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Activity of CD4^{+} T-Cell Clones of Type 1 and Type 2 in Generation of Influenza Virus-Specific Cytotoxic Responses In Vitro

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The activity of distinct CD4^{+} T-helper cell (Th) clones in promoting secondary A/PR/8/34/Mt.S.(H1N1) (A/PR8) influenza virus-specific, class I-restricted cytotoxic T-lymphocyte (CTL) responses in vitro was examined. CD8^{+} T cells which had been purified by fluorescence-activated cell sorter from spleen cells of A/PR8-primed mice were used as responders. On their own, purified CD8^{+} T cells were unable to generate cytotoxic activity upon in vitro culture with A/PR8-infected stimulator cells. Significant cytotoxic activity was generated in cultures that were additionally supplemented with A/PR8-specific Th clones or cell-free supernatant from these clones. Although there were large differences among individual Th clones in this function, Th clones of type 1 (Th1) promoted, on average, significantly stronger cytotoxic responses than Th clones of type 2 (Th2). The differences in promotion of a cytotoxic response correlated with the amount of interleukin-2 (IL-2) or IL-4 secreted by individual Th clones. These two lymphokines accounted for the CTL-promoting activity of the respective Th clones, since addition of recombinant IL-2 (IL-2) or rIL-4 to Th-free cultures substituted fully for the respective Th clones. As observed with Th clones, rIL-2 was significantly more effective than rIL-4 in promoting a cytotoxic response. When used in combination, Th2 clones had an antagonistic effect on the generation of a CTL response by Th1 clones. This effect could be partially transferred with cell-free supernatant from activated Th2 clones and could be reversed by addition of excess rIL-2. Both consumption of IL-2 by Th2 and secretion of an inhibitory factor(s) appear to be involved in this phenomenon.

CD8^{+} lymphocytes are the progenitors of major histocompatibility complex class I-restricted cytotoxic T lymphocytes (CTL), which have been shown to play an important role in recovery from virus infection both by secreting cytokines such as gamma interferon and by lysing virus-infected cells (1, 10). The generation of CTL responses has two basic requirements: (i) the recognition of the specific class I antigen complexes on stimulator cells, which induces responsiveness to cytokines in the precursor CTL, and (ii) the interaction with various cytokines that promote proliferation and differentiation into effector T cells. The dependence of CD8^{+} CTL on cytokines secreted by class II-restricted CD4^{+} T-helper (Th) cells during this second phase of the response has been studied extensively. Current evidence indicates that CD8^{+} lymphocytes, or a subpopulation thereof, are capable of secreting cytokines required for this second phase of the response, allowing them to function in a Th-independent manner (15, 24, 45). This activity has been observed particularly when the CTL precursors are present at high frequency, as in anti-class I alloresponses (24, 35, 36), or when they are triggered by special stimulator cells (5, 37, 48). The Th dependence of antiviral CTL responses is less clear. With some exceptions (13, 18), most studies have found that virus-primed CD8^{+} lymphocytes usually give strongly reduced (23) or undetectable (4, 6, 31) CTL responses upon restimulation in vitro in the absence of CD4^{+} Th or Th-derived factors. On the other hand, several recent observations that primary antiviral CTL responses can be obtained in CD4-depleted mice (2, 7, 20, 23, 27) have been taken as evidence of the Th independence of this response in vivo. These findings cannot be interpreted unambiguously, however, because CD4-depleted mice contain residual Th cells. Furthermore, the responder CD8^{+} lymphocytes in these CD4-depleted mice may differ significantly in terms of composition from the CD8^{+} lymphocytes present in normal naive mice and thus may not reveal the degree of Th involvement in the CTL response of normal mice.

We have previously generated a large panel of influenza virus-specific Th clones and have shown that these clones display helper functions for antiviral B-cell responses in vivo following adoptive transfer into athymic mice (33, 34). Before conducting analogous studies addressing the function of Th in the induction or modulation of influenza virus-specific CTL responses in vivo, we wished to first compare various Th clones for activity in promoting secondary virus-specific CTL responses in vitro. We find that (i) culture conditions which are strictly dependent on the presence of activated Th or Th-derived factors can be established; (ii) Th clones of type 1 (Th1) are, on average, significantly more effective than Th clones of type 2 (Th2) in promoting a CTL response; (iii) Th1 and Th2 mediate their functions mainly through interleukin-2 (IL-2) and IL-4, respectively; and (iv) when present simultaneously with Th1, Th2 have an antagonistic effect on the CTL-promoting activity of Th1.

