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THE LT SYSTEM IN EXPERIMENTAL ANIMALS

III. Physicochemical Characteristics and Relationships of Lymphotoxin (LT) Molecules Released *in Vitro* by Activated Lymphoid Cells from Several Animal Species[1]

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High levels of material with lymphotoxin- (LT) like activity, cytolytic for L-929 cells *in vitro*, were rapidly released by lymphoid cells obtained from rat, guinea pig, hamster, and rabbit after co-culture for 7 to 10 hr with lectin-coated L-929 cells *in vitro*. These supernatants were all rapidly fractionated under similar conditions by molecular sieving, ion exchange chromatography, and polyacrylamide gel electrophoresis, and the results were compared to those previously obtained for human and murine LT molecules. The activity in all supernatants was separated by gel filtration into multiple m.w. classes that were strikingly similar to those previously assigned to human and murine LT molecules, i.e. complex (>200,000 d), α (70 to 150,000 d), β (35 to 50,000 d), and γ (12 to 20,000 d). The α m.w. class was resolved on Ultrogel AcA 44 into two distinct m.w. forms, termed α heavy (α_H) (110 to 150,000 d), and α light (α_L) (70 to 100,000 d). All supernatants contained LT forms of the Cx, α_H, and α_L m.w. classes. The smaller β and γ m.w. forms were not as evident in supernatants induced in this fashion. The percentage of activity in each m.w. class varied, but the majority of activity was generally due to α_H and α_L forms. However, the relative percentage of activity in any given m.w. class was dependent upon the ionic strength of the separating column buffer. The actual molecular dimensions of materials within the α m.w. class varied somewhat between species, however, they did not vary within the smaller m.w. β and γ forms when detected. The α_H and α_L m.w. forms could each be further resolved into multiple charge subclasses. Although the α_H and α_L forms in guinea pig and rabbit have been shown by immunologic and/or physical data to be related forms, they can be distinguished by charge from one another. This evidence supports the concept that LT molecules detected in supernatants from experimental animals, although heterogeneous, represent a system of related subunits similar to that defined for the human and mouse. Moreover, this system of cell toxins appears to be conserved in evolution and has common features in each of the animals examined in this study.

Studies by other laboratories on lymphotoxin (LT)[2] molecules released by lymphocytes from various animal species *in vitro* have been difficult to relate to one another because of differences in m.w. of the various LT activities studied. For example, three separate groups found m.w. heterogeneity of LT activity in supernatants from activated guinea pig lymphocytes (1-3), whereas two different groups found material(s) with LT activity in only one m.w. range (4, 5). In a similar situation, one group studying murine LT molecules found only one activity at 70 to 150,000 daltons (d) (6), and another group found activity at three different m.w. ranges (>200,000 d, 150,000 d, and 45,000 d) (7). It is, however, difficult to compare these results directly, for each of these studies differed in a number of technical aspects. The present studies were initiated to examine the possibility that lectin-activated lymphoid cells from various experimental animals release material(s) with LT activity *in vitro* that are similar in physical characteristics to those described for human and murine LT molecules. The assumption was that the LT system of effector molecules may be broadly similar in all animals, however, for a variety of technical reasons, various investigators only detected portions of the molecules which comprise this system. We employed the newly developed LT release system described in the first manuscript of this series (8). The nonglass-adherent lymphoid cells from each animal species tested were stimulated *in vitro* under identical conditions. The supernatants were handled rapidly and fractionated by identical procedures to permit direct comparisons. The results support the concept that the m.w. forms of LT detected are strikingly similar in all animal species tested and probably represent a subunit system as described for human and murine LT molecules. The present results are compared with those previously obtained by other investigators.

MATERIALS AND METHODS

Target cells and culture media. Target cells were obtained from stock cultures of mouse L-929 fibroblasts maintained as

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[2] Abbreviations used in this paper: BS, boiled serum, a heat stable serum fraction; Cx, complex, a high m.w. LT form; DEAE, diethylaminoethyl cellulose - 11; HS, high salt; LS, low salt; LT, lymphotoxin; PAGE, polyacrylamide gel electrophoresis; PS, physiologic salt; PWM, pokewood mitogen; d, dalton.

described previously (8).

