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CD8⁺ T CELLS CAN BE PRIMED IN VITRO TO PRODUCE IL-4

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IL-4 production by T lymphocytes from naive mice in response to stimulation by plate-bound anti-CD3 is concentrated among CD4⁺ T cells. In vitro stimulation of lymph node T cells with anti-CD3 plus IL-2 and IL-4 strikingly increases the frequency of cells that produce IL-4 in response to subsequent stimulation with anti-CD3 plus IL-2. Separation of these primed cell populations into CD4⁺ and CD8⁺ T cell by cell sorting reveals that the frequency of IL-4-producing cells in both population is similar. Verification that CD8⁺ T cells produce IL-4 is provided by the capacity of anti-IL-4 mAb to inhibit the response of the indicator cell line to the growth factor produced by the primed cells and by detection of IL-4 by an IL-4-specific ELISA. The in vitro "priming" of CD8⁺ T cells to produce IL-4 is not dependent on the presence of CD4⁺ T cells because highly purified CD8⁺ T cells can be stimulated to develop into cells capable of producing IL-4 by culture with plate-bound anti-CD3 plus IL-2 and IL-4.

IL-4 is a member of a set of lymphokines produced by T cells and by mast cells as a result of receptor-mediated stimulation (1-3). Among long term cloned mouse T cell lines, only a subset of CD4⁺ T cells have been demonstrated to produce IL-4 (4). These cells, termed Th2 cells, also produce IL-3, IL-5, IL-6, IL-10, and GM-CSF² but fail to produce IL-2, IFN- γ , or LT. By contrast, another set of such clones (Th1 cells), produce IL-2, IFN- γ , LT, IL-3, and GM-CSF but not the other lymphokines produced by Th2 cells.

It is recognized, however, that the Th1/Th2 clonal dichotomy may represent a late stage polarization of lymphokine production (5). The production of both IFN- γ and IL-4 by short term mouse T cell clones (6) has been observed and is frequent among human T cell clones (7, 8). Information regarding production of lymphokines by individual normal T cells is still lacking.

Lymphokine production is not limited to CD4⁺ cells. In the mouse, alloreactive CD8⁺ T cell clones have been reported to have a pattern of lymphokine production

similar to Th1 cells (9). That is, such cells produce IL-2, IFN- γ , and LT and fail to produce IL-4 or IL-5, although a small minority of CD8⁺ T cell clones with mRNA for IL-4 have been reported (6). Recently, intraepithelial CD8⁺ T cells, isolated from the small intestine of mice, have been shown to produce IL-5 in response to Con A (10). In addition, CD8⁺ human T cell clones that produce small amounts of IL-4 have been described (7, 8), and recently it was shown that among human peripheral blood T cells stimulated with PMA and ionomycin, a minority of cells with cytoplasmic IL-4 were CD8⁺ (11). Among T cells prepared from naive mice, IL-4 production is observed principally in CD4⁺ cells (12). We show that stimulation of CD8⁺ T cells from naive mice with immobilized anti-CD3 in the presence of IL-2 and IL-4 prepares them to produce IL-4. On restimulation, these CD8⁺ cells are as active as comparably primed CD4⁺ T cells in the production of IL-4. These results raise the possibility that in the course of certain types of immune responses, CD8⁺ T cells may be major contributors to the production of IL-4.

METHODS AND MATERIALS

Animals. Virus-free female BALB/c mice, 8 to 12 wk of age, were obtained from Division of Cancer Therapy Animal Program, National Cancer Institute.

Culture medium. RPMI 1640 (Biofluids, Rockville, MD) supplemented with 10% FCS (HyClone Labs, Logan, UT), L-glutamine (2 mM), 2-ME (0.05 mM), sodium pyruvate (1 mM), penicillin (100 U/ml), and streptomycin (100 U/ml) was used for culturing cells.