MATERIALS AND METHODS

Viruses. Influenza viruses A/PR/8/34/Mt.S.(H1N1) (A/PR8) and B/Lee/40 were grown in the allantoic cavities of 10-day-old embryonated chicken eggs. After 2 days of incubation at 37°C, the allantoic fluid was harvested, aliquoted, and stored at −70°C until used.

Antibodies and lymphokines. Sterile filtered supernatants

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from hybridomas 3.16.88 (32), S4B6 (8), and 11B11 (28) were used as sources of monoclonal antibody to Lyt-2 (CD8), IL-2, and IL-4, respectively. Fluorescein isothiocyanate-conjugated affinity-purified $F(ab')_2$ fragment of mouse anti-rat immunoglobulin G (Pel-Freez, Rogers, Ark.) was used as second antibody for cell-sorting experiments. Sterile filtered supernatants from X63-Ag8-653 plasmacytoma cells transfected with a BMGNeo vector containing cDNA of either IL-2 or IL-4 (19) were used as sources of recombinant ILs (rILs).

$CD4^+$ Th clones. The Th clones were generated, characterized, and maintained in tissue culture as described previously (14, 33).

Purification of $CD8^+$ cells. Spleens were obtained from BALB/c mice that had been primed 3 to 8 weeks previously by intraperitoneal injection of 0.2 ml of A/PR8-containing allantoic fluid ($\approx 2 \times 10^8$ 50% egg infective doses). A spleen cell suspension was prepared, and erythrocytes were lysed by 2 min of incubation in NH_4Cl (0.83 g/100 ml, pH 7) at room temperature. The cells were centrifuged through a cushion of fetal calf serum (FCS), washed with phosphate-buffered saline (PBS), resuspended at $\approx 30 \times 10^6$ cells per ml in neat culture medium from the rat hybridoma 3.16.88 (anti-CD8), and incubated for 45 min on ice. The cells were washed once in PBS and incubated at $\approx 20 \times 10^6$ cells per ml for 45 min on ice in PBS containing 10 μg of fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of mouse anti-rat immunoglobulins per ml. The cells were washed twice in PBS, resuspended at $\approx 20 \times 10^6$ cells per ml in Iscove's modified Dulbecco's medium (ISC)–2% FCS (GIBCO, Grand Island, N.Y.) supplemented with transferrin (Sigma Chemical Co., St. Louis, Mo.) at 0.005 mg/ml, 2-mercaptoethanol at 5×10^{-5} M, gentamicin at 0.05 mg/ml, and 2% FCS (HyClone, Logan, Utah). The cells were sorted in a Becton Dickinson FACS IV cell sorter (Becton Dickinson, Braintree, Mass.). The sorted cells were washed in ISC–2% FCS and counted.

Preparation of stimulator cells. Spleen cells from naive BALB/c mice were irradiated (2,200 rads), washed, and resuspended at $\approx 10^7$ cells per ml in ISC as above but without serum. The cells were then infected by addition of A/PR8 containing allantoic fluid (0.1 ml/10^7 cells corresponding to ≈ 10 50% egg infective doses per cell) and incubated for 1 h at 37°C. The cells were washed twice prior to use. P815 cells were maintained in vitro in ISC–5% FCS. For preparation as stimulators, they were washed in ISC and infected with PR8 as described for the splenic stimulators. The cells were then washed, resuspended at 2×10^7 cells per ml in ISC containing 0.5 mg of mitomycin (Sigma) per ml, incubated for 20 min at 37°C, and washed four times prior to use as stimulators.

Generation of secondary CTL response in vitro. Replicate cultures of 1 ml each were set up in 24-well plates. Each culture contained 2×10^5 fluorescence-activated cell sorter (FACS)-purified $CD8^+$ cells, 2×10^6 irradiated (2,200 rads) A/PR8-infected splenic stimulator cells, and 4×10^5 irradiated (2,200 rads) cells from individual Th clones. Cultures in which the effects of ILs and Th-derived cytokines were tested contained the same number of $CD8^+$ responder cells and 5×10^5 A/PR8-infected mitomycin-treated P815 cells as stimulators. After 6 days of incubation, cells within each culture were resuspended, and 100-μl aliquots of neat culture were transferred to 96-well round-bottom plates and tested for cytotoxic activity in a standard Cr release assay as described previously (47), by using 10^4 infected or uninfected P815 cells per well as targets.