Production of LT-containing supernatants from experimental animals. Supernatants from activated nonglass-adherent lymphocytes obtained from the spleens of animal species were generated as described previously (8). The following animal species were used for these studies: C57BL/6 and DBA/2 mice, albino rats, Golden Syrian hamsters, outbred Hartley guinea pigs, and New Zealand White rabbits.

Production of LT-containing supernatants from human lymphoid cells. The details of these methods have been reported previously (9). Briefly, lymphocyte suspensions were obtained from tonsils and adenoids and established in culture in RPMI 1640, containing 20 μg/ml phytohemagglutinin-P (PHA, Difco, Detroit, Mich., 20 μg/ml of boiled serum (BS), a heat stable serum fraction, and antibiotics. Supernatants from activated lymphocytes were collected after 3 to 5 days, cleared of cells by either centrifugation at 300 × G for 10 min, or passage through one layer of glass fiber filter paper (Gelman, Ann Arbor, Mich.) in a Buchner Suction Funnel. Supernatants were produced in this fashion because we found that human lymphoid cells from tonsils and adenoids did not respond well in the rapid production system employed for animal lymphoid cells. Human spleen cells were not tested.

Cell-lytic (LT) assay. Two types of assays were used in this study: One that determines quantitatively the amount of LT activity present in a given supernatant, and a second that indicates qualitatively its presence or absence in a fraction obtained from a separation procedure. These techniques are identical to those described in the first and second manuscripts in this series and elsewhere (8, 10, 11).

Physicochemical separation methods. The physiochemical separation methods employed for the fractionation of cell-lytic activity in supernatants from activated lymphocytes are identical to the methods published previously (12, 13), and are presented in detail in the second manuscript of this series (10). It should be strongly emphasized that there was a minimum time delay between each step in these separation procedures and all procedures were conducted at 4°C. This is very important and facilitates detection of unstable LT components. We found a large percent of the activities in these supernatants decays rapidly upon fractionation.

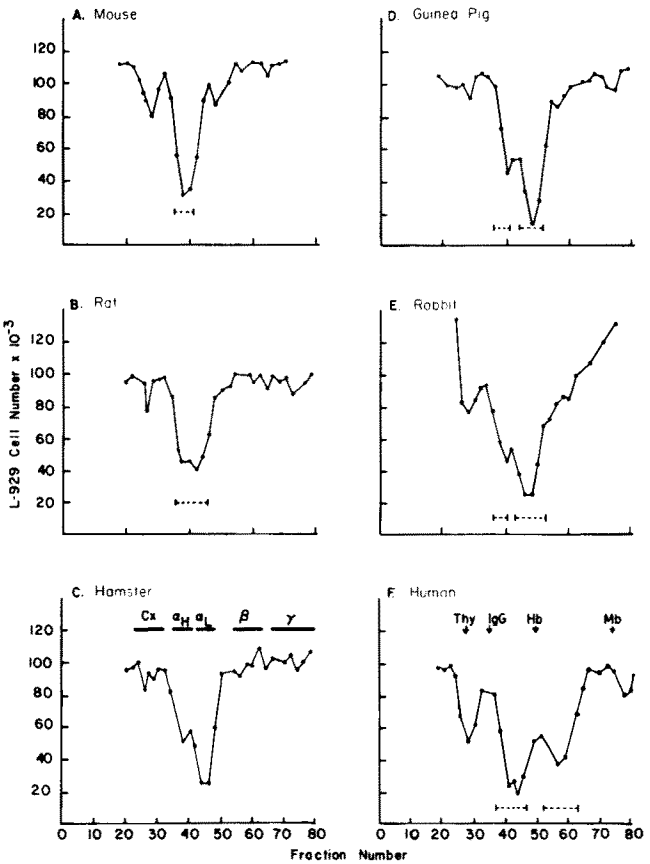

Figure 1. Identification of multiple m.w. classes of materials with cell lytic activity released *in vitro* by lectin activated lymphoid cells from various experimental animals and man. Supernatants from lectin-stimulated cultures of lymphoid cells were collected, concentrated, and fractionated over AcA 44 Ultrogel columns. Alternate fractions were tested in duplicate on L-929 cells for cell lytic activity. Profiles shown are typical of multiple separations made with different supernatants. *A*, *B*, *E*, and *F* are representative of supernatants fractionated in LS buffer, whereas *C* and *D* are from supernatants fractionated in PS buffer. Elution of m.w. markers are indicated in *F* by arrows. The heavy bars in *C* denote the elution areas of the various classes as defined in the *Results* section. Fractions pooled for further characterization are indicated by dashed bars.

