

DIRECT TRANSFER OF ANTIGEN-SPECIFIC
CYTOLYTIC ACTIVITY TO NONCYTOLYTIC CELLS
UPON FUSION WITH LIPOSOMES DERIVED FROM
CYTOLYTIC T CELL CLONES

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Despite extensive research during the past few years, the mechanism of lysis by cytolytic T lymphocytes (CTL)¹ is poorly understood (1). Even less well elucidated is the nature of the antigen-specific T cell receptor (2, 3). Although both of these activities must be present for a CTL to specifically recognize and lyse a target cell, the cellular requirements for their expression remain obscure. Recently, preliminary studies from this laboratory (4) have shown that "cybrids" obtained by fusion of enucleated cloned CTL with noncytolytic EL4 cells could display specific cytolytic activity. Although this activity was low and somewhat variable, it seemed to indicate that participation of the CTL nucleus was not necessary.

An alternative approach to investigate the putative role of membrane components in cytolytic activity would be to transfer CTL-derived material to noncytolytic recipient cells via synthetic liposomes. In this context, work from Jakobovits et al. (5) demonstrated that T or B lymphoid cells acquired the ability to respond to normally nonstimulatory mitogens upon fusion with liposomes containing B or T lymphocyte membrane components. In other words, B cells fused with T cell membrane components could now respond to concanavalin A (Con A); likewise, T cells fused with B cell membrane components could be stimulated by lipopolysaccharide (LPS). These results indicated that the inability of a particular lymphocyte population to respond to a specific mitogen was due to the lack of suitable membrane receptors, but not to an inherent cellular (nuclear) defect.

In the present study, we investigated the requirements for the expression of antigen specificity and cytolytic activity by constructing liposomes composed of detergent-solubilized CTL clones (separated from nuclear constituents), exogenous lipids, and Sendai virus envelope proteins, and fusing these liposomes with various noncytolytic cell lines. The resultant fusion products were observed to

¹ *Abbreviations used in this paper:* Con A, concanavalin A; CTL, cytolytic T lymphocyte; DPPC, dipalmitoylphosphatidylcholine; DMEM, Dulbecco's modified Eagle's medium; E/T, effector/target ratio; F, Sendai virus fusion protein; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; HN, Sendai virus hemagglutinin/neuraminidase protein; IL-2, interleukin 2; LPS, lipopolysaccharide; mAb, monoclonal antibody; MoLV, Moloney leukemia virus; NP-40, Nonidet P-40; PBS, phosphate-buffered saline.

be highly cytolytic and appeared to exhibit the same specificity as the original CTL clone. The process of liposomal transfer was found to be very efficient and could be applied to cellular recipients other than those of T cell origin. These experiments demonstrated operationally that both the antigen-specific T cell receptor and the CTL lytic machinery could be solubilized and transferred to recipients that did not display these properties.

Materials and Methods

Reagents. Dipalmitoylphosphatidylcholine (DPPC) (98% pure) was obtained from Sigma Chemical Co., St. Louis, MO and stored as a stock solution in chloroform (Merck Sharp & Dohme AG, Zurich, Switzerland) and 100 mg/ml under nitrogen. Cholesterol (99% pure; Calbiochem-Behring Corp., La Jolla, CA) was stored at 50 mg/ml in chloroform under nitrogen. Both reagents were used without further purification. Con A and EGTA were from Pharmacia Fine Chemicals, Uppsala, Sweden and Sigma Chemical Co., respectively. Radiolabeled chromium (^{51}Cr) was obtained from IRE, Fleurus, Belgium as sodium chromate with a specific activity of 1 mCi/ml.

Mice. Adult DBA/2 (H-2^d), C57BL/6 (H-2^b), and BALB/c (H-2^d) mice were obtained from the mouse colony at the Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland. The original breeding pairs were obtained from The Jackson Laboratory, Bar Harbor, ME.

Interleukin 2 (IL-2) Source. Supernatants collected from a subline of EL4 thymoma cells (kindly provided by J. Farrar, Bethesda, MD) that were stimulated (10^6 cells/ml) for 40 h in the presence of 10 ng/ml phorbol-12-myristate-13-acetate (6) were used as a standard source of IL-2.

Virus Stocks. Sendai virus (original stocks were generously donated by Dr. L. Roux, Geneva, Switzerland) was grown in LLC-MK2 cells or in 10-d-old embryonated chicken eggs as described by Scheid and Choppin (7) and Roux and Holland (8).

Cell Lines. The following tumor cell lines were used: P815 (H-2^d, DBA/2 mastocytoma), EL4 (H-2^b, thymoma), BW5147 (H-2^k, thymoma of AKR origin), M12.4.1 (H-2^d, B cell lymphoma of BALB/c origin), LSTRA (H-2^d, a Moloney leukemia virus (MoLV)-transformed line of BALB/c origin), and MBL-2 (H-2^b, a MoLV-transformed line of C57BL/6 origin). All cell lines were maintained in vitro except for LSTRA and MBL-2, which were passaged (weekly) in vivo in their respective strains of origin.