Determination of IL-2 and IL-4 concentrations by CTLL bioassay. Replicate samples of supernatant from activated Th cultures were mixed 1/1 with ISC–1% FCS or neat culture supernatant from hybridoma 11B11 (anti-IL-4). The mixtures were kept for 30 min at room temperature and then serially diluted in ISC–1% FCS in 96-well flat-bottom microtiter plates to give 50 μl of sample per well. Serial dilutions of rIL-2 and rIL-4 were included as standards in each experiment. Fifty microliters of ISC–1% FCS containing 2×10^5 CTLL cells per ml was added to each well. After 1 day of incubation at 37°C, the number of viable CTLL cells was assessed by the MTT assay (25). The sample dilution giving 50% of the maximum number of viable CTLL (defined as 1 U of IL-2 [or IL-4]) was determined, and the IL content per milliliter of neat supernatant was calculated. No inhibition of CTLL proliferation was observed when supernatants from activated Th1 clones were tested after incubation with anti-IL-4 monoclonal antibody, while the inhibition was complete in the case of supernatants from Th2 clones. The CTLL cells were maintained by continuous passage in ISC–5% FCS supplemented with rIL-2 (≈ 30 U/ml) and rIL-4 (≈ 20 U/ml).

RESULTS

Characterization of influenza virus-specific Th clones as Th1 and Th2. Previous studies have demonstrated that mouse Th clones can be subdivided into three subclasses on the basis of their lymphokine production (38) as follows: (i) Th1, which are characterized by production of, among other cytokines, IL-2, gamma interferon, and lymphotoxin; (ii) Th2, which produce IL-4, IL-5, and IL-10; and (iii) Th0, which secrete both Th1- and Th2-related cytokines. Several other cytokines, such as IL-3, IL-6, and granulocyte-macrophage colony-stimulating factor, appear to be secreted by most or all Th. As shown in Table 1, all but two clones could be classified unambiguously as Th1 or Th2 on the basis of their production of IL-2 or IL-4, respectively. Clones V1.2 and V2.1 secreted very small amounts of IL-2 but could be assigned to the Th1 group because of gamma interferon secretion (data not shown). IL-2 and IL-4 secretion by these Th clones was confirmed in several independent experiments.

Both Th1 and Th2 can provide help for secondary virus-specific CTL responses in vitro. To examine the role played by Th cells in the generation of virus-specific CTL responses, we wanted to establish a culture system that showed maximum dependency on the presence of Th. Depletion of Th cells from virus-primed spleen cells by treatment with anti-CD4 monoclonal antibodies and complement, which eliminated approximately 90% of the $CD4^+$ T cells as judged by FACS analysis, failed, in our study, to reduce significantly the ability of the residual spleen cells to generate a virus-specific CTL response after 6 days of culture (data not shown). However, when FACS-purified $CD8^+$ splenocytes were cultured with irradiated virus-infected spleen cells from naive BALB/c mice, CTL responses were significantly reduced from approximately 60% specific lysis to <10% (Fig. 1). This background response was completely eliminated when FACS-purified $CD8^+$ splenocytes were stimulated with A/PR8-infected P815 cells, a class II-negative mastocytoma cell line of DBA (*H-2^d*) origin. Addition of $CD8^-$ cells from the spleen of A/PR8-primed BALB/c mice to cultures of $CD8^+$ cells and irradiated spleen cells completely restored the CTL response. Thus, for the remainder of the study, FACS-purified $CD8^+$ splenocytes from virus-primed mice were used as responders and irradiated spleen

TABLE 1. Summary of the CD4+ Th clones used in this study

Clone	Specificity[a]	Concentration (U/ml) of[b]:		Type[c]
		IL-2	IL-4	
V1.2	HA	1	0	1
V2.1	HA	0.7	0	1
8.1-6	HA	13	0	1
7.1-2	HA	66	0	1
5.1-1	HA	81	0	1
5.1-5	HA	24	0	1
8.1-10	HA	84	0	1
7.1-5	HA	40	0	1
MT2B11.1	HA	19	0	1
MT2B7.1	HA	0	54	2
TL2/1-1	M	19	0	1
7.1-3	M	74	0	1
8.1-14	M	25	0	1
T2.5-41	M	75	0	1
6.2-13	M	71	0	1
T2.5-26	M	303	0	1
LD_313-10	M	0	48	2
LD_38-8	NA	0	41	2
8.1-9	NA	81	0	1
TL1/4-1	NA	59	0	1
5.1-7	NA	119	0	1
7.1-1	NP	22	0	1
TL2/4-3	NP	116	0	1
7.1-6	NP	122	0	1
TL1/3-1	NP	103	0	1
TL1/1-1	NP	66	0	1