RESULTS

A. Comparison of the m.w. of materials with cell lytic activity in supernatants from lectin-activated lymphocytes from experimental animals. Supernatants from activated lymphocyte cultures from various experimental animals were collected, concentrated (20 to 40 ×), and chromatographed on Ultrogel AcA 44 columns. All fractions were assayed in duplicate, and the entire procedures were performed at least twice for each animal species. Three different buffers were employed during gel filtration chromatography of these supernatants, 0.01 M phosphate, pH 7.2, 10^{-4} M EDTA with 0.5 M (HS), 0.15 M (PS), or 0.0 M (LS) NaCl.

Individual profiles from each species are shown in Figure 1. These data clearly indicate that there are multiple m.w. classes of cell lytic activity released *in vitro* by lymphocytes from various animal species. For the sake of comparison, the elution profile of LT activity present in supernatants from 5-day lectin-stimulated human lymphocyte cultures is also shown in Figure 1 (12). The size classes are defined as in the human system, cell lytic activity eluting in the void volume, i.e., greater than 200,000 d, is termed complex (Cx); toxic activity in the 70 to 150,000 d range is termed α; in the 30 to 50,000 d range, β; and in the 10 to 25,000 d region, γ (12). There are two m.w. forms of α released by cells from these animals. The heavier form, 110 to 150,000 d, is termed α heavy (α_H), and the lighter form, 70 to 100,000 d, termed α light (α_L). Whereas Figure 1 is the result of a single column separation for each species, the results of numerous separations in various ionic strength buffers are shown in Table I. Although Cx activity is present in profiles of supernatants obtained from all animal species, the amounts of activity range from 0 to 40% and in most cases, Cx accounts for only a minor percentage of the total activity. It should be noted that the amount of activity due to Cx exceeds 21% in these studies only when the supernatants are chromatographed under conditions of low ionic strength. In particular, hamster supernatants contain high levels of Cx activity only under conditions of low ionic strength and low levels in the high ionic strength buffer. Since Cx elutes in the void volume of Ultrogel, variations in the m.w. of Cx derived from the various animals can not be detected by these methods.

The α class is the major lytic activity in the Ultrogel profiles of each species. Although α_H can be detected in each experi-

TABLE I

Relative amounts of cell lytic activity in various molecular weight (m.w.) classes released in vitro by lectin-stimulated lymphocytes from various animal species and man[a]

Species	Expt. No.	Buffer[b]	Class of Cell Lytic Activity[c]								
			% Cx (>200d)	m.w. of α_H	% α_H	m.w. of α_L	% α_L	m.w. of β	% β	m.w. of γ	% γ
Mouse	1	LS	11	150	72	n.d.	0	n.d.	0	23	11
	2	LS	4	160	40	90	41	n.d.	0	n.d.	0
	3	LS	16	125	69	72	12	n.d.	0	n.d.	0
	4	PS	5	110	70	72	19	n.d.	0	n.d.	0
	5	PS	20	140	36	98	21	n.d.	0	18	8
	6	PS	14	125	59	68	14	n.d.	0	n.d.	0
	7	HS	10	135	1	90	77	49	3	17	15
Rat	1	LS	7	140	22	100	68	n.d.	0	n.d.	0
	2	LS	4	120	39	100	54	n.d.	0	n.d.	0
	3	LS	8	140	22	100	65	42	2	n.d.	0
	4	PS	8	n.d.	0	100	80	42	5	n.d.	0
	5	PS	3	130	25	90	61	42	6	n.d.	0
	6	PS	4	n.d.	0	100	76	42	8	n.d.	0
Hamster	1	LS	6	120	22	90	64	n.d.	0	n.d.	0
	2	LS	40	n.d.	0	80	37	52	17	n.d.	0
	3	LS	29	n.d.	0	80	37	52	19	n.d.	0
	4	PS	6	125	29	85	60	n.d.	0	n.d.	0
	5	PS	0	110	31	80	57	n.d.	0	n.d.	0
	6	PS	0	110	26	72	68	n.d.	0	n.d.	0
Guinea pig	1	LS	7	110	31	72	42	44	3	27	4
	2	LS	6	110	28	72	64	n.d.	0	n.d.	0
	3	PS	3	110	26	72	57	n.d.	0	17	3
Rabbit	1	LS	4	110	24	76	71	n.d.	0	n.d.	0
	2	PS	12	110	25	72	50	n.d.	0	n.d.	0
Human[d]	1	LS	16	n.d.	0	90	43	45	25	12	5
	2	LS	27	n.d.	0	80	43	60	21	12	3
	3	LS	10	n.d.	0	80	52	50	16	12	10
	4	PS	19	n.d.	0	85	49	45	16	12	9
	5	PS	21	n.d.	0	70	45	50	14	12	13