CTL Clones. CTL clones used in these experiments were derived by micromanipulation as described in detail elsewhere (9, 10). Briefly, populations enriched in antigen-specific cells were obtained by immunization in vivo and/or in vitro with allogeneic or MoLV-transformed tumor cells. These populations were cloned in the presence of irradiated, antigenically relevant cells together with a source of IL-2. For routine maintenance, the clones were passaged once weekly by plating 5×10^5 – 1×10^6 cloned cells together with irradiated feeder cells and/or antigen in 40 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS) (Seromed, Munich, Federal Republic of Germany [FRG]), 5×10^{-5} M 2-mercaptoethanol, 10 mM Hepes, penicillin/streptomycin, additional amino acids (11), and a source of IL-2 in 150-mm petri dishes. Clones A9 and B3 were derived from 5-d primary BALB/c anti-DBA/2 mixed lymphocyte cultures. Both of these clones are anti-Mls^a-specific, noncytolytic, and have been described elsewhere (12). CTL clones 7, 10, 11, and 12 were derived from peritoneal exudate lymphocytes isolated from C57BL/6 mice immunized against P815 cells. Clone 11 has a known specificity of anti-H-2K^d (data not shown). Clone 14 is a C57BL/6-derived CTL clone directed against MoLV-associated antigenic determinants.

Cytolytic Assay. Effector cells and ^{51}Cr -labeled target cells (generally 8,000/well) were mixed at various effector/target (E/T) ratios in round-bottom, 96-well microtiter plates (Greiner, Nurtigen, FRG), centrifuged (1,000 rpm, 2 min), and incubated at 37°C for 2 h as previously described (11). At this time, the plates were centrifuged (2,000 rpm, 5 min), the supernatant of each well was collected, and the radioactivity measured in a gamma counter (LKB multigamma counter; LKB, Bromma, Sweden). Target cells incu-

bated in medium alone or in 1% Nonidet P-40 (NP-40) detergent were used to obtain spontaneous and maximum ^{51}Cr -release values, respectively. Percent specific lysis was calculated as described (11). All E/T data points were performed in replicates of three to six with the standard deviation of any series of replicates not >12%.

For assays in which lectin was present, Con A was added to the effector/target cell mixtures at 5 $\mu\text{g}/\text{ml}$ and remained in the assay for its duration. During the cytolytic assays in which EGTA was used, effector and target cells were mixed, centrifuged as described above, and incubated for 30 min at 23°C. EGTA (20 mM/well final concentration) was then added and the cells incubated an additional 2 h at 37°C before determination of the ^{51}Cr -release. In the antibody-blocking experiments, target cells, monoclonal antibody (mAb) (1:500 final dilution), and effector cells were added in that order, and the assay performed as described above. mAb was present throughout the course of the assay. The mAb used (S13-11, anti-H-2K^d) was the kind gift of Dr. S. Tonkonogy, North Carolina State University, Raleigh, NC.

Preparation of Liposomes. Liposomes were prepared by a modification of previously published procedures (13–15). Briefly, donor cells were extensively washed in phosphate-buffered saline (PBS) to remove medium and serum contaminants, followed by a 30 min incubation at 4°C in 0.5% NP-40-Tris-HCl-saline buffer, pH 7.4. After centrifugation at 3,000 rpm for 10 min (to remove nuclei and mitochondria), the supernate was collected and added to Sendai virus hemagglutinin/neuraminidase (HN) and fusion (F) proteins that had been prepared as described (7). The cellular/viral protein mixture (ratio of 5–10:1) was then added to lipids (DPPC and cholesterol, 1:1 molar ratios; protein/lipid ratio, 1:1) previously dried from chloroform by nitrogen in a small, round-bottom flask and incubated for 5 min at 37°C. The flask was vortexed briefly and the solution dialyzed against a 1,000-fold excess of PBS at 4°C for 48 h. In a typical experiment, 50×10^6 donor cells were solubilized in 1.0 ml of detergent-containing buffer, resulting in a supernate containing ~1 mg of cellular protein. This material was then added to 100 μg of purified HN and F proteins (in 1.0 ml), and the cellular/viral protein mixture added to 1.1 mg of dried lipids (DPPC and cholesterol, 1:1 molar ratio). The 2.0 ml solution was dialyzed against 2 liters of PBS. At the end of 48 h, the liposomes were collected by high-speed centrifugation (50,000 g, 1 h) and washed with PBS in this manner. The resuspended liposomal pellet would then be defined as containing 50×10^6 cell equivalents. This method resulted in the formation of multilamellar liposomes ranging in size from 0.5 to 10 μm , with the majority in the 1–4 μm range. Approximately 50% of the original cellular protein is incorporated into the liposomes.

Cellular Reconstructions. Liposomes and recipient cells were incubated together in 1.0 ml of 0.14 M NaCl, 10 mM Tris-HCl, 3 mM KCl, 0.8 mM MgSO₄, pH 7.4, buffer for 60 min at 4°C to allow liposome/cell binding. The cells were washed by centrifugation (1,500 rpm, 5 min, twice) in ice-cold PBS and resuspended in 1.0 ml of DMEM without FBS. The cells were then incubated for 30 min at 37°C to allow liposome/cell fusion, washed twice by centrifugation in DMEM containing FBS, and resuspended in DMEM-FBS for the assays. Unless otherwise stated, all fusions were performed at a ratio of two cell equivalents of liposomes to recipient cells; i.e., in a typical experiment 1×10^6 recipient cells were fused with liposomes derived from the equivalent of 2×10^6 donor cells. Designation of the experimental protocol is "F(donor)/recipient", which indicates that liposomes derived from a specific donor were fused to the indicated recipient.

