Rescue of Anti-Influenza A Virus Cytotoxic T-Lymphocyte Responses in Chemotherapy-Suppressed Mice

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The administration of cyclophosphamide (50 to 100 mg/kg) at 48 to 72 h before removal of murine lung or spleen mononuclear cells for culture rendered DBA/2 mice incapable of generating an effective cytotoxic T-lymphocyte response to influenza A virus-infected cells. The cytotoxic T-lymphocyte precursor frequency to influenza A virus in lung and spleen cells from cyclophosphamide-treated mice was significantly decreased when compared with that of normal littermate controls. The low cytotoxic T-lymphocyte activity in the lungs and spleens of cyclophosphamide-treated mice could be partially restored in vitro by human interleukin 2.

The use of influenza A virus as a prototype model for infectious disease complications during chemotherapy is well founded (1, 5-7, 9, 17, 19, 33, 43, 44, 46, 47). Evidence has shown that although influenza A virus induces a protective antibody response (42), cytotoxic T lymphocytes (CTL) play a more functional role in increasing recovery from infection and resolution of pneumonia (19, 44). These and other studies have shown that CTL are associated with recovery of mice with influenza pneumonia (1, 6, 7, 17, 19, 43, 46, 47). The CTL response to influenza A virus is dependent on Lyt 1⁺ helper T lymphocytes (1, 6, 7, 9), as are other viral models (21, 48). In addition, supernatants from concanavalin A-rat spleen cell cultures can replace the need for Ly 1⁺ helper T cells in vitro for the generation of CTL against influenza virus-infected cells (7). This has also been demonstrated in another system by Effros and Walford (18), who have shown that aged mice are incapable of generating normal CTL responses to influenza A virus-infected cells. These low CTL responses were elevated to normal levels by the addition of crude lymphokine preparations to the culture system.

It is apparent from our previous studies as well as others that cyclophosphamide (CY) possesses a degree of selective toxicity with respect to subpopulations of lymphoid cells (24, 26, 28-31, 35, 37, 39, 41). Recent results have shown that CY, at therapeutic doses, spares some (but not all) CTL precursors to allogeneic and syngeneic stimulator cells (24, 26, 39). Helper T lymphocytes (28) or undefined accessory cells needed for helper T-cell responses in vitro (41) may be limiting in CY-treated mice and thus render the mouse incapable of generating normal CTL responses. Both in vitro and in vivo CTL responses to tumor cells can be restored by interleukin 2 (IL2) after CY therapy (24, 28-31).

The purpose of this study was to enumerate the CTL precursors to influenza A virus in the lungs and spleens of CY-treated mice and attempt to restore low anti-influenza virus CTL activity using human IL2. Human IL2 was used because the preparations are recombinant DNA-derived materials produced in *Escherichia coli*. In addition, we are developing experimental systems as relevant models for the analysis of human IL2 efficacy in clinical trials for immune

deficiencies and in the reversal of immunosuppression by antineoplastic therapy.

MATERIALS AND METHODS

Mice. Male DBA/2 (*H-2^d*) mice (6 to 8 weeks old) were obtained from the Jackson Laboratory, Bar Harbor, Maine. The mice were housed in a sterilgard hood (Baker Co., Sanford, Maine; model UBM-600). The air flow balance prevents ingress and egress of airborne contamination. Individual cages were also covered with biohazard filter tops. Mice were fed routine mouse chow and water ad libitum.

CY. The CY-NaCl preparation (Mead Johnson, Evansville, Ind.) was dissolved in sterile water to a concentration of 20 mg/ml. Individual mice were weighed and given intraperitoneal injections of CY (50 to 100 mg/kg) 48 to 72 h before removal of lymphoid tissue. Generally, a 40 to 75% reduction in lung and splenic cellularity was observed. Lymphoid cells were >80% viable as judged by trypan blue exclusion.