[a] Supernatants were collected, concentrated, chromatographed over Ultrogel AcA 44 columns, and fractions were tested for cell lytic activity. The m.w. of each class of activity was determined according to various known m.w. protein markers and is reported as daltons $\times 10^{-3}$. The percentage of activity in each class was determined by graphing L-929 cell number *vs* fraction number and calculating the area under each peak.

[b] Three different buffers were employed during gel filtration, 0.01 M phosphate, pH 7.2, 10^{-4} M EDTA with 0.5 M (HS), 0.15 M (PS), or 0.00 M (LS) NaCl.

[c] n.d., Activity not detectable.

[d] Human supernatants were obtained from 5-day lectin-stimulated human lymphocyte cultures.

mental animal tested, it does not appear in the human profiles of 5-day supernatants. In the mouse, the α_H peak contains the largest percentage of LT activity. In the rat, α_H is sometimes absent from the high ionic strength Ultrogel profiles. Conversely, the hamster profiles reveal that α_H is absent only under conditions of low ionic strength. The m.w. of α_H in the hamster, guinea pig, and rabbit is uniformly from 110 to 125,000 d. However, the m.w. appears higher in the mouse and rat, averaging around 130 to 140,000 d. With the exception of the mouse profiles, α_L comprises the major fraction of cell lytic activity eluting off Ultrogel and contains from 37 to 80% of the total activity. The size of α_L generally averages from 70 to 80,000 d. However, in the rat, α_L is larger, and averages about 100,000 d. The smaller classes, β and γ, are most visible in the human 5-day profiles. In the experimental animals, they are not as reproducibly detectable as the other forms of lytic activity, and only comprise from 0 to 19% of the total activity in the case of β and 0 to 11% for γ. These studies reveal that, as with the human and murine LT system, the relative amounts of LT activity present in the various m.w. classes can vary, depending upon the ionic strength of the buffer employed in these columns.

B. Separation of various cell lytic m.w. classes into subclasses by ion exchange chromatography. Supernatants from activated lymphocyte cultures that had been separated into α m.w. classes were subsequently tested for heterogeneity with respect to charge. Selected fractions from the Ultrogel columns containing combinations of α_H and/or α_L lytic activity, indicated by horizontal bars in Figure 1, were collected, pooled, concentrated by ultrafiltration, dialyzed, and chromatographed on diethylaminoethyl cellulose-11 (DEAE) columns. Fractions from DEAE columns were assayed in duplicate and representative profiles are shown in Figure 2-*a-f*. The data clearly show that multiple charge subclasses exist within both the α_H and α_L classes from each animal species examined. Since it was difficult to resolve rat α_H from α_L, the entire rat α class was used in these studies. Multiple charge subclasses were also found to exist in this rat α preparation (Fig. 2*b*). As in the human LT system, lytic activity that does not bind to the