Results

Characterization of the Transfer System. The experimental procedure we used to examine the requirements for the expression of cytolytic activity and immunologic specificity by CTL consisted of the following steps. Cytolytic T cell clones were solubilized in the nonionic detergent NP-40 and the matrix and plasma membrane components separated from nuclear and mitochondrial constituents. Matrix proteins (detergent-insoluble fraction primarily consisting of actin) were

retained to increase liposome stability (14). To this cellular protein mixture were added Sendai virus envelope proteins that had been purified from detergent-solubilized viral preparations. These envelope proteins were of two types: a hemagglutinin-neuraminidase protein (HN) and a fusion protein (F). The HN is required for virus binding to cells, while F mediates the fusion of viral/cell membranes. The viral/cellular protein mixture was added to exogenous lipids to construct liposomes. Exogenous lipids were included to reduce the requirements for large amounts of cloned CTL cells and Sendai virus, whose natural lipids would otherwise be the only source of lipids for liposome construction. Liposomes were formed by dialysis of the lipid-protein mixture against PBS and harvested by high speed centrifugation. This procedure yielded only large multilamellar liposomes, the average size being $\sim 1-4 \mu\text{m}$. To fuse liposomes with recipient cells, we incubated the two together at 4°C for 60 min to allow Sendai HN protein-mediated binding of liposomes to the cells. The cells were then pelleted by centrifugation, resuspended in medium without FBS, and incubated at 37°C for 30 min to allow F protein-mediated fusion of the bound liposomes with the cells. At the end of this time, the fused cells were again pelleted and washed by centrifugation. The fused cells were then used immediately in assays to test for cytolytic activity and immunologic specificity.

When liposomes were derived in this manner from C57BL/6 anti-P815 CTL clones and fused with noncytolytic anti-Mls^a T cell clones, it was found that specific cytolytic activity could be transferred to cells that lacked this property (Table I). The presence of this activity in the fusion products was evidenced by the ability of the cells to lyse P815 target cells but not the irrelevant EL4 target cells. The reconstruction of cytolytic cells seemed to be possible using liposomes prepared from a single CTL clone or a mixture of several C57BL/6 anti-P815

TABLE I
Transfer of Cytolytic Activity by Liposomes to Noncytolytic T Cell Clones

Experiment	Effector population	Percent specific lysis*	
		P815 (H-2 ^d)	EL4 (H-2 ^b)
1 [‡]	Liposomes alone [§]	9	0
	Clone A9 alone	8	0
	F(7)/A9	65	7
2 [¶]	Liposomes alone [§]	6	0
	Clone A9 alone	5	0
	Clone B3 alone	6	0
	F(7,10,11,12)/A9	72	0
	F(7,10,11,12)/B3	67	0

* Data are expressed as the percent specific lysis obtained after 2-h incubation at an E/T cell ratio of 10:1.

[‡] Liposomes were derived from CTL clone 7 (C57BL/6 anti-P815) and fused with noncytolytic clone A9 (anti-Mls^a).

[§] Equivalent number of liposomes were used as would be present in the fusion products.

[¶] Liposomes were derived from a mixture of CTL clones 7,10,11, and 12 (all C57BL/6 anti-P815) and fused with either clone A9 or clone B3, both noncytolytic (anti-Mls^a).

TABLE II
Acquisition of Specific Cytolytic Activity by Noncytolytic Clones Requires Fusion of Liposomes Derived from Cytolytic Clones with Intact Cells

Experiment	Effector population	Percent specific lysis	
		P815 (H-2 ^d)	EL4 (H-2 ^b)
1*	Clone A9 alone	5	ND [‡]
	Clone A9 + Con A	5	ND
	Liposomes alone	2	ND
	Liposomes + Con A	2	ND
	F(10)/A9	88	1
2 [§]	Clone A9 alone	3	0
	EL4 alone	0	0
	Liposomes (7) alone	1	0
	Liposomes (EL4) alone	1	1
	Clone 7	97	6
	F(EL4)/7	91	0
	F(EL4)/A9	1	0
	F(7)/A9	85	0
3	Clone A9 alone	1	ND
	Clone 10	56	ND
	F(10)/A9	65	ND
	Clone 10 + EGTA	0	ND
	F(10)/A9 + EGTA	1	ND

Experiments 1, 2, and 3 represent three independent experiments.

* Liposomes were derived from CTL clone 10 (C57BL/6 anti-P815) and fused with noncytolytic clone A9 (anti-Mls^{*}). Cytotoxicity assays were performed at an E/T cell ratio of 10:1 for 2 h at 37°C. Whenever indicated, Con A (5 µg/ml) was present throughout the assay.

[‡] Not done.

[§] Liposomes were derived from either CTL clone 7 (C57BL/6 anti-P815) or EL4 tumor cell line and fused with either clone 7 or clone A9. ⁵¹Cr-release assays were performed at an E/T cell ratio of 10:1.