Influenza A virus. Influenza A/HK/8/68-X-21 (H3N2) virus was obtained from R. O'Reilly (Sloan-Kettering Institute). The virus was propagated in allantoic sacs of 11-day-old chicken embryos for 72 h, and the allantoic fluid was removed aseptically and stored at -80°C. Hemagglutinating units were determined by titration of the virus on fresh chicken erythrocytes (40).

In vivo immunization. DBA/2 mice were immunized intranasally with 4 hemagglutinating units on 2 successive days. The mice were anesthetized with chloral hydrate (325 mg/kg), the virus suspension was placed on the nosepad using a 100- μ l pipette, and the inoculum was allowed to be inhaled. Mice were used at 3 weeks to 2 months after priming.

In vitro generation of CTL. Culture medium consisted of RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated (56°C; 45 min) fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 μ g/ml), and 5×10^{-5} M 2-mercaptoethanol.

Spleen cells from primed mice or normal DBA/2 mice were used as a source of stimulator cells. Spleens were removed aseptically, teased apart, and washed in balanced salt solution (BSS), and the erythrocytes were lysed with ammonium chloride lysing buffer. The spleen cells were then

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washed in culture medium, counted in trypan blue, and adjusted to 2×10^7 /ml in culture medium with 100 hemagglutinating units of influenza A virus. The cells were incubated at 37°C in a shaking water bath for 90 min, washed three times in BSS, and γ -irradiated at 1,200 R. These stimulator cells were then adjusted to 5×10^6 /ml in complete culture medium and dispensed at 2.5×10^5 per culture well in Linbro flat-bottomed microtest plates (no. 76-004-05).

Splenic responder cells were derived from influenza A virus-primed mice. The cells were removed, washed three times in BSS, and adjusted to 2×10^7 /ml in culture medium. The cells were then dispensed at 10^6 per culture well in flat-bottomed microtest plates with the γ -irradiated stimulator cells for 5 to 7 days at 37°C in a 5% CO₂-air humidified atmosphere. The total volume per well was 0.2 ml.

Lung responder cells were prepared using an adaptation of the method described by Liu et al. (27). The mice were exsanguinated by retroorbital bleeding. The lungs were removed aseptically, rinsed in BSS, and chopped into 1-mm³ fragments using a McIlwain tissue chopper (Brinkman Instruments Inc., Westbury, N.Y.). The fragments were placed in 100 U of collagenase (Worthington Diagnostics, Freehold, N.J.) per ml in culture medium for 90 min at 37°C. The fragments were then passed through a 35-mesh screen and filtered through sterile gauze to remove large debris. The cells were pelleted ($400 \times g$), washed three times in BSS plus 10% FCS, layered over a Ficoll-Isopaque (Sigma Chemical Co., St. Louis, Mo.) gradient (density = 1.090), and centrifuged at $400 \times g$ (room temperature) for 20 min. The cells at the interface were collected, pelleted, washed once in culture medium, and adjusted to 5×10^6 /ml in culture medium. There was total mononuclear cell recovery of 0.5×10^6 to 2.7×10^6 per lung which were >80% viable as judged by trypan blue exclusion. The cells were then dispensed into round-bottomed microtest plates (Linbro; no. 76-013-05) at 2×10^5 to 2.5×10^5 per well. Stimulator cells were added (2.5×10^5 per well) as described above. The total volume per well was 0.2 ml. The plates were incubated for 5 to 7 days at 37°C in a 5% CO₂-air humidified atmosphere.

Measurement of CTL activity. (i) Preparation of influenza A virus-infected targets. For uninfected targets, P815 mastocytoma cells (*H-2^d*) obtained from weekly intraperitoneal passage in DBA/2 mice were harvested from ascites fluid, washed twice in BSS, and cultured at 2×10^7 /25 ml in complete culture medium (75-cm² flask; Corning Glass Works, Corning, N.Y.) overnight at 37°C in 5% CO₂-air. For influenza A virus-infected P815 target cells, 2×10^7 P815 tumor cells were incubated for 90 min with 100 hemagglutinating units of influenza A virus in culture medium in a 37°C shaking water bath before culturing overnight as described above. The cells were washed twice in BSS and cultured as described above. After incubation overnight, the targets were washed in BSS and incubated with 250 μ Ci of ⁵¹Cr (New England Nuclear Corp. Boston, Mass.) in a volume of 0.6 ml of RPMI tissue culture medium for 1 h. After washing and counting, 5×10^3 ⁵¹Cr-labeled P815 or influenza A virus-infected P815 were mixed with in vitro-generated CTL effector cells as described below.