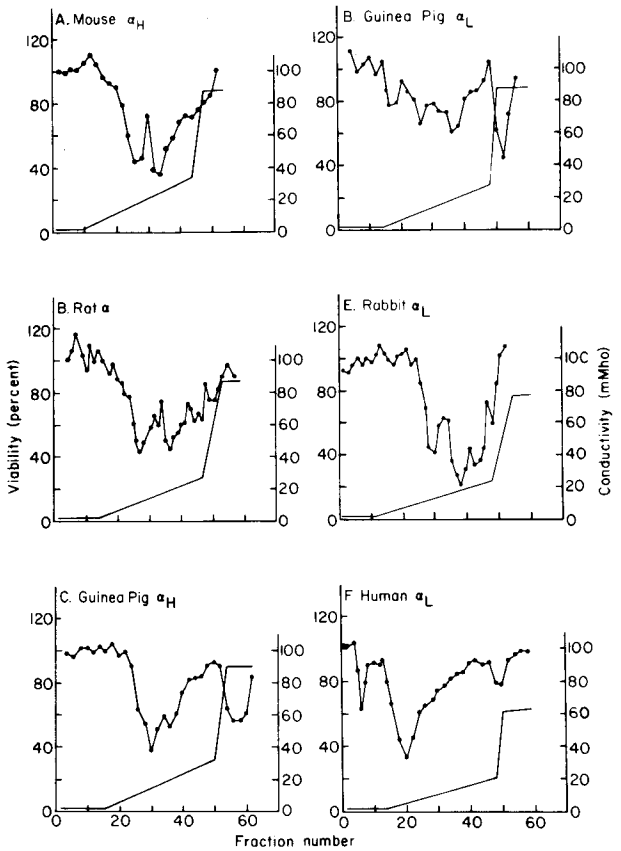

Figure 2. Separation of α m.w. classes from various experimental animals and man into subclasses by ion exchange chromatography. Supernatants were first separated into various m.w. classes by passage over Ultrogel AcA 44 columns. Fractions containing α class activity were pooled, as indicated in Figure 1, concentrated, chromatographed over DEAE columns, and tested for cell lytic activity on L-929 cells. Elution profiles shown are individual column separations but, with the exception of the rabbit, are representative of duplicate columns performed on different supernatant LTs. ——, conductivity; ●——●, per cent viability.

DEAE column in 0.0 M NaCl is termed subclass$_1$. All cell lytic activity that elutes in the salt gradient (0 to 0.3 NaCl) is termed subclass$_2$, and the activity that elutes in the 1 M salt wash is termed subclass$_3$ (11). We will employ this terminology when referring to subclasses of cell lytic activity from experimental animals separated on DEAE. The mouse α_H and rat α profiles showed a small amount of activity in the α_3 region, and the majority of activity in the α_2 region. Similarly, the guinea pig profile showed most of the activity in the α_{H2} region, yet there was a significant amount of activity in the α_{H3} region. With regard to the α_L forms, only the human and guinea pig profiles exhibited appreciable activity in the α_{L3} region. The majority of activity was present in the α_{L2} region of each profile, and only the human α_L showed activity in the α_{L1} region.

C. Separation of various m.w. classes by polyacrylamide gel electrophoresis, (PAGE). To verify further the heterogeneity of lytic activity seen in the DEAE profiles, we chose a second method of separation, PAGE. The Ultrogel fractions, containing either α_H and/or α_L activity, which were chromatographed on DEAE, were also subjected to PAGE electrophoresis. Major peaks from Ultrogel columns were collected, pooled, concentrated, and applied to standard 7% polyacrylamide disc gels. After electrophoresis at 4°C, the gels were sliced into 2-mm sections, eluted in 0.3 ml RPMI for 24 hr at 4°C, and tested in duplicate for toxic activity. Selected profiles showing PAGE of α_H and/or α_L class activity are shown in Figure 3. Since all the data are plotted with respect to R_f values, the migration profiles can be directly compared. It is again evident that each class of cell lytic activity examined is heterogeneous when analyzed by PAGE. The only α class species to show large amounts of activity with R_f values >0.8 were the mouse α_H and rat α. All of the profiles, with the exception of the rat α profile, contain activity in the moderately charged region of R_f values between 0.4 and 0.8. Furthermore, slowly migrating lytic activity with R_f values <0.4 was present in the guinea pig α_H, rabbit α_L, and human α_L profiles. A careful comparison of the guinea pig α_L and α_H profiles reveals that although they are similar, the guinea pig α_L consists mainly of moderately charged materials, whereas, guinea pig α_H has predominantly lesser charged materials. A similar comparison of the rabbit profiles indicates that rabbit α_L contains lytic activity with R_f values between 0.2 and 0.6, whereas, rabbit α_H contains activity in the region of R_f values from 0.3 to 0.9. In general, it appears that although heterogeneous, murine, rat, and guinea pig toxins are more highly charged than human LT molecules, as indicated by their high R_f values on PAGE gels.