^{||} Liposomes were derived from clone 10 (C57BL/6 anti-P815) and fused with clone A9. Cytotoxicity assays were performed at an E/T cell ratio of 1:1. Whenever indicated, the effector and target cells were incubated at 23°C for 30 min, EGTA was added (20 mM/well final concentration), and the cells incubated for an additional 2 h at 37°C.

CTL clones.

In additional experiments, we determined that neither the constructed liposomes nor the recipient cells were able to induce cytolysis when the lectin Con A was included in the cytolytic assay (Table II, experiment 1). Cytolysis was observed only when the liposomes had been fused with the recipient cells. This observation excluded the possibility that lysis of the target cells was the result of unfused liposomes present in the assay or a latent cytolytic capacity of the recipient cells. This experiment also showed that the liposomes by themselves were not toxic to the target cells.

It is possible, however, that the cytolytic activity could be due to a functional alteration in the recipient cells as a result of the fusion such that the antigenic specificity would be contributed by the donor liposomes and the cytolysis by the

recipient cells. To examine this possibility, liposomes were constructed from either cytolytic or noncytolytic cell lines and fused with either cytolytic or noncytolytic recipients (Table II, experiment 2). When liposomes from a noncytolytic T cell line (EL4) were fused with a cytolytic CTL clone, the fused clone was unaltered in its cytolytic capacity. When the liposomes from noncytolytic T cells were fused with noncytolytic recipients, the fusion products remained noncytolytic. Only when the liposomes were derived from cytolytic donors were cytolytic fusion products obtained. Thus, it appears that to obtain cytolytic fusion products, the donor cells must be cytolytic and the liposomes must be introduced into cells.

We also examined the effect of EGTA on the cytolytic activity displayed by the fusion products (Table II, experiment 3). It has been previously demonstrated (16) that Mg^{2+} is required for CTL binding to target cells, while Ca^{2+} is essential for the cytolytic phase of the lytic process. The cytolytic assay was performed as usual except that a 30 min preincubation period at 23°C was included. Under these conditions, the fusion products bound to the target cells (as observed by light microscopy) but lysis was negligible. The mixture of fusion products and target cells was then incubated for 2 h at 37°C in the presence or absence of EGTA, which chelates Ca^{2+} . The fusion products behaved exactly as the CTL clone from which the liposomes had been derived. That is, cytolysis was completely inhibited when Ca^{2+} was not available in the assay.

In the experiments described above, the cytolytic capacity of the fusion products was examined at only one E/T ratio. Additional experiments were performed in which the lytic capacity of the fusion products was compared with that of the donor CTL clones. The results of a representative experiment are shown in Fig. 1. It can be seen that over a wide range of E/T ratios (1:3–10:1), the lytic activity of the fusion products was similar to that of the donor CTL clone. It thus appears that upon transfer of components from a CTL into a suitable recipient cell (such as A9 or B3), the cytolytic capacity of such a fusion product is highly efficient.

In an attempt to quantitate the liposomal transfer of cellular constituents to the various recipient cells, we performed the following experiment. Viable donor CTL were labeled with fluorescein isothiocyanate (FITC) (17) and liposomes constructed as described above. These liposomes were then fused to various recipient cells and examined for fluorescence by fluorescence-activated cell sorter (FACS) analysis. Positive controls consisted of donor cells freshly labeled with FITC. Negative controls were recipient cells incubated with identically constructed liposomes lacking the Sendai virus HN and F proteins. Examination of three different recipient cells demonstrated that indeed transfer of FITC had occurred during the fusion process. It was observed that 100% of the recipient cells (for each of the three different cell types) had undergone fusion as assessed by this criterion. Calculation of the amount of fluorescent material transferred revealed that each of the recipient cell types examined had acquired ~5% of the labeled cellular components present in the positive controls (data not shown).

Antigenic Specificity of Fusion Products. To further verify the specificity of the lytic activity of the fusion products, we derived liposomes from a C57BL/6 (H-2^b) anti-P815 CTL clone directed against H-2K^d alloantigens (that is, its lytic