Preparation of T cells. Cell suspensions were obtained from pooled axillary, inguinal, and mesenteric lymph nodes. Single cell suspensions were prepared, washed three times in HBSS, and suspended in staining buffer (culture medium containing 5 mM EDTA). They were incubated at 4°C for 1 h with a mixture of FI antibodies containing 10 μ g/ml of anti-B220 (6B2) and anti-Ia^d (MKD6), to stain B cells. For preparation of CD4⁺ T cells, anti-CD8 (2.43) was added to above cocktail; for preparation of CD8⁺ cells, anti-CD4 (GK1.5) was added to the cocktail. At the end of the incubation, the cells were washed twice in staining buffer and mixed for 1 h at 4°C with a suspension of magnetic beads coated with sheep anti-FI antibodies (Advanced Magnetics Inc., Cambridge, MA; 12 ml/10⁶ stained cells). Positively staining cells were depleted by two 20-min cycles of exposure to a magnetic field. The remaining cells were washed twice in culture medium and examined for the removal of the FI-stained B cells by cytometric analysis on a FACScan (Becton Dickinson, Mountain View, CA). In general, contamination with residual B cells was less than 2%. CD4⁺ and CD8⁺ cells were also obtained by cell sorting. Lymph node T cells were prepared as above using anti-B220 and anti-Ia followed by magnetic bead depletion. Cells were then stained with FI anti-CD8 (anti-CD8-FITC) (Becton Dickinson) and phycoerythrin labeled anti-CD4. The cells were then sorted into CD4⁺ and CD8⁺ using an EPICS 753 (Coulter Electronics, Hialeah, FL) with an Argon laser (Coherent Inc., Palo Alto, CA).

Lymphokines and cytokines. Human rIL-2 was a gift of Cetus Corporation (Emeryville, CA). IL-2 activity was defined in terms of "Cetus units." One Cetus unit is equivalent to 0.3 ng and to 6 WHO

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² Abbreviations used in this paper: GM-CSF, granulocyte-macrophage colony stimulating factor; LT, lymphotoxin; FI, fluoresceinated.

IU. Mouse rIL-4 was obtained from a baculovirus expression system, using a vector into which the IL-4 gene had been inserted by C. Watson, Laboratory of Immunology, NIAID. IL-4 activity was measured using the CT.4S cell line (13), comparing it with a standard that had been initially calibrated on the basis of 10 U/ml being equal to the amount of IL-4 required for half maximal stimulation of [³H]TdR uptake by resting B cells stimulated with 5 µg/ml of goat anti-IgM antibody (14). One U is equal to 0.5 pg of protein.

Ig and mAb. The following antibodies were prepared by a combination of ammonium sulfate precipitation, DE-52 ion exchange chromatography, and Sephadex G200 gel filtration (15); anti-CD3 (2C11) (16); anti-B220 (6B2) (17); anti-Ia^d (MKD6) (18); anti-CD4 (GK1.5) (19); and anti-CD8 (2.43) (20). Purified Ig was fluoresceinated with FITC, as previously described (21). Purified monoclonal rat anti-mouse IL-4 (11B11) (22) was prepared by Verax Corporation (Hanover, NH).

Measurement of lymphokine production from cells stimulated by plate-bound antibodies. Flat-bottomed 96-well microtiter Immulon 2 plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with purified anti-CD3 antibody (2C11; 10 µg/ml in 50 µl borate buffered saline, pH 8.5) for 4 h at room temperature. Wells were washed three times with 200 µl HBSS. Cells were added to each well (in triplicate) in 0.2 ml culture medium. After 36 h in culture, the plates were exposed to 3000 rad in an irradiator (Gamma Cell 40; Atomic Energy of Canada Ltd., Ontario, Canada). Lymphokine-dependent cell lines (CT.4S), an IL-4-dependent line, and CTLL, an IL-2-dependent line were added (5000 cells/well) to measure the secretion of lymphokines. Anti-IL-4 antibody (11B11; 5 µg/ml) was added with the CTLL cells to block any effect of IL-4 on the CTLL line. Then 48 h later, 1 µCi of [³H]TdR (ICN, Irvine, CA) was added, and after 6 h the cells were harvested. The amount of lymphokine was expressed as the equivalent of the amount of purified lymphokine required to support the same level of thymidine incorporation in the indicator cell line (equivalent units).

IL-4 and IFN-γ in 36-h culture supernatants were assayed by highly specific two-site ELISA (23, 24) with reference to standard curves constructed using known amounts of recombinant lymphokines. The IL-4 ELISA was performed using a kit supplied by Endogen (Boston, MA).