(ii) CTL assay. Cytotoxic activity was measured by two methods. For lung CTL, a modification of the ⁵¹Cr release assay as described by Baum and Pilarski (8) was used. This assay was necessary because of the relatively few lymphocytes obtainable from murine lungs. This procedure reflects results obtained using the classical ⁵¹Cr release assay (30). Briefly, the ⁵¹Cr-labeled P815 or influenza A virus-infected P815 targets (5×10^3) were added (20 μ l) to the cultures of

lung effector cells on day 5 or 7 of incubation. The culture wells containing both effectors and ⁵¹Cr-labeled targets were then mixed with a multichannel pipettor, centrifuged for 3 min at $30 \times g$, and incubated at 37°C for 5 h in a humidified atmosphere with 5% CO₂. At the end of the 5-h incubation period, the plates were centrifuged at $400 \times g$ for 5 min, and the amount of radioactivity released into 0.1 ml of the supernatant of each culture well was determined in a Nuclear Chicago gamma counter. The percentage of specific ⁵¹Cr release on triplicate or quadruplicate cultures was calculated in the following manner:

$$\frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100$$

Total releasable counts were determined by dilution of the ⁵¹Cr-labeled target cells in distilled water.

For quantitative measurement of splenic cytotoxic effector cells, a modification of the method of Cerottini and Brunner (13) was followed. The effector cells were harvested, washed, counted, and suspended in RPMI 1640-FCS culture medium. The effector cells and target cells were plated in V-bottomed culture wells (Linbro; no. 76-321-05) at several effector to target cell ratios in triplicate or quadruplicate. The plates were centrifuged for 3 min at $30 \times g$ and incubated at 37°C for 5 h in 5% CO₂-air. After 5 h, the amount of radioactivity released into 0.1 ml of the supernatant of each culture well was determined as described above.

(iii) Limiting dilution analysis for CTLp. Limiting dilution analysis of CTL precursor (CTLp) frequency was performed by an adaptation of the method described by Taswell et al. (39). Limiting numbers of splenic or lung mononuclear cells were cultured in RPMI 1640-FCS culture medium with stimulator cells and 25 to 50% (vol/vol) secondary mixed lymphocyte reaction supernatant (30, 36). The cell mixtures were cultured in round-bottomed microtest plates containing a total volume of 0.2 ml. Twelve to twenty-four microcultures were set up per dilution; at least five doubling dilutions were run per group. The cultures were incubated for 7 days at 37°C in 5% CO₂-air. The plates were covered with foil to minimize evaporation. Cytotoxic activity was measured against ⁵¹Cr-labeled influenza A virus-infected P815 targets by the method described above for lung CTL. This system allows for the expression of CTL directed against only influenza A virus-infected targets providing that the secondary mixed lymphocyte reaction supernatant has an IL2 activity of <5 U per culture well. Microcultures were scored as positive for cytotoxic activity when the ⁵¹Cr release exceeded the mean spontaneous release by 3 standard deviations. Estimates of CTLp frequency were calculated by an iterative procedure based on maximum likelihood analysis (34) using a program written by E. Kenney (Miami University).

(iv) Anti-Thy 1.2 and complement. Lymphoid cells (2×10^5 to 2×10^7) were treated with monoclonal anti-Thy 1.2 ascites (1/100; obtained from U. Hämmerling, Sloan-Kettering Institute) for 45 min at room temperature. Rabbit complement (1/16; Cederlane Laboratories, Hornby, Ontario, Canada) was then added for 45 min at 37°C. The cells were washed in BSS, counted, and suspended in RPMI 1640-FCS culture medium.