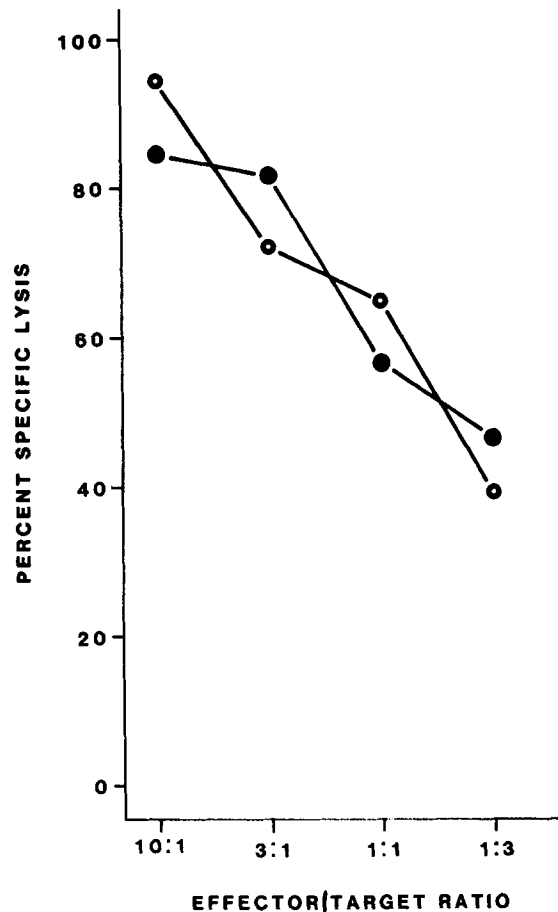


FIGURE 1. Cytolytic efficiency of fusion product. The CTL donor clone 10 (C57BL/6 anti-P815) (○) and the fusion product F(10)/A9 (liposomes constructed from clone 10 and fused with clone A9) (●) were assayed for cytolytic activity on P815 target cells in a 2-h, ^{51}Cr -release assay. Incubation with liposomes or clone A9 alone resulted in <3% lysis of P815 target cells.

activity was inhibited by mAb against H-2K^d but not mAb against H-2D^d or H-2L^d, fused them with (anti-Mls^a) noncytolytic clone A9, and then tested whether the lytic activity of the fusion products could be blocked by anti-H-2K^d mAb (Fig. 2). It was found that the lytic activity of the fusion products could indeed be inhibited by this mAb, indicating that the liposomal transfer had conferred to A9 cells the immunologic specificity of the donor CTL.

The antigenic specificity of the fusion products also was assessed by using a panel of target cells bearing different antigens. For these experiments we used as liposome donors two C57BL/6 CTL clones with distinct antigenic specificities, namely anti-P815 clone 7 and anti-MoLV clone 14. As shown in Fig. 3, clone 7 displayed lytic activity against P815 (H-2^d) target cells and another H-2^d (MoLV-induced) cell line, LSTRA. However, it was nonreactive with H-2^b (MoLV-induced) MBL-2 target cells. In contrast, clone 14 lysed MBL-2 target cells but had no activity against P815 or LSTRA tumor cells. When liposomes derived

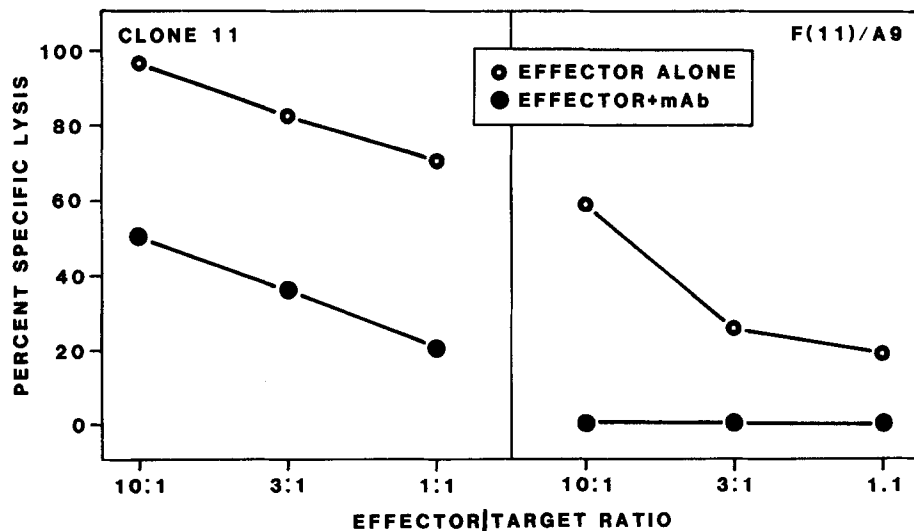


FIGURE 2. Ability of monoclonal antibody to inhibit the acquired lytic activity of fusion products. Cytolytic assays were performed either in the presence (solid symbols) or absence (open symbols) of mAb S13-11 (anti-H-2K^d). ⁵¹Cr-labeled P815 cells were used as target cells and either clone 11 (C57BL/6 anti-H-2K^d) or the fusion product F(11)/A9 (liposomes constructed from clone 11 and fused with clone A9) were used as effector cells.

from clone 7 were fused with A9 cells, the fusion products exhibited the same specificity pattern as CTL clone 7 (Fig. 3). Reciprocally, when clone 14 was used as the liposome donor, the fusion products obtained with A9 cells exhibited the same lytic reactivity as CTL clone 14. These results thus indicate that immunological specificity was related to the source of liposomes and not to the recipient cells.

Dose-Response Analysis of Liposomal Transfer. To examine the efficiency of liposomal transfer, varying numbers of liposomes from clone 7 or clone 14 were fused with the noncytolytic clone A9, and the lytic activity of the fusion products was tested on the corresponding target cells. The results of two such experiments are shown in Fig. 4. It can be seen that the solubilization of the CTL and subsequent liposomal transfer to the recipient cells was a highly efficient process, since significant cytolytic activity was imparted to the A9 recipient cells after fusion with one cell equivalent of liposomes from either CTL clone. In these experiments, maximal efficiency of transfer occurred with five cell equivalents of liposomes. In other experiments, transfer of two to five cell equivalents resulted in comparable lytic activity. Surprisingly, a further increase in the amount of liposomes used for fusion resulted in a concomitant decrease in cytolytic activity. Further experiments in which unfused liposomes were titrated into a cytolytic assay demonstrated that such free liposomes did not directly inhibit cytolysis (data not shown). Whether this decrease of activity was due to the introduction of excess lipids and/or cellular proteins has not been ascertained, but recipient cell viability was equivalent at all cell equivalent ratios tested (>90%; data not shown).