Generation of IL-4-producing T cells in vitro. Unfractionated T cells from lymph nodes of naive mice were incubated on anti-CD3-coated tissue culture dishes (2–3 × 10⁵ cells/dish) in 3 ml culture medium supplemented with IL-2 and IL-4. After 3 days in culture, 2 ml of fresh culture medium containing IL-2 and IL-4 was added. Two days later the cells were collected, washed three times with HBSS, and resuspended in culture medium in the presence of IL-2 and plated either on anti-CD3-coated Terasaki wells (for limiting dilution analysis) or on anti-CD3-coated flat bottom Immulon 2 plates and then tested for their IL-4-producing capacity.

Limiting dilution analysis. Terasaki plates (Robbins Scientific, Sunnyvale, CA) were coated by incubation for 4 h at room temperature with purified anti-CD3 antibody (2C11; 10 µg/ml in 50 µl borate buffered saline, pH 8.5). Wells were washed three times with 200 µl HBSS. Graded numbers of cells were plated in replicates of 36 to 72 in 10 µl culture medium in the presence of IL-2 (5 U/ml). After 36 h in culture, 1000 CT.4S cells were added to each well and 48 h later the wells were scored visually for viability of CT.4S cells. As a negative control, IL-2 in media alone was added to wells at day 0 and 36 h later CT.4S cells were added. Frequencies were calculated according to maximum likelihood method (25). *p* values were >0.1 for all frequencies calculated.

RESULTS

IL-4 production by CD8⁺ T cells from naive mice is generally undetectable. T cells from naive mice produce very little IL-4 in response to polyclonal stimulators (26, 27). We have recently developed a limiting dilution assay capable of detecting IL-4-production by a single cell thus allowing the enumeration of the frequency of IL-4-producing cells in a population (28). We showed that the frequency of cells capable of producing IL-4 in response to plate-bound anti-CD3 was ~1/1000 among T cells isolated from lymph nodes of naive donors. Previous work in the mouse had shown that IL-4 production was limited to the CD4⁺ subset of T cells (12). The improved sensitivity offered by the single cell assay allowed us to examine the frequency of IL-4-producing cells in both CD4⁺ and

CD8⁺ T cell populations. Initially, we tested lymph T cells prepared by negative selection using FITC-conjugated anti-Ia, anti-B220, and anti-CD4 to prepare CD4-depleted T cells (CD4⁻ T cells) and FITC-conjugate anti-Ia, anti-B220, and anti-CD8 to prepare CD8-depleted T cells (CD8⁻ T cells).

Table I shows results from four such experiments (experiments 1–4) using limiting dilution analysis. The frequency of anti-CD3-stimulated IL-4-producing cells among unfractionated T cells ranged from 1/555 to 1/2700, with a mean of 1/1500. For CD8-depleted T cells (CD8⁻), the frequencies were similar. In three experiments, IL-4-producing cells in the CD4-depleted T cell population (CD4⁻) were rare (<1/10,000). However, in one case, a relatively high frequency (1/1000) was observed. The lymph nodes of the donor mice in this experiment were larger than in the others, the donors were older and the frequency of IL-4-producing cells was also greater in the unseparated population. These results suggest that environmental stimulation may have increased the frequency of IL-4-producing cells and raise the possibility that in "primed" populations, CD8⁺ T cells capable of producing IL-4 may be found.

To verify these results, CD4⁺ and CD8⁺ T cells were purified by FACS. In one experiment (Table I, experiment 5) the frequency of IL-4-producing T cells was 1/4000 among CD4⁺ T cells and undetectable among CD8⁺ T cells. In a second experiment (Table I, experiment 6) using varying numbers of cells in "bulk" culture, rather than the limiting dilution assay, IL-4 production could be detected from 5000 CD4⁺ T cells stimulated with PB-anti-CD3 plus IL-2 whereas no IL-4 was detected from 20,000 CD8⁺ T cells stimulated similarly.