(v) IL2. A recombinant DNA-derived human IL2 produced in *E. coli* was obtained from Amgen (Thousand Oaks, Calif.). Both the recombinant preparation and a highly purified human IL2 preparation react similarly in the CTL systems described above as well as in other systems de-

TABLE 1. Effect of CY on the generation of spleen CTL to influenza A virus-infected cells

Pretreatment of mice ^a	Length of incubation (days)	% Specific ⁵¹ Cr release ^b (mean ± SEM)	
		Influenza A virus P815	P815
None	5	29 ± 1.3	4 ± 0.3
CY (150 mg/kg), 48 h	5	4 ± 0.4	<1
None	5	31 ± 2.0	4 ± 0.5
CY (100 mg/kg), 48 h	5	3 ± 1.5	<1
None	5	67 ± 1.2	9 ± 1.2
CY (100 mg/kg), 72 h	5	29 ± 0.8	4 ± 0.5
None	7	34 ± 0.8	5 ± 0.9
CY (100 mg/kg), 72 h	7	19 ± 1.6	5 ± 0.9

^a DBA/2 mice were injected with the CY dose at the specified number of hours before removal of spleen cells for culture. All cultures were stimulated with influenza A virus-infected syngeneic spleen cells. Responder cells were spleen cells.

^b Five-hour assay. Effector to target cell ratio, 25/1.

scribed previously by our laboratory (31, 45; K. Welte, R. Mertelsmann, A. Einzig, V. Merluzzi, Y. Stabinsky, T. Boone, and L. Souza, Blood 62(Suppl. 1), abs. 377, 1983). Semipurified murine and rat IL2 also are active but are not as extensively purified nor available to us in recombinant DNA preparations. The use of human IL2 therefore is necessary to rule out lymphokine contamination products present in IL2 preparations from other sources and provides necessary data to progress from these experimental animal models to clinically relevant in vitro and in vivo testing.

RESULTS

Initial experiments were designed to test the effect of CY pretreatment on anti-influenza A virus CTL activity in vitro. Splenic CTL responses were consistently inhibited by CY doses of ≥100 mg/kg given 48 to 72 h before removal of responder cells for culture (Table 1). Generally, for splenic responder cells, CY doses of <100 mg/kg were more variable in the induction of immunosuppression. No significant killing of P815 target cells was seen in these experiments nor in any subsequent experiments. Similar to the splenic responder cells, lung CTL responses were also inhibited by CY; however, CY doses of <100 mg/kg were suppressive (Table 2). In both the spleen and lung CTL assays, incubation periods of 5 and 7 days were used. The peak CTL response ranged between 5 and 8 days. Experiments are now in

TABLE 2. Effect of CY on the generation of lung CTL to influenza A virus-infected cells

Pretreatment of mice ^a	Length of incubation (days)	% Specific ⁵¹ Cr release ^b (mean ± SEM)	
		Influenza A virus P815	P815
None	5	45 ± 2.4	4 ± 1.1
CY (100 mg/kg), 72 h	5	3 ± 0.8	<1
None	5	58 ± 3.8	<1
CY (50 mg/kg), 48 and 24 h	5	<1	<1
None	7	72 ± 4.0	4 ± 1.0
CY (50 mg/kg), 72 h	7	11 ± 1.9	<1

^a DBA/2 mice were injected with the CY dose at the specified number of hours before removal of lung lymphocytes for culture. All cultures were stimulated with influenza A virus-infected syngeneic spleen cells. Responder cells were lung cells.

^b Five-hour assay.

progress to assess the effects of CY given at times >72 h before culture. In addition, experiments are planned to examine CTL responses past the peak response date.