Liposomal Transfer of Cytolytic Activity Is Not Restricted to Noncytolytic T Cell

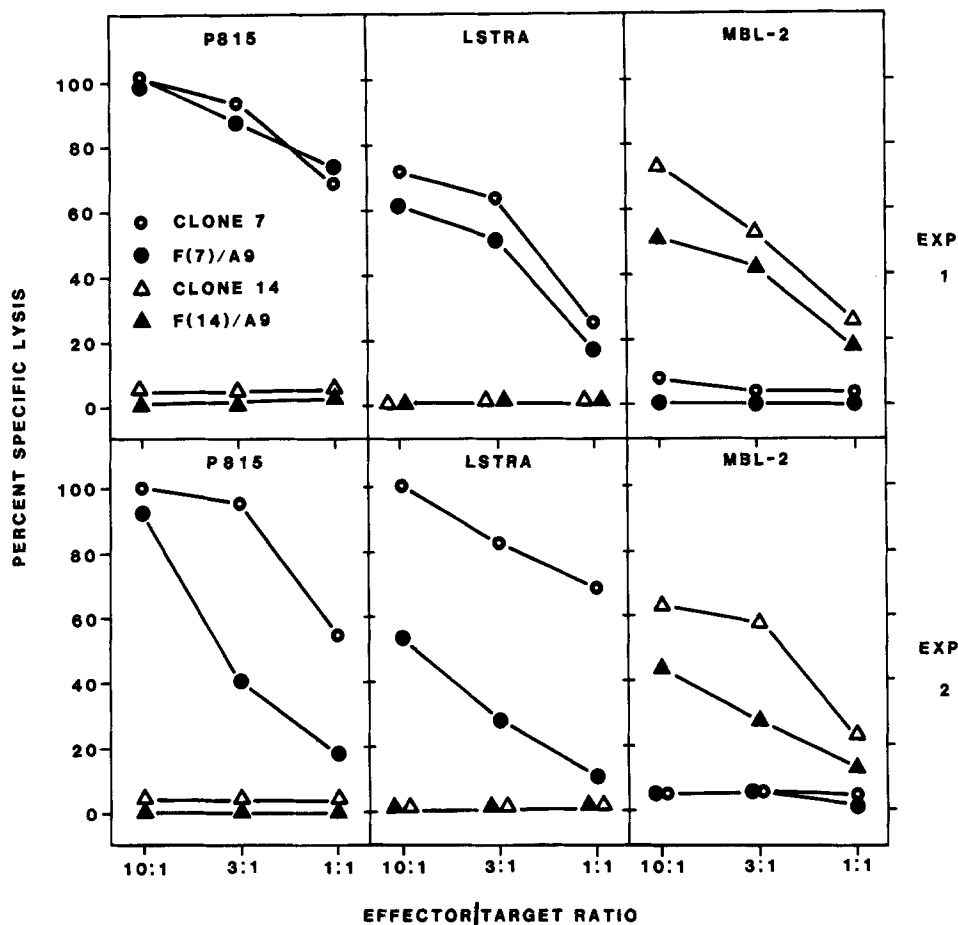


FIGURE 3. Specificity of fusion products. Liposomes were constructed from either clone 7 (C57BL/6 anti-P815) or clone 14 (C57BL/6 anti-MoLV) and fused with clone A9 (anti-Mls^d). The original CTL clones and the fusion products were assayed for cytolytic activity in a 2-h, ⁵¹Cr-release assay. Target cells were either P815 (H-2^d), LSTRA (H-2^d-MoLV), or MBL-2 (H-2^b-MoLV). Effector populations consisted of clone 7 (○), clone 14 (△), F(7)/A9 (●), and F(14)/A9 (▲). Lysis of any one of the target cells by either of the liposome populations or clone A9 alone was <2% at an E/T cell ratio of 10:1. Experiments 1 and 2 represent two independent experiments.

Clones. In the previous experiments, we had only investigated the possibility of transferring the cytolytic activity and/or the antigen specificity from CTL clones to noncytolytic T cell clones. The feasibility of using transformed T cell lines as well as non-T cell lines as recipients for liposomal fusion was also examined. As shown in Table III, it was possible to obtain cytolytic fusion products with a variety of recipient cells, although a variable degree of cytolytic activity was observed. Fusion products obtained with two T cell lines, the BW5147 thymoma and a subline of EL4 thymoma cells, expressed low but significant cytolytic activity. However, a separate subline of EL4 cells yielded fusion products that expressed high cytolytic activity. Surprisingly, fusion products obtained with the B cell line M12.4.1 were also able to express lytic activity, thus indicating that