TABLE I
Frequency of IL-4-producing CD8⁺ T cells derived from naive mice^a

Purification Method	Expt.	T Cells	IL-4-Producing Cells
			(Frequency)
Magnetic bead depletion	1	Unsep.	1/2700
		CD8 ⁻	1/1000
		CD4 ⁻	1/16,700
Magnetic bead depletion	2	Unsep.	1/1610
		CD8 ⁻	1/1000
		CD4 ⁻	1/14,300
Magnetic bead depletion	3	Unsep.	1/833
		CD8 ⁻	ND
		CD4 ⁻	1/1000
Magnetic bead depletion	4	Unsep.	1/555
		CD8 ⁻	ND
		CD4 ⁻	1/14,285
Cell sorting	5	Unsep.	ND
		CD4 ⁺	1/4000
		CD8 ⁺	Undetectable
Cell sorting	6	Unsep.	ND
		CD4 ⁺	Pos at 5,000 cells/well
		CD8 ⁺	Neg at 20,000 cells/well

^a CD4⁻ and CD8⁻ T cells were purified from lymph nodes of naive mice by magnetic bead separation in experiments 1 to 4. Experiment 5 used CD4⁺ and CD8⁺ T cells purified by FACS. Unsep. represents lymph node cells depleted of B cells by magnetic bead separation. Frequency of cells capable of producing IL-4 in response to anti-CD3 plus IL-2 was measured as described in *Materials and Methods* using the maximum likelihood method of Taswell (25).

In vitro priming causes induction of IL-4-producing cells from CD4⁺ and CD8⁺ T cells. We and others have recently reported *in vitro* priming methods (29–31) leading to the appearance of T cells capable of producing IL-4. In our approach, normal T cells are stimulated for 5 days with anti-CD3 plus IL-2 and IL-4 and then restimulated with anti-CD3 plus IL-2 (29). This priming led to a 50-fold increase in the frequency of IL-4-producing cells (28). To study the capacity of CD4⁺ and CD8⁺ T cells to give rise to cells capable of producing IL-4, purified T cells were cultured for 5 days on plates coated with anti-CD3 in the presence of IL-2 and IL-4. The starting cell population was also sorted into CD4⁺ and CD8⁺ cells to assess the capacity of the unprimed cells of the two types to produce IL-4 in response to stimulation with anti-CD3 plus IL-2. As shown in Figure 1, the CD4⁺ T cells had a low frequency of cells capable of producing IL-4 and no IL-4-producing cells were detected among the unprimed CD8⁺ T cells. The unseparated T cell population that had been cultured for 5 days with anti-CD3 plus IL-2 and IL-4 was then separated by cell sorting into CD4⁺ and CD8⁺ T cell populations. The purity of these cell populations is illustrated in Figure 2. Figure 2, *left panel* shows the profile of the unseparated T cells after 5 days of culture; the *middle panel* shows the postsorting profile of the

CD4⁺ population whereas the *right panel* shows the profile for the CD8⁺ T cell populations.

The CD4⁺ and CD8⁺ T cells that had been purified by sorting after 5 days of culture were then assessed for their IL-4-producing capacity in response to anti-CD3 plus IL-2 using limiting dilution analysis. Figure 1 demonstrates that both cell populations contained significant numbers of IL-4-producing T cells, with a frequency of 1/97 among CD4⁺ cells and 1/17 among CD8⁺ T cells. This experiment has been repeated several times with generally similar results although the frequency of IL-4-producing T cells in the CD8⁺ T cell population does not usually exceed the frequency in the CD4⁺ T cell population.

CD8⁺ T cells produce IL-4 after primary in vitro stimulation in absence of CD4⁺ cells. We demonstrated above that CD8⁺ T cells purified after 5 days of *in vitro* stimulation with anti-CD3, IL-2, and IL-4 were excellent producers of IL-4. The possibility that CD4⁺ T cells were important for the acquisition of IL-4-producing capacity of CD8⁺ T cells was studied by removing CD4⁺ or CD8⁺ T cells by magnetic bead depletion. The cells were stimulated independently for 5 days with anti-CD3, IL-2, and IL-4. At the end of the culture the CD4⁺ and CD8⁺ cells were repurified by cell sorting, leading to more than 99% purity for both populations. The frequency of IL-4-producing T cells in the CD8⁺ population was compared to that of the CD4⁺ T cells.

The frequency of IL-4-producing T cells among CD8⁺ cells primed in the absence of CD4⁺ cells is 1/200 whereas the frequency of IL-4-producing CD4⁺ was 1/90, indicating that CD4⁺ cells are not required for CD8⁺ T cells to develop into IL-4-producing cells under these conditions (Fig. 3). In a subsequent experiment, CD4⁺ and CD8⁺ T cells were purified by sorting before culture. After stimulation for 5 days with anti-CD3, IL-2, and IL-4, the frequency of CD4⁺ cells that produce IL-4 was 1/42 whereas for CD8⁺ cells it was 1/29 (data not shown).