The CTLp frequency of both spleen and lung cells in normal and CY-treated DBA mice was determined (Tables 3 and 4). It can be seen that there is a higher CTLp frequency in the lung as compared with the spleen. This is most likely due to the initial route of priming (intranasal) and perhaps increased lymphocyte traffic to the lung after priming. There was a significant reduction in the CTLp frequency in both spleens and lungs of CY-pretreated mice. This reduction in CTLp frequency may be one of the causes of the low CTL responses seen in spleen and lung cells after CY treatment. There is evidence to suggest, however, that the reduction in CTLp frequency in allogeneic or syngeneic CTL responses by CY may be compensated for by the expansion of existing CTLp with IL2-containing supernatants (24, 30). Tables 5 and 6 show the ability of human IL2 to restore both spleen and lung CTL activity to influenza A virus-infected target cells. In CY-treated responder cells, both splenic (Table 5) and lung (Table 6) CTL responses to influenza virus were enhanced by IL2. The effector cells for splenic and lung CTL anti-influenza A virus responses were Thy 1.2⁺ regardless of whether IL2 was present or not in the culture system. The P815 targets used were insensitive to classical natural killer cell activity (unpublished observations), and previous experiments have shown that IL2 doses of <12.5 U/ml do not induce nonspecific cytotoxicity but rather enhance specific CTL responses (31).

DISCUSSION

Chemotherapeutic agents currently used clinically for neoplastic and autoimmune diseases have an inherent problem of selectivity. Modalities directed toward treatment of these conditions or suppressing advanced inflammatory disease subject the host to the complications of infectious disease (2–4, 10). Immune deficits occurring during chemotherapy might limit the host's potential to eradicate residual antigenic tumor cells and, in addition, the immune response necessary to eliminate complicating infections. A complete understanding of the defects in immune responsiveness,

TABLE 3. CTLp frequency to influenza A virus in spleen cells of control and CY-treated mice

Group ^a	Cells/well (×10 ⁴)	Fraction responding	Reciprocal mean CTL frequency ^b (95% confidence interval)
DBA	25	24/24	81,898 ^c (62,475–107,358)
DBA	12.5	23/24	
DBA	6.25	10/24	
DBA	3.125	7/24	
DBA	1.5625	0/24	
DBA	0.7813	0/24	
DBA-CY	25	20/24	233,779 ^d (167,979–325,353)
DBA-CY	12.5	8/24	
DBA-CY	6.25	3/24	
DBA-CY	3.125	2/24	
DBA-CY	1.5625	2/24	
DBA-CY	0.7813	0/24	

^a Influenza A virus-primed DBA/2 mice (DBA) or influenza A virus-primed DBA/2 mice injected with CY (100 mg/kg) (DBA-CY) 72 h before removal of spleen cells for culture.

^b Assay after 7 days of incubation; 5-h ⁵¹Cr release.

^c Regression coefficient, 0.212.

^d Regression coefficient, 0.061.

TABLE 4. CTLp frequency to influenza A virus in lung cells of control and CY-treated mice

Group ^a	Cells/well ($\times 10^4$)	Fraction responding	Reciprocal mean CTL frequency ^b (95% confidence interval)
DBA	12.5	24/24	19,662 ^c (14,926–25,899)
DBA	6.25	24/24	
DBA	3.125	22/24	
DBA	1.5625	12/24	
DBA	0.7813	3/24	
DBA	0.3906	3/24	
DBA-CY	12.5	23/24	78,201 ^d (58,327–104,846)
DBA-CY	6.25	16/24	
DBA-CY	3.125	4/24	
DBA-CY	1.5625	1/24	
DBA-CY	0.7813	0/24	
DBA-CY	0.3906	0/24	

^a Influenza A virus-primed DBA/2 mice (DBA) or influenza A virus-primed DBA/2 mice injected with CY (50 mg/kg) (DBA-CY) 72 h before removal of lung cells for culture.

^b Assay after 7 days of incubation; 5-h ⁵¹Cr release.

^c Regression coefficient, 0.695.

^d Regression coefficient, 0.227.

arising during chemotherapy, would allow immediate specified correction by currently available biological response modifiers.