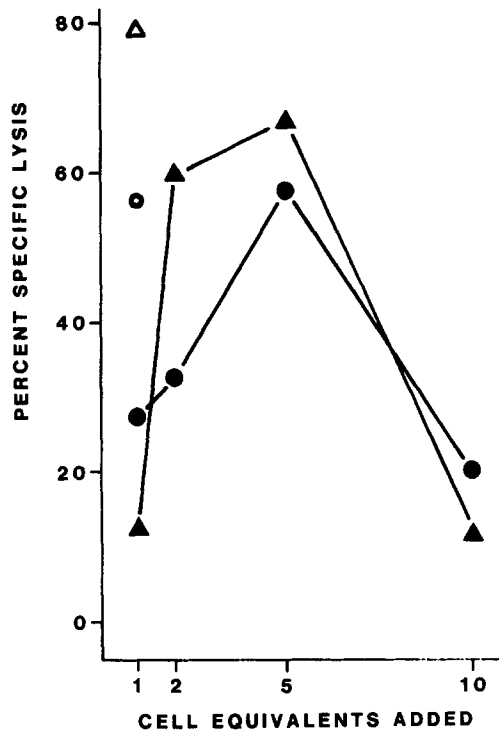


FIGURE 4. Efficiency of cytolytic transfer between CTL and noncytolytic cells by liposomes. Liposomes were constructed and fused with recipient cells at the indicated cell equivalents as described in Materials and Methods. ^{51}Cr -release assays were then performed at an E/T cell ratio of 1:1 using either P815 or MBL-2 target cells. (○) clone 7 (C57BL/6-anti-P815); (●) F(7)/A9; (△) clone 14 (C57BL/6 anti-MoLV); (▲) F(14)/A9.

cells other than those of T cell origin could serve as suitable recipient cells. In these experiments, the clones A9 and B3, normally used as recipients in the experiments described above, again demonstrated high cytolytic activity after fusion with the same liposomes. Since liposome-mediated transfer of FITC-labeled material to each of three different recipients was equivalent (see above), these observations suggest that the differences in cytolytic expression among the fusion products were not due to different extents of liposomal fusion and transfer to the recipient cells, but rather were related to some inherent factor(s) in the recipient cells. Moreover, it is also apparent that the cytolytic activity of the fusion products was specific irrespective of the origin of the recipient cell, since no lysis of irrelevant EL4 target cells was observed with any of the fusion products tested (Table III).

Discussion

In this study, we investigated some of the requirements for the expression of specific CTL activity by transferring this activity from cloned CTL to various noncytolytic recipient cells. In particular, material derived from detergent-solubilized CTL clones was inserted into synthetic liposomes and, with the aid of Sendai virus envelope proteins, fused with various recipient cells. These experi-

TABLE III
Liposomal Transfer of Specific Cytolytic Activity from Cytolytic Clones Is Not Restricted to Noncytolytic T Cell Clones

Experiment	Effector population	Percent specific lysis*	
		P815 (H-2 ^d)	EL4 (H-2 ^b)
1 [‡]	Clone A9 alone	1	ND
	M12.4.1 alone	0	ND
	BW5147 alone	2	ND
	EL4 alone	0	ND
	F(10)/A9 [‡]	94	ND
	F(10)/M12.4.1	62	ND
	F(10)/BW5147	18	ND
F(10)/EL4.1	17	ND	
2 [§]	Clone A9 alone	3	0
	Clone B3 alone	1	0
	M12.4.1 alone	1	0
	EL4.2 alone	0	0
	F(7)/A9 [‡]	85	0
	F(7)/B3	70	2
	F(7)/M12.4.1	77	0
F(7)/EL4.2	80	0	

* Data are presented as percent specific lysis at an E/T cell ratio of 10:1 during a 2-h assay. ND, not done.

[‡] Liposomes were derived from CTL clone 10 (C57BL/6 anti-P815) and fused with either noncytolytic clone A9, B cell line M12.4.1, T cell line BW5147, or T cell line EL4.1 (a ouabain-thioguanine-resistant subline of EL4).

[§] Liposomes were derived from CTL clone 7 (C57BL/6 anti-P815) and fused with either clone A9, clone B3, B cell line M12.4.1, or T cell line EL4.2 (a H-2K^b D^b subline of EL4).

[‡] Liposomes derived from either clone 7 or clone 10 resulted in <3% specific lysis when tested alone against either P815 or EL4 target cells.

ments demonstrated that both specificity and lytic activity could be imparted to recipient cells. Liposomes constructed without Sendai virus proteins did not bind efficiently to recipient cells and were unable to transfer cytolytic activity. Liposomes containing both cellular and viral proteins had no direct lytic activity even in the presence of Con A. Furthermore, only when liposomes were derived from cytolytic donors and integrated into intact cells was specific cytolytic activity expressed.

Noncytolytic T cell clones were highly suitable as recipient cells for the introduction of specific CTL components. Also suitable were various noncytolytic tumor T cell lines such as EL4 and BW5147. Although the expression of CTL activity varied among the fusion products obtained with these different recipient cells, each one of the three lines tested displayed significant cytolytic capability. It does not seem that this variability in cytolytic expression was due to the ability of the different cell types to be fused with the liposomes. Possibly, this difference is related to undetermined nuclear/cytoplasmic factors. If some sort of recipient cell contribution is required for the expression of cytolytic activity after liposomal