[a] Specificity for one of the four major structural proteins of influenza virus (hemagglutinin [HA], neuraminidase [NA], nucleoprotein [NP], or matrix [M]).

[b] Concentration of IL-2 and IL-4 in culture supernatant 24 h after stimulation with antigen, measured in the experiment whose results are shown in Fig. 2.

[c] Type of the clone according to the classification of Mosmann et al. (47).

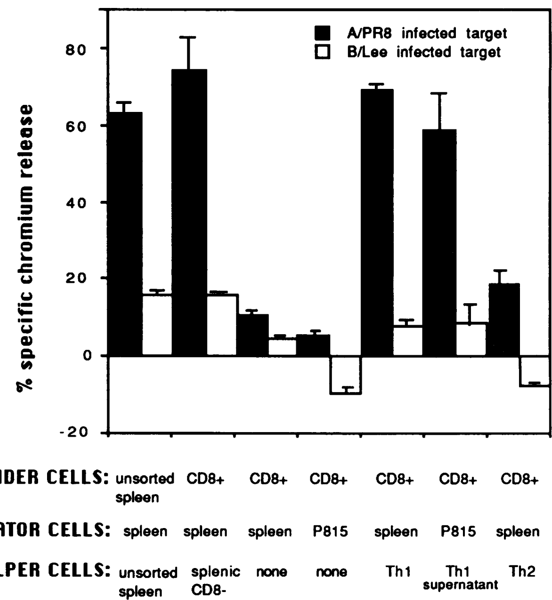

FIG. 1. Requirement of CD4+ T cells or derived factors for the generation of virus-specific CTL activity from CD8+ T cells in vitro. CD8+ splenocytes from A/PR8-infected mice were positively sorted by FACS and incubated in vitro with virus-infected stimulator cells in the presence or absence of CD4+ Th1 or Th2 clones specific for A/PR8 influenza virus or cell-free supernatant from activated cultures of a Th1 clone. Results for unsorted spleen cells are shown for comparison. Virus-specific cytotoxic activity was tested after 6 days of culture by ^{51}Cr release assay by using A/PR8-infected (solid bars) or B/Lee/40-infected (open bars) P815 cells as targets. The average lysis generated by Th1 clones is significantly different from the lysis generated by Th2 clones ($P < 0.05$ in the Student t test).

cells from naive mice were used as stimulators in the CTL cultures. Addition of irradiated Th1 or Th2 cells to the CTL cultures generated clearly detectable A/PR8-specific CTL responses (Fig. 1).

The activity of Th1 and Th2 in promoting CTL responses correlates with secretion of IL-2 and IL-4, respectively. Initial experiments, in which distinct Th clones were tested for CTL-promoting activity, revealed large differences among clones. Since IL-2 and IL-4 had been shown to play crucial roles in CTL responses in various experimental systems, we first wished to investigate whether the differences among Th clones might be related to differences in amounts of secretion of the above lymphokines. To avoid variability due to different batches of responders and stimulators, all Th clones were tested at the same time in parallel cultures that contained responders from the same pool of FACS-purified CD8+ T cells and stimulators from the same pool of virus-infected splenocytes. Some of the cultures received various amounts of rIL-2 or rIL-4 instead of the Th cells. After 1 day of incubation, samples of the culture supernatants were withdrawn for subsequent determination of IL-2 and IL-4 concentrations. CTL activity was tested after 5 additional days of incubation by resuspending each culture and testing undiluted culture aliquots for cytotoxicity, by using the same pool of A/PR8-infected and uninfected target cells. Data for the means of two independent experiments are shown in Fig. 2. It is evident that a significant correlation existed between the amounts of IL secreted by individual Th clones by day 1 and the specific cytotoxicity generated by day 6. More important, because a similar relationship was displayed in Th- and IL-supplemented cultures, the different CTL-promoting activities of the various Th clones appear to be explained adequately by differences in amounts of IL-2 or IL-4 secreted. A possible exception may be clone TL2/1-1 (marked with an asterisk in Fig. 2), which produced in five different experiments an average of 19 U of IL-2 per ml and promoted CTL cultures displaying an average of 8% specific lysis. It is possible that, in the case of this clone, other cytokines known to modulate CTL responses play a more significant role in the generation of cytotoxicity.