Verification that CD8⁺ T cells produce IL-4. To verify that the lymphokine produced by CD8⁺ T cells that we classified as IL-4 is actually that molecule, two confirmatory assays were used. In the experiment illustrated in

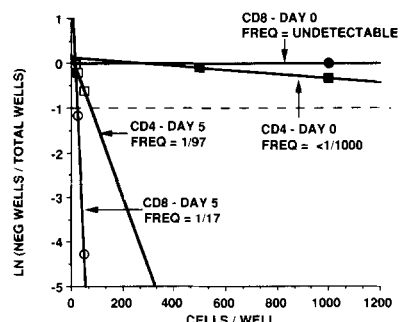


Figure 1. *In vitro* priming causes induction of IL-4-producing cells from CD4⁺ and CD8⁺ T cells. Purified T cells were separated into CD4⁺ and CD8⁺ cells by fluorescence activated cell sorting and their frequency of IL-4-producing cells measured by limiting dilution analysis, in the presence of IL-2, in Terasaki wells coated with anti-CD3. Another portion (5×10^5) of the initial purified T cell population was cultured, in the presence of IL-2 and IL-4, in a 60×15 mm dish that had been coated with anti-CD3. After 5 days, CD4⁺ and CD8⁺ were purified by FACS and frequency of IL-4-producing cells measured. The CD4⁺ starting populations were tested at 500 and 1000 cells/well in replicates of 48 and 72, respectively. The CD8⁺ starting populations were tested at 1000 and 2000 cells/well in replicates of 72 each. For the CD8⁺ cells, no positive wells were observed at either point. The 5-day primed CD4⁺ cells were tested at 12.5, 25, and 50 cells/well at replicates of 48 or more. The 5-day primed CD8⁺ cells were tested at 25, 50, and 100 cells/well at replicates of 48 or more. The result at 100 cells/well (not shown) for CD8⁺ cells were 48 positive wells of 48 plated.

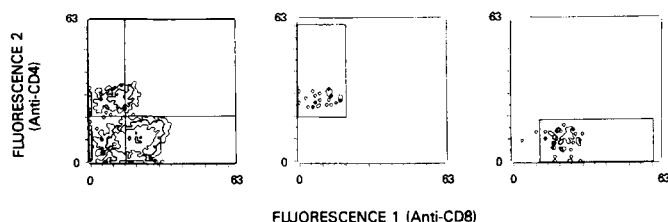


Figure 2. Fluorescence analysis and sorting of T cells primed *in vitro* with anti-CD3, IL-2, and IL-4. Cells from the experiment described in Figure 1 were obtained after 5 days of culture on anti-CD3-coated dishes, in the presence of IL-2 and IL-4. They were stained with anti-CD4-PE and anti-CD8-FITC and analyzed on an EPICS 753 (*left panel*). The cells were then sorted into CD4⁺ and CD8⁺ populations and reanalyzed (*middle and right panels*).

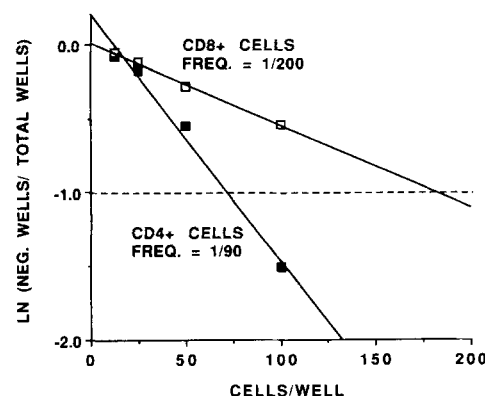


Figure 3. CD4⁺ and CD8⁺ T cells were prepared from lymph node cells of naive mice by magnetic bead depletion. The cells were stimulated with immobilized anti-CD3 plus IL-2 and IL-4 for 5 days and purified by FACS. The purity of the cells at 5 days was verified post sorting and found to be >99% for both CD4⁺ and CD8⁺ T cell populations. The cells were then washed and added, at limiting dilution, in replicates of 36 or more to Terasaki wells coated with anti-CD3, in the presence of IL-2. Then 36 h later, 1000 CT.4S cells were added. Wells were scored for the presence of viable CT.4S cells 48 h later.