DISCUSSION

The present results reveal that lymphoid cells obtained from various animal species possess the capacity to rapidly release *in vitro*, upon co-culture with lectin-treated allogeneic or xen-

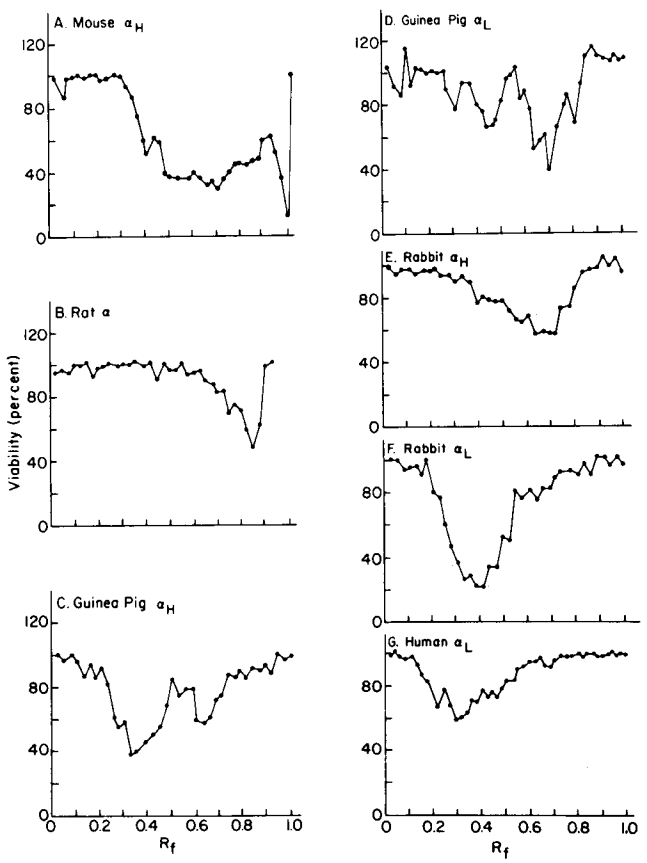

Figure 3. Separation of α m.w. classes from experimental animals and man into multiple subclasses by PAGE. Supernatants were separated into various m.w. classes by chromatography over Ultrogel AcA 44 columns. Fractions containing α class activity were pooled, as indicated in Figure 1, concentrated, and 100 μl of material subjected to electrophoresis in standard PAGE. Individual gels were then sliced into 2-mm sections, and materials in each section were eluted and tested for cell lytic activity.

ogeneic cells, materials with cell lytic activity. Although the m.w. of cytolytic materials detected in these studies were heterogeneous, both within and between species, they could all be ordered into five common m.w. classes as shown in Table I. These m.w. classes had been previously established and defined by several investigators to help classify human and murine LT activities (10, 12, 14). Complex (>200,000 d) and α (70 to 150,000 d) m.w. classes were evident in supernatants from all species tested. The amount of complex-induced lysis detectable ranged from 0 to 40% of the total cell lytic activity eluted from the column. In addition, there were two forms of α m.w. class activity detected in supernatants from each experimental animal examined in these studies. The two forms have been termed α_H for activity eluting at 110 to 150,000 d, and α_L for activity eluting at 70 to 100,000 d. The smaller m.w. classes, β (30 to 50,000 d), and γ (10 to 25,000 d), were either not detected or only present at low levels in these 7- to 10-hr supernatants. The α forms were clearly the predominant molecular species present under these *in vitro* conditions. These studies were conducted under identical conditions, and the supernatants were fractionated and handled rapidly. Although these were high activity supernatants, these were still difficult studies because certain supernatants rapidly lost activity. The various murine forms were the most labile, the guinea pig and rat forms were less labile, and the human forms from 5-day lectin-stimulated human lymphocyte cultures were quite stable. The instabilities pose a serious problem, and to date, our attempts to stabilize this activity has had very limited success. The molecular basis of this instability is not clear but must be resolved before these cell toxins can be further examined.