That IL-2 and IL-4 were capable of promoting, on their own, an antiviral CTL response was additionally verified by adding rILs to cultures that contained FACS-purified CD8+ responder cells and A/PR8-infected P815 cells as stimulators. P815 cells were used as stimulators to avoid secondary effects resulting from activation of T cells or other cell types present in the splenic stimulator population by the exogenously added ILs. As shown in Fig. 3, no virus-specific CTL response was generated in the absence of exogenous lymphokines. The addition of rIL-2 to these cultures, however, promoted a strong response, while rIL-4 gave a clearly detectable but three- to fourfold-weaker CTL response. Interestingly, the combination of IL-2 and IL-4, either added simultaneously at the onset of culture (Fig. 3) or added in sequence (IL-2 on day 0 and IL-4 on day 3) (data not shown), did not improve the CTL response. Mastocytoma cells have been reported to secrete IL-4 upon stimulation with IL-2 (3); we found no evidence, however, that P815 cells secreted

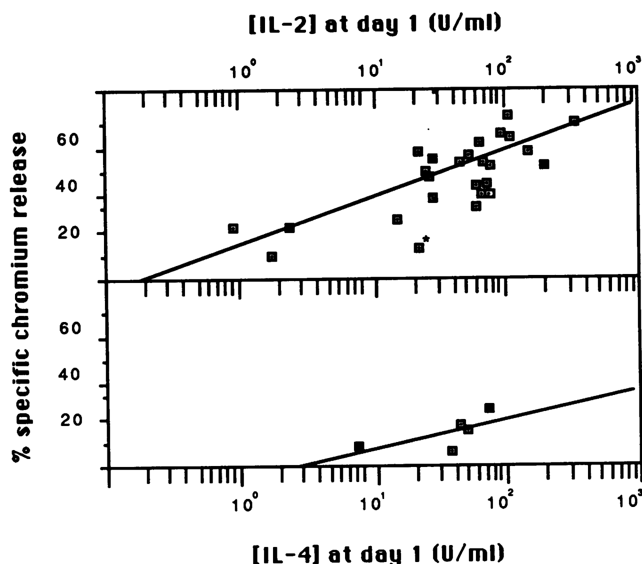

FIG. 2. Relation between IL-2 and IL-4 release by Th clones and promotion of CD8$^+$ CTL activity. Purified CD8$^+$ splenocytes were cultured in the presence of irradiated A/PR8-infected splenocytes and irradiated cells from distinct virus-specific Th1 (top) or Th2 (bottom) clones. After 1 day of culture, a sample of supernatant from each culture was withdrawn and tested by bioassay for concentration of IL-2 (top) or IL-4 (bottom). After 5 further days of incubation, the cultures were tested for A/PR8-specific cytotoxic activity. Symbols: ⊡, data from cultures containing a distinct Th clone; ■, data from cultures to which exogenous rIL-2 (top) or rIL-4 (bottom) was added instead of Th cells; *, clone TL2/1-1 (see text). In the cases of both Th1 and Th2 clones, a significant correlation ($P < 0.05$ of the Pearson product-moment correlation coefficient) was observed between IL-2 and IL-4 secretion at day 1 and cytotoxic activity of the culture at day 6.

IL-4 upon incubation with IL-2, at least not in sufficient quantity to be detected in the CTLL bioassay (data not shown).

Taken together, the data show that both Th1 and Th2 can promote, on their own, a secondary antiviral CTL response in vitro and that this activity appears to be mediated by IL-2 and IL-4, respectively. On average, Th1 promote more vigorous CTL responses than Th2 (Fig. 2). In the case of the most effective Th1 clones, as few as 4×10^3 Th per 1-ml culture promoted near-maximal response (data not shown).