Figure 3, the cells that had been primed in vitro with anti-CD3, IL-2, and IL-4 and then sorted were stimulated at $1 \times 10^5/0.2$ ml with immobilized anti-CD3, in the presence of IL-2, for 36 h. Supernatants were harvested and IL-4 content was measured in an ELISA assay. Supernatant from stimulated CD4⁺ T cells contained 460 U/ml while that from CD8⁺ T cells contained 105 U/ml, consistent with the relative frequency of IL-4-producing cells shown in Figure 3. The lower limit of detection of this assay was 10 U/ml.

The second line of evidence is based on the capacity of an anti-IL-4 mAb to inhibit the growth stimulatory activities of primed CD8⁺ cells for the IL-4 selective CT.4S cell line. CD4⁺ T cells were isolated by magnetic bead depletion to remove CD4⁺ cells and shown by FACS analysis to be 99% CD8⁺ cells. These cells were stimulated for 5 days with anti-CD3, IL-2, and IL-4 and then restimulated in 96-well plates coated with anti-CD3 in the presence of IL-2. Then 36 h later, the T cells were irradiated and 5000 CT.4S cells were added alone or in the presence of anti-IL-4 (5 μ g/ml). The results shown in Table II illustrate that CT.4S proliferation in response to stimulated CD8⁺ T cells is completely inhibited by anti-IL-4 antibody, demonstrating that the stimulatory factor is IL-4.

IFN- γ and IL-2 production by CD8⁺ T cells primed in vitro. We have demonstrated above that CD8⁺ T cells primed in vitro and then restimulated were capable of producing IL-4 and were interested to know their relative capacity to produce IFN- γ and IL-2. CD4-depleted lymph node T cells were isolated by magnetic bead separation and cultured in the presence of IL-2 and IL-4 on dishes coated with anti-CD3 for 5 days and then restimulated with IL-2 at 100,000 cells/well in microtiter plates coated with anti-CD3. Then 36 h later, supernatants were harvested and tested for the presence of IFN- γ by ELISA. Table III shows that 100,000 CD8⁺ cells in 0.2 ml produce more than 30 ng/ml of IFN- γ . The unseparated T cells also produced more than 30 ng/ml, consistent with the fact that on day 5, when the in vitro primed cells are analyzed, the majority of the cells are CD8⁺. We were unable to detect any IFN- γ production from the CD4⁺ T cells.

The production of IL-2 and IL-4 in these cells were determined using coculture assays. To measure IL-4 production, primed cells were plated on anti-CD3-coated microtiter wells in the presence of IL-2 at various cell concentrations for 36 h, then irradiated and CT.4S cells (5000/well) added. For detection of IL-2, primed cells were stimulated on anti-CD3-coated microwells in the absence of IL-2. After irradiation at 36 h, CTLL cells (5000/well)

TABLE III
Lymphokine production by CD4-depleted T cells primed in vitro

Cell Type	Lymphokine		
	IL-2 (Equiv U/ml)	IL-4 (Equiv U/ml)	IFN- γ (ng/ml)
CD8 ⁺	<0.01	41.0	<0.4
CD4 ⁺	<0.01	15.6	>30
Unseparated	<0.01	26.4	>30

^a CD4⁺ and CD8⁺ T cells were separately depleted from lymph nodes of naive mice. The resultant cells were stimulated for 5 days on anti-CD3-coated dishes in the presence of IL-2 and IL-4. The cells were washed and restimulated under the following conditions. For the assay of IFN- γ , "primed" cells were restimulated in microtiter wells coated with anti-CD3 at 100,000/well in the presence of IL-2. IFN- γ was assayed by ELISA. For the assay of IL-2, 100,000 "primed" cells were restimulated in microtiter wells coated with anti-CD3 for 36 h, irradiated and CTLL (5000/well) were added in the presence of monoclonal anti-IL-4. Then 48 h later, [³H]TdR was added and cells were harvested 6 h later. For IL-4, 30,000 primed cells were restimulated in the presence of IL-2 in microtiter wells coated with anti-CD3 for 36 h, irradiated and CT.4S cells (50,000/well) were added. Then 48 h later, [³H]TdR was added and cells were harvested 6 h later. Results for IL-2 and IL-4 are expressed as equivalent units/ml per 100,000 and per 30,000 cells, respectively. For IFN- γ , results are expressed as ng/ml for 100,000 cells.