Our results indicate that materials with cell lytic activity from various animal species appear to be quite similar with respect to m.w., however, when we compare the work of previous investigators to each other and to these results, they did not find the same similarities. The various m.w. LT forms reported by previous investigators are shown in Table II. For the sake of comparison, we have placed them into the five basic m.w. classes that we have defined in the present manuscript. Some investigators studying supernatants from the same animal species apparently were only able to detect a single m.w. form, whereas others detected multiple m.w. forms. A somewhat similar situation exists when we compare the various m.w. forms of LT detected in supernatants from activated human lymphocytes shown in Table III. It was not clear in these studies whether this heterogeneity was due to different molecules, as suggested by Walker *et al.* (14) for human α and β LT forms, or perhaps related molecules with a capacity to assemble into various composites (7). However, when we assemble the data in this fashion, they reveal that although different investigators did not find all the same m.w. LT forms, all activities detected to date can be assigned to one or more of the presently defined m.w. classes. We feel these data lend support to the concept of a common "set" of m.w. LT forms that may be present in a supernatant from a given animal species. However, which forms will be present under a given set of conditions cannot yet be predicted.

There is now convincing evidence to support the concept that LT molecules, although heterogenous in size and charge, are interrelated forms composed of a system of common subunits. As shown in Tables II and III, forms similar in size to α_L were previously detected by other investigators in 1 to 3-day lectin or antigen-induced supernatants from mouse (6), rat (15), guinea pig (1–3), and human (12). Although material similar in size to the α_H form was previously detected in supernatants derived from mouse (7), guinea pig (2) and human (18, 20), the relationship of these two LT forms was unknown. There is quite

TABLE II

Classification of materials with cell lytic activity detected in supernatants from experimental animals into previously defined m.w. classes[a]

Animal Species	Method of: Induction	Method of: Separation	Buffer	Days in Culture	m.w. Class Detected: Cx	α_H	α_L	β	γ	Investigator
Mouse	PHA	Sephadex G-200	T.C.M.[b]	3	+	+	−	++	−	Trivers *et al.* (7)
Mouse	PHA-treated L cells	Sephadex G-100	Low salt	2–3	−	−	++	−	−	Kolb and Granger (6)
Mouse[c]	PHA-treated L cells	Ultrogel AcA 44	LS, PS, or HS	7–10 hr	+	+	+	±	±	This manuscript
Rat	Antigen	Sephadex G-100	T.C.M.	2	−	−	++	±	−	Namba and Waksman (15)
Rat	PHA-treated L cells	Ultrogel AcA 44	LS or PS	7–10 hr	+	+	++	+	−	This manuscript
Guinea pig	Con A	Sephadex G-100	PBS	1	±	−	+	++	±	Coyne *et al.*, (1)
	Antigen				±	−	+	++	+	
Guinea pig	Antigen	Sephadex G-200	0.1 M NaCl, 0.002 M Tris	1	−	++	+	−	−	Heise and Weiser (2)
Guinea pig	PHA, Con A	Sephadex G-100	PBS	2	−	−	−	++	−	Sawada *et al.* (4)
Guinea pig	Antigen	Sephadex G-100	PBS	1	−	−	−	++	−	Gately and Mayer (5)
Guinea pig	Antigen	Sephadex G-100	0.05 M NH_5CO_3	1	±	±	++	−	−	Dumonde *et al.* (3)
Guinea pig	PHA-treated L cells	Ultrogel AcA 44	LS or PS	7–10 hr	+	+	++	±	+	This manuscript

[a] The m.w. classes of cell lytic activity are defined as described in the *Results* section. The relative levels of cytotoxic activity are indicated as follows: ++, major peak of activity; +, activity present; ±, possible activity; −, no activity detectable. The results of the present study are indicated as + when activity is detected in more than 50% of the experiments.

[b] Tissue culture medium.

[c] The major peak of activity changes with variations in the ionic strength of the buffer.