Th2 inhibit the promotion of a CTL response by Th1. Since both Th1 and Th2 could promote a CTL response in vitro, though with different efficiencies and mediated through different lymphokines, it was of interest to examine whether the two types of Th would act additively or even synergistically. This possibility was tested by adding Th2 cells to cultures containing graded numbers of Th1 cells and a constant number of virus-infected splenic stimulator cells. The pairs of Th clones were of the same antigen specificity to ensure equal activation in culture. CTL activity was measured after 6 days of incubation. Figure 4 shows that addition of Th2 to cultures strongly interfered with the generation of CTL activity and, in the case of cultures containing suboptimal numbers of Th1, abrogated generation of CTL. This inhibitory effect was also detectable, though at a reduced level, when cell-free supernatant from activated Th2, instead of the Th2 cells, was added to the cultures. The inhibitory effect could be overcome by the addition of rIL-2.

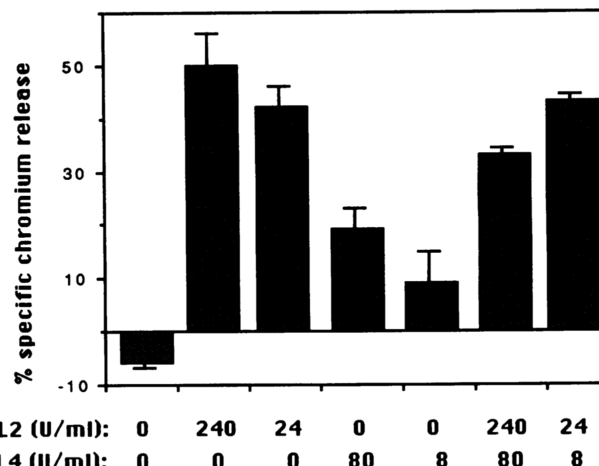

FIG. 3. Generation of virus-specific CTL activity in vitro in the presence of IL. CD8$^+$ splenocytes from A/PR8-immunized mice were purified by FACS and incubated in vitro with mitomycin-treated A/PR8-infected P815 mastocytoma cells and the indicated IL. Virus-specific cytotoxic activity was tested after 6 days of culture by ^{51}Cr release assay by using A/PR8-infected and B/Lee/40-infected (data not shown) P815 cells as targets.

DISCUSSION

In our experimental system, in which class I-restricted CTL responses against influenza virus were generated in vitro from in vivo-primed murine splenocytes, CD8$^+$ cells proved not to be autonomous in their response. Thus, not even trace amounts of specific cytotoxic activity were observed when FACS-purified CD8$^+$ splenocytes from virus-primed mice were cultured for 6 days with virus-infected P815 cells, while vigorous cytotoxic responses were obtained from the same cultures after the addition of cell-free supernatant from activated Th clones. However, when virus-infected spleen cells from naive mice were used as stimulators, small cytotoxic background responses were usually observed. These background responses could be due to an infrequent stimulator cell, such as a virus-infected dendritic cell (5, 37, 48) that may be able to provide a Th-independent stimulus to virus-specific CD8$^+$ lymphocytes. Alternatively, activation of a few Th cells in the naive spleen cell population, which are specific for virus or medium components (FCS), may suffice for generating a marginal CTL response. That only few activated Th cells may be required for such a background CTL response can be deduced from the finding that the most effective Th1 clones promoted near-maximal CTL responses when present at 4×10^3 cells per culture.

When tested for their ability to promote antiviral CTL responses in vitro, individual Th clones differed greatly from each other with regard to this activity. It appears that these differences were mainly due to differences in amounts of IL-2 or IL-4 secreted by individual Th clones. This conclusion was supported by the findings that (i) the concentration of IL-2 or IL-4 in the supernatant at day one of culture correlated fairly well with the CTL activity observed at day 6; and (ii) the CTL-promoting activity of individual Th clones could be substituted by the addition of a corresponding amount of rIL-2 (or rIL-4 in the case of Th2) to these cultures. Thus, as has been reported for other experimental systems (6, 11, 17, 29, 46), these cytokines appear to be required and limiting factors for generating a virus-specific