in the presence of monoclonal anti-IL-4 were added. For both assays, [³H]TdR was added 48 h later and cells were harvested 6 h thereafter. We were able to detect IL-4 production from all three cell populations with CD4⁺ T cells producing somewhat more than CD8⁺ T cells. We failed to detect IL-2 production by either CD4⁺ or CD8⁺ cells. This is consistent with recent evidence that demonstrates that prolonged stimulation with anti-CD3 inhibits IL-2 production (32).

DISCUSSION

IL-4 production by T cells from naive mice and among long term murine T cell clones has largely been limited to CD4⁺ T cells (4, 12, 26). However, it is known that cells other than CD4⁺ T cells can produce lymphokines. Thus, NK cells produce IFN- γ in response to stimulation by IL-2 or through cross-linkage of FC γ RIII (33). IL-3-dependent mast cell lines produce IL-3, IL-4, IL-5, and GM-CSF in response to cross-linkage of Fc ϵ RI (3) and Fc ϵ R⁺ cells from bone marrow and spleen (mast cells and/or basophils) produce large amounts of IL-4 in response to cross-linkage of a high affinity Fc ϵ R (34). This indicates that production of "T cell-derived" lymphokines is, in fact, not restricted to T cells. Furthermore, in the mouse, CD8⁺ T cell lines have been shown to produce IFN- γ and small amounts of IL-2 and IL-3. Intraepithelial CD8⁺ murine T cells have been reported to produce both IFN- γ and IL-5 in response to Con A (10). Some human CD8⁺ T cell clones have been reported to produce both IL-2, IL-4, and IFN- γ (11). Recently it was reported that CD8⁺ T cell clones isolated from patients with lepromatous leprosy predominantly produced IL-4 (35).

In our report, we confirm that IL-4 production by T cells from naive donors is largely found in the CD4⁺ T cell population and that few if any CD8⁺ T cells from such mice produce IL-4 in response to stimulation with anti-CD3 plus IL-2. However, it is clear that upon appropriate stimulation, some CD8⁺ T cells acquire the capacity to produce IL-4 in response to immobilized anti-CD3 in the presence of IL-2. Indeed, in the vitro priming system we have used, which involves culture for 5 days with anti-CD3 plus IL-2 and IL-4, the frequency of IL-4-producing cells in the CD8⁺ populations is comparable to

TABLE II

Anti-IL-4 inhibits CT.4S proliferation in response to "primed" CD4-depleted T cells stimulated with anti-CD3 plus IL-2^a

Cells/Well	³ H]TdR by CT.4S Cells	
	-	+
10,000	35,589	700
5,000	32,791	700
2,500	26,507	ND
625	3,685	240

^a CD4-depleted T cells were cultured for 5 days on anti-CD3-coated plates in the presence of IL-2 and IL-4. They were washed and replated at the indicated densities in the presence of IL-2 in microwells coated with anti-CD3. Then 36 h later, the cells were irradiated and CT.4S cells (5000/well) were added in the presence (+) or absence (-) of anti-IL-4 (5 μ g/ml). [³H]TdR was added 48 h later and cells were harvested after 6 h.

that in the CD4⁺ population. Inasmuch as these culture conditions lead to a T cell population in which the number of CD8⁺ cells exceeds that of CD4⁺ cells (R. A. Seder, unpublished observations), the majority of IL-4-producing cells in these cultures is often of the CD8 phenotype. This in vitro priming of CD8⁺ T cells is not dependent on the action of CD4⁺ T cells; purified CD8⁺ T cells cultured with anti-CD3 plus IL-2 and IL-4 develop into cells capable of producing IL-4.

Our results clearly establish that murine CD8⁺ T cells can acquire the capacity to produce IL-4 as a result of in vitro priming. A key question that remains to be established is the relative importance of such cells as sources of IL-4 in physiologic situations and in disease states and the functions mediated by such cells as a result of IL-4 production.

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