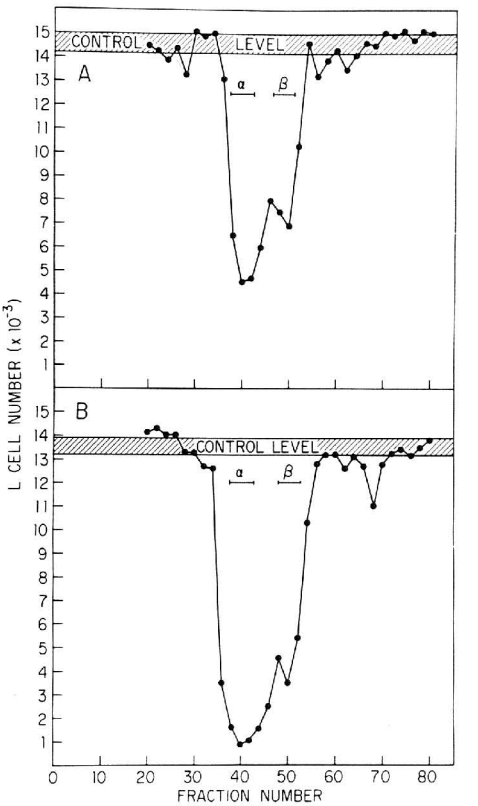



FIG. 4 (Right). Cytotoxic elution profiles of 24-h (A) and 72-h (B) RPMI-LAH PMA-stimulated IR 3.4 supernatant chromatographed on an AcA 44 molecular sieving column.

however, the levels of material were very low, and these cells could not be induced by stimulation with lectins to increase levels of LT secretion. In the present studies, seven continuous B cell lines were screened for constitutive LT release, and of these seven, four released higher levels of LT than the other three. The selection of a cell line from the four best LT releasers was determined by two criteria: (a) the lytic product was identifiable as an α, both biochemically and by its immunological cross-reactivity with rabbit anti-human $\alpha_2$-LT serum; and (b) there was optimal production of the α using PMA under serum-free conditions. The GM3104A line, while meeting both requirements, offered an additional advantage over the other cell lines: the HLA genotype had been established (HLA, A:3,3; B:35,35; C:4,4; D:1,1; DR:1,1).

The GM3104A was cloned and a high-LT–producing subpopulation was selected from this cell lined termed IR 3.4. The IR 3.4, when cultured at a cell density of $5 \times 10^5$ cells/ml in the presence of 20 ng PMA/ml in RPMI-LAH for 72–96 h, consistently produces 1,000–2,000 units/ml. LAH was used as a serum

**TABLE 7.** *Percent neutralization of α- and β-lymphotoxin (LT) classes by anti-WS and anti-$\alpha_2$-LT sera* in vitro[a]

| Culture condition | LT class | PMA IR 3.4 supernatants collected at: 24 h Anti-α | 24 h Anti-WS | 72 h Anti-α | 72 h Anti-WS |
|---|---|---|---|---|---|
| RPMI-0% | α | 113.82 ± 16.46 | 90.15 ± 1.44 | 82.89 ± 2.67 | 97.63 ± 6.69 |
| | β | 18.62 ± 3.45 | 71.83 ± 3.77 | 1.66 ± 1.16 | 43.67 ± 1.12 |
| RPMI-LAH | α | 82.08 ± 0.06 | 96.34 ± 2.87 | 99.05 ± 6.74 | 104.21 ± 3.25 |
| | β | 37.46 ± 2.02 | 67.38 ± 3.16 | 35.99 ± 0.94 | 55.85 ± 0.16 |

[a] α- and β-LT molecular weight classes were obtained from PMA IR 3.4 supernatants chromatographed over an AcA 44 molecular sieving column.
Abbreviations as in Table 6.

substitute because (a) it appears to prevent α-LT molecules in these supernatants from aggregation or degradation; (b) it can be easily removed from the α-LT form by molecular sieving; and (c) there is more α-LT produced in RPMI-LAH (70–90% α) than in RPMI-0% (40–50% α). It was interesting that the β-MW class is more predominant (60%) under RPMI-0% culture conditions after 72–96 h than the α-MW class (30%). However, the α is the predominant form produced constitutively in the absence of PMA stimulation. These are the first studies to show that the type of inducing stimulus may influence the type of LT form released. This β-MW class demonstrates no cross-reactivity with the anti-$\alpha_2$-LT serum and appears to be stable. However, it is neutralized by the anti-WS serum, indicating that it is present in supernatants from the tonsil and adenoid cultures. These are the first studies to show that a β-LT MW class can be synthesized and secreted by a continuous B cell line. Previous reports detected only the α form(s) (12).

The present studies reveal that the α-LT MW class obtained from the PMA-stimulated IR 3.4 cells has similar biochemical and immunological characteristics to the α-LT class obtained from lectin-stimulated tonsil and adenoid cultures. Separation of IR 3.4 supernatants on AcA 44 molecular sieving columns indicates that the lytic activity elutes in the MW range of α-LT. Immunological studies employing a rabbit antiserum raised against purified α-LT from normal lymphocytes (16) revealed that this antiserum can totally abrogate the α lytic activity from the IR 3.4 cells. In a subsequent report the purification to homogeneity of the α-LT in these supernatants in quantities for biochemical and functional studies will be described.

The use of PMA has made possible the production of high-activity supernatants from continuous human B lymphoblastoid cells. These high-activity supernatants provide the means to obtain single LT forms in quantities adequate for purification and functional studies.

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