CY has been shown to be a potent agent against several types of malignancies (12, 16, 22, 25, 27, 38). Several animal models demonstrate that the effectiveness of this compound in the treatment of cancer is greatly enhanced when the host's immune system is not compromised (14, 15, 23, 32).

Recent work in our laboratories and others has shown that both CY and 5-fluorouracil, at therapeutic doses, spare most allogeneic CTLp but inhibit allogeneic CTL responses by removing (limiting) IL2-producing Lyt 1⁺2⁻ T cells or alternatively an undefined accessory cell necessary for CTLp differentiation to effector cells (24, 28–30, 39, 41). It has been shown that both in vitro and in vivo CTL responses can be restored with IL2-containing supernatants (24, 31). It should be noted that syngeneic CTL precursors may be more sensitive to CY than allogeneic CTL precursors (24, 26). The syngeneic CY-resistant precursors can still be expanded, however, with IL2 (24). The fact that CY spares some cytotoxic precursors and IL2 allows for their differentiation and/or proliferation can lessen this drug's negative impact on the cellular immune system and allow for a better resolution of chemotherapy-induced complications to infectious disease. Many immunological responses to viral infections are dependent on T cells (11). This is quite evident in the case of

TABLE 6. Restoration of lung anti-influenza A virus CTL activity after CY treatment by human IL2

Responders ^a	IL2 ^b	% Specific ⁵¹ Cr release ^c (mean $\pm$ SEM)	
		Influenza A P815	P815
Expt 1			
DBA	–	44 $\pm$ 3.9	2 $\pm$ 0.9
DBA	+	71 $\pm$ 1.7	8 $\pm$ 1.6
DBA-CY	–	21 $\pm$ 2.9	<1
DBA-CY	+	39 $\pm$ 3.0	3 $\pm$ 1.9
Expt 2			
DBA	–	72 $\pm$ 4.0	4 $\pm$ 1.0
DBA	+	85 $\pm$ 3.0	8 $\pm$ 2.9
DBA-CY	–	10 $\pm$ 1.9	<1
DBA-CY	+	26 $\pm$ 2.9	<1

^a Influenza A virus-primed DBA/2 mice (DBA) or influenza A virus-primed DBA/2 mice injected with CY (50 mg/kg) (DBA-CY) 72 h before removal of lung cells for culture. All cultures were stimulated with influenza A virus-infected syngeneic spleen cells.

^b Recombinant DNA human IL2 (6 U/ml).

^c Five-hour ⁵¹Cr release assay. Expt 1, day 7 of incubation; expt 2, day 5 of incubation.

influenza A virus, which is highly dependent on CTL and helper T cells for elimination by the host's immune system.

The experiments presented here show that a chemotherapeutic agent (CY) decreases the CTLp frequency to influenza A virus target cells in both the spleen and lung (Tables 3 and 4), corroborating earlier studies suggesting that syngeneic CTLp may be more susceptible to CY than allogeneic CTLp (24, 26). The CY-resistant CTLp to influenza A virus, however, can be expanded and CTL activity can be restored to normal levels by IL2 in both the spleen and lung lymphoid populations (Tables 5 to 7). The effector cells are T cells, as judged by their susceptibility to anti-Thy 1 and complement (Table 7). These results resemble those of Effros and Walford (18), who have shown that the decline in CTL production to influenza A virus in aged mice can be corrected by crude IL2-containing lymphokine supernatants.

The experiments outlined here show that because some precursor cells are spared by cytoreductive therapy, the immunosuppressive effect of these agents (CY) may be counteracted by naturally occurring purified lymphokines (IL2). If systems for delivering purified lymphokines in vivo (20, 31) can be further developed and refined, it is possible that the in vivo use of purified lymphokines, such as IL2, will aid in the restoration of immune reactivity and be useful as clinical adjunct therapies. In this model system, it is possible that influenza A virus, a highly T-helper-dependent

TABLE 5. Restoration of splenic anti-influenza A virus CTL activity after CY treatment by human IL2