TABLE III

Classification of materials with cell lytic activities detected in supernatants from human lymphoid cells into previously defined m.w. classes[a]

Method of Induction	Type of Gel Filtration	m.w. Class Detected[b]					Investigator
		Cx	α_H	α_L	β	γ	
PHA	Sephadex G-100	−	−	+	+	−	Walker *et al.* (14)
PHA	Sephadex G-150	+	−	+	+	+	Hiserodt *et al.* (16)
PHA	Ultrogel AcA 44	+	−	+	+	+	Granger *et al.* (12)
Continuous lymphoid cell line	Sephadex G-150	−	−	+	−	−	Fair *et al.* (17)
PHA, PWM, Con A	Sephadex G-150	−	+	+	−	−	Peter *et al.* (18)
PHA	Sephadex G-150	−	−	+	−	−	Russell *et al.* (19)
Continuous lymphoid cell line	Sephadex G-200	−	+	+	−	−	Amino *et al.* (20)

[a] The m.w. classes of cell lytic activity are defined as described in the *Results* section.

[b] +, Activity present; −, no activity detected.

clear evidence in both the human and mouse that these two forms are interrelated (10, 21). Furthermore, studies with the guinea pig α_H forms chromatographed in high ionic strength buffer reveal they also dissociate into α_L and β LT forms (unpublished observations). The results of preliminary studies using rabbit anti-guinea pig β LT sera, kindly provided by Drs. M. Gately and M. Mayer (22), are shown in Table IV. It is quite clear that both α forms of guinea pig LT carry similar immunologic determinants to each other and to the β forms. However, the present results reveal there are charge differences between the α_H and α_L forms obtained from the guinea pig or rabbit supernatants. These latter observations suggest the α_H form contains a noncovalently bound subunit, perhaps nontoxic, which is not present on the α_L form. It is quite apparent that α_H, α_L, and β LT m.w. forms in mouse, man, and guinea pig are interrelated. Moreover, the present results indicate LT molecules from rat and hamster can associate or dissociate into subunit forms that suggest that similar subunit forms exist in these two species.

The relationship(s) between the various soluble LT forms detected within a supernatant from a single species are complex, yet are beginning to clarify. Extensive studies on human and mouse LT forms indicate that the system consists of at least three types of materials: 1) the LT subunits α, β, and γ; 2) condensing molecule(s) (23); and 3) an antigen-binding receptor(s) (24, 25). It appears that the smaller m.w. LT forms can be assembled, via condensing molecule(s), into high m.w. complexes that can then be identified in association with antigen-binding receptor(s). Furthermore, the higher m.w. forms, α_H and Cx, have greatly increased cytolytic capacity over the smaller m.w. α_L and β forms (26). In addition, they can cause an apparent "specific" and rapid ^{51}Cr release from targets when the Cx contains a receptor capable of recognizing the target cell(s). Association of the smaller m.w. LT subunits with receptors via condensing molecules can both direct and enhance the lytic effects of the LT subunits.

We feel the data collectively are in support of a common system of cell toxins, probably similar in many animal species. However, there are other alternatives. One possibility is that the different m.w. classes represent small m.w. cell lytic molecules that have nonspecifically attached to other macromolecules. We feel this is not a tenable concept for the following reasons: a) the same discrete m.w. LT classes are detectable in the human and mouse whether the media are serum-free or contain serum or selected protein carriers such as BSA or BS; b) the same basic classes are detected when human or animal lymphoid cells are stimulated via different methods; c) we have shown that similar m.w. forms are seen in various animal species studied under various conditions; and d) there are functional differences now reported for the Cx and α_H forms and the smaller m.w. α_L, β, and γ forms in human and mouse (25, 26). The precise molecular relationships of these various m.w. forms will require further study. However, the findings of similar forms in various animal species and of similar subunit structures in these species strongly suggest that LT molecules represent a similar system of lymphocyte-derived cell lytic molecules in all animal species.

TABLE IV

Reactivity of rabbit anti-guinea pig β LT antiserum[a] *with higher m.w. forms of guinea pig LT*[b]

m.w. Class Tested	% Lysis of L-929 Cells in the Presence of:		% Neutralization
	Preimmune Serum (50 µl)	Anti-β LT (50 µl)	
α_H	59	0	100
α_L	71	0	100

[a] Obtained from Dr. Manfred Mayer. See Reference 22 for characterization of this serum.

[b] At 4°C, 50 µl of serum were added to 2.5 units of LT. After 30 min, this was added to a tube culture of L-929 cells to be counted 22 hr later.

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