fusion (since liposomes containing CTL components are themselves inactive), then it is reasonable to expect that various recipient cell types could exhibit different levels of cytolytic capacity. This contribution could be either nuclear, such as a signal from the fusion product to the target cell as proposed by some investigators for the mechanism of CTL-mediated lysis (18–20), or could merely be a cytoplasmic interaction that, upon receptor occupancy, exposes an enzyme-like activity that causes target cell lysis (21, 22). However, it should be noted that regardless of the cell type used as a recipient, there was no difference in the antigenic specificity exhibited by the fusion products. Surprisingly, even a lymphoid cell line of non-T cell origin, the B cell line M12.4.1, could serve as a suitable functional recipient for liposomes containing CTL components. This finding suggests that metabolic activities necessary for the expression of CTL function are present in B cells but that these cells lack the membrane and/or other cytoplasmic components that are essential for binding to, and/or lysis of target cells. Examination of recipient cells other than those of lymphoid origin for their ability to express CTL function upon fusion with liposomes is currently underway. If indeed nonlymphoid cells are suitable recipients, the implication would be that all cells are potentially able to express such a specialized function, given the proper CTL-derived components.

Experiments involving the ability of mAb to inhibit specifically the lytic activity of fusion products, as well as the analysis of the patterns of lytic reactivity displayed by recipient cells of liposomes containing membrane proteins from CTL of two different specificities confirmed the high degree of specificity conferred by this transfer system. Since no attempt was made to separate the function of target cell recognition from that of lysis, it remains to be determined whether these two activities can be dissociated or not.

In contrast to the high cytolytic activity of liposome-derived fusion products described here, previous work from our laboratory (4) indicated that cytolytic "cybrids" obtained by fusing enucleated CTL clones (which were noncytolytic) with EL4 tumor cells expressed low and variable lytic activity. The discrepancy may in part be due to the fact that the lytic activity of the CTL clones used in the latter experiments was susceptible to inhibition by mAb against Lyt-2 (Lyt-2-dependent CTL), while Lyt-2-independent CTL clones were used as liposome donors in the experiments presented here. CTL that are inhibited in their activity by anti-Lyt-2 antibodies appear to possess relatively low affinity and/or few antigen receptors (23, 24). Further work is needed, however, to ascertain whether the effectiveness of liposome-mediated transfer of CTL activity depends on the availability of CTL with high affinity or more numerous receptors. It also is noteworthy that enucleated CTL clones were not cytolytic even in the presence of Con A, but had to be fused with noncytolytic recipient cells to exhibit functional activity (4). These findings, which are in agreement with the present results, support the contention that the noncytolytic recipient cell provides some (nuclear and/or cytoplasmic) contribution that is essential for the expression of specific cytolytic activity by fusion products.

Jakobovits et al. (5) have demonstrated that the unresponsiveness of lymphocyte populations to particular mitogens was not due to an inherent lack of the necessary intracellular machinery, but rather was due to the absence of appro-

priate membrane components. Upon transfer of B cell membrane components to T cells, or vice versa, the fused cells could now respond to a normally nonstimulatory mitogen. Our ability to construct cytolytic cells using a B cell line as a fusion recipient is in accord with these observations. It should be noted that the transfer system we used here is similar to that used by Jakobovits et al. (5), in that we also used Sendai virus envelope proteins to mediate fusion. This system appears to be preferable to that using polyethyleneglycol because it is less toxic (>90% cell viability after fusion) and highly efficient (90–100% of cells can be fused). However, our system differs from that of Jakobovits et al. (5) in that our liposomes were constructed from synthetic lipids, thus enabling the use of smaller quantities of cellular and viral components.

Finally, although the participation of cytoplasmic CTL constituents (including granules) cannot be ruled out, the present study provides suggestive evidence that the cellular component(s) responsible for specific CTL activity resides solely in (or is associated with) the plasma membrane and can be fully functional after transfer into recipient cells that lack this property. It is not known, however, whether other activities that are linked with membrane-associated receptors, such as antigen-dependent lymphokine production and cellular proliferation, can be expressed by fusion products. If antigen-specific function in fusion products can be shown to be maintained for sufficiently long periods of time (and preliminary experiments indicate that near optimal cytolytic activity persists for at least 6 h), then the liposomal transfer system described here should provide a powerful tool to examine these questions. Moreover, it may allow a direct testing of the functional activity of purified membrane constituents such as the putative T cell receptors recently described by several groups (25–27).

Summary

Murine cytolytic T lymphocytes (CTL) clones were solubilized in Nonidet P-40 detergent, and the matrix and membrane proteins separated from the nuclear constituents. These proteins, in combination with exogenous lipids and Sendai virus envelope proteins, were used to construct liposomes that were then fused with noncytolytic cloned T cell recipients. The resultant fusion products were found to be highly cytolytic and appeared to express the same specificity as the original donor clone. Further analysis showed that the liposomal transfer process was extremely efficient. Moreover, in addition to noncytolytic T cell clones, three transformed T cell lines and one B cell line were found to express specific cytolytic activity after fusion with appropriate liposomes. Inhibition experiments using monoclonal antibodies against target cell antigens, as well as analysis of the lytic reactivity pattern of the fusion products, confirmed the high degree of specificity conferred to the recipient cells. This study thus indicates that the two characteristics typically associated with CTL, namely antigen-specific recognition and cytolytic activity, can be solubilized from CTL and transferred to recipient cells that do not express these characteristics.

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