Responders ^a	IL2 ^b	% Specific ⁵¹ Cr release ^c ($\bar{x} \pm$ SEM)					
		Influenza A P815			P815		
		25/1 ^d	12.5/1	6.25/1	25/1	12.5/1	6.25/1
DBA	–	61 $\pm$ 3.1	49 $\pm$ 1.4	39 $\pm$ 1.3	2 $\pm$ 0.8	2 $\pm$ 0.9	1 $\pm$ 0.9
DBA-CY	–	6 $\pm$ 0.3	4 $\pm$ 0.6	2 $\pm$ 0.4	1 $\pm$ 0.6	2 $\pm$ 0.7	1 $\pm$ 0.5
DBA-CY	+	35 $\pm$ 1.6	29 $\pm$ 0.4	22 $\pm$ 1.7	4 $\pm$ 1.3	3 $\pm$ 0.3	1 $\pm$ 0.6

^a Influenza A virus-primed DBA/2 mice (DBA) or influenza A virus-primed DBA/2 mice injected with CY (100 mg/kg) (DBA-CY) 72 h before removal of spleen cells for culture. All cultures were stimulated with influenza A virus-infected syngeneic spleen cells.

^b Recombinant DNA human IL2 (6 U/ml).

^c Five-hour ⁵¹Cr release assay on day 7 after culture initiation.

^d Effector to target cell ratio.

TABLE 7. Effect of anti-Thy 1.2 and complement on splenic and lung cytotoxic effector cells

Responders ^a	IL2 ^b	Treatment of effector cells ^c	% Specific ⁵¹ Cr release ^c (mean ± SEM)	
			Influenza A P815	P815
Expt 1 (spleen)				
DBA	—	None	68 ± 2.4	1 ± 0.1
DBA	—	Complement	46 ± 1.7	1 ± 0.4
DBA	—	Θ + complement	4 ± 0.7	<1
DBA	+	None	52 ± 2.8	<1
DBA-CY	—	None	14 ± 1.5	<1
DBA-CY	+	None	30 ± 1.7	<1
DBA-CY	+	Complement	23 ± 1.5	1 ± 0.8
DBA-CY	+	Θ + complement	5 ± 1.8	<1
Expt 2 (lung)				
DBA	—	None	37 ± 1.1	<1
DBA	—	Complement	44 ± 4.7	<1
DBA	—	Θ + complement	<1	<1
DBA	+	None	55 ± 5.3	1.8 ± 1.0
DBA-CY	—	None	5 ± 2.5	<1
DBA-CY	+	None	29 ± 2.1	<1
DBA-CY	+	Complement	28 ± 5.8	<1
DBA-CY	+	Θ + complement	1 ± 1.2	<1

^a Responder cells from influenza A virus-primed DBA/2 mice (DBA) or influenza A virus-primed DBA/2 mice injected with CY (DBA-CY). Expt 1, spleen responders, CY (100 mg/kg), 72 h; expt 2, lung responders, CY (50 mg/kg), 72 h.

^b Recombinant DNA human IL2 (6 U/ml).

^c For expt 1 (spleen) cells were treated with anti-Thy 1.2 (Θ) and complement as described in the text and adjusted to an effector to target cell ratio of 25/1. For expt 2 (lung) cells were treated directly in the culture plates.

^d Five-hour ⁵¹Cr release assay.

virus, may be eliminated by IL2-driven CTL. The use of an influenza A virus model may be a basis for extending these types of adjunct therapies to other T-cell-dependent viral pathogens.

Because lymphokine therapy for neoplasia is becoming potentially important, it may be possible to use these naturally occurring biological response modifiers in clinical trials. Preliminary studies in humans suggest a role for IL2 in the restoration of the chemotherapy-suppressed mitogen response *in vitro* as well as *in vivo* enhancement of immunological parameters in acquired and congenital immunodeficiency syndromes (R. Mertelsmann, K. Welte, C. Sternberg, R. J. O'Reilly, M. A. S. Moore, B. D. Clarkson, and H. F. Oettgen, submitted for publication).

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