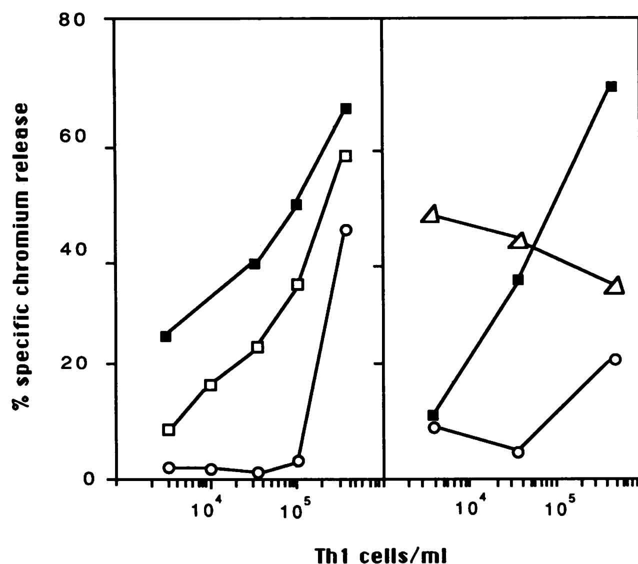

FIG. 4. Th2 clones inhibit the promotion of CTL activity by Th1. Purified CD8$^+$ splenocytes were cultured with A/PR8-infected splenic stimulator cells and graded numbers of a Th1 clone (■). Replicate cultures were further supplemented with 4 × 10^5 irradiated cells from a Th2 clone of the same antigen specificity (○) or the cell-free supernatant of the same Th2 clone (□) at a final dilution of 2:5 (left half). Cell and factors were added to the culture at the beginning of antigen stimulation, and the virus-specific cytotoxic activity was tested after 6 days of culture. The right half shows an analogous experiment in which additional rIL-2 (240 U/ml) was added to the culture containing cells from both Th1 and Th2 clones (△).

CTL response from primed CD8$^+$ cells when either virus-infected spleen or P815 cells are used as stimulators. We cannot exclude, however, the possibility that additional cytokines (30, 31, 40, 41, 44) are involved in the present CTL response but are never limiting, because they are produced in sufficient quantities either by activated CD8$^+$ lymphocytes (e.g., gamma interferon) or by virus-infected stimulator cells.

Although both Th1 and Th2 clones were capable of promoting a CTL response, on average, Th1 clones were roughly four times more effective than Th2. This appears to be a reflection of the higher CTL-promoting activity of IL-2 compared with that of IL-4 in this system, since a similar difference was observed when Th cells were substituted by rIL-2 and rIL-4. This difference in activity of IL-2 and IL-4 is interesting because it is in contrast to results of several other studies that found rIL-4 to be as effective as rIL-2 (or more so) in promoting CTL responses (9, 21, 22, 29, 42, 46). On the other hand, the CTL response to Ia-negative lymphoma tumor cells in vitro was found to require the addition of IL-2, while IL-4 could synergize with IL-2 but was ineffective on its own (43). Taken together, the various studies are compatible with the notion that IL-2 and IL-4 can promote CTL responses on their own but that IL-2 may emphasize growth and IL-4 may emphasize differentiation. Accordingly, the relative efficacies of IL-2 and IL-4 may differ among experimental systems, depending on the frequency of the responder cell and the extent to which proliferation is required for detection of cytotoxic effector cells. Additional factors that may play a role are the type and concentration of residual cytokines that are synthesized within the culture system, the type and strength of the activation signal delivered to the responder CTL, and the differences between naive and primed CTL precursors.

Several recent studies have indicated that IL-2 and IL-4 act synergistically in the promotion of CTL responses (17, 42). It was surprising, therefore, to find that the simultaneous presence of Th1 and Th2 in a culture strongly decreased the CTL response compared with the presence of the Th1 alone. Two distinct mechanisms appear to contribute to this phenomenon. First, because cell-free supernatant from activated Th2 clones induced partial inhibition, it is clear that a cytokine(s) secreted by activated Th2 is involved, at least in part. A good candidate is the recently described IL-10, which has been shown to suppress cytokine secretion by Th1 (12, 26). Alternatively, a Th2-derived cytokine could directly act on the responder CD8$^+$ CTL, e.g., by inhibiting their responsiveness to IL-2. Second, since full inhibition was observed only in the presence of Th2 cells and could be overcome by exogenous IL-2, it is likely that Th2 additionally compete by consumption of IL-2. Suppression of mixed lymphocyte responses by CD8$^+$ bystander T cells has been attributed to this mechanism (16, 39, 43). The importance of Th in the induction and regulation of the antiviral CTL responses in vivo remains to be defined.

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