

## HETEROGENEITY OF HELPER/INDUCER T LYMPHOCYTES

### I. Lymphokine Production and Lymphokine Responsiveness

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Helper/inducer T lymphocytes recognize protein antigens in association with class II MHC (Ia) determinants on APC. Proliferative responses of such T cells to antigen + Ia, antireceptor antibodies, alloantigens, or lectins also require "accessory" signals that, for different T cell populations, have been shown to be provided by a secreted or membrane-associated form of the monokine, IL-1 (1–4), or by undefined stimuli provided by accessory cells which can often be replaced by phorbol esters (5–8). Little is known about how such accessory signals permit or enhance T cell proliferation. In fact, detailed analysis of this question has been difficult because of variability among different T cell clones and bulk populations in their use of different accessory stimuli. Thus, it is unclear why some T cells are IL-1-responsive and others are not.

Recent work from several laboratories (9–11) has shown that murine helper/inducer T lymphocytes are composed of at least two nonoverlapping subsets that can be distinguished on the basis of their patterns of lymphokine secretion. One subset, provisionally termed Th1, produces IL-2 and IFN- $\gamma$  in response to antigen receptor-mediated or lectin-mediated stimulation, and exclusively uses IL-2 as its autocrine growth factor. The other T cell subset, called Th2, secretes B cell stimulatory factor 1 (BSF-1)<sup>1</sup>/IL-4 and uses IL-4 as its autocrine growth factor but does not secrete IL-2.

At present these subsets cannot be distinguished phenotypically. It is reasonable to postulate that Th1 cells are important in cell-mediated immunity (delayed hypersensitivity, cytotoxic T cell generation), whereas the Th2 subset may play a major role in the induction of antibody responses, but such functional differences have not been definitively established and are the subject of active investigation.

In the course of studies analyzing the functions of various T cell subsets, we have examined the proliferative responses of cloned lines representing Th1 and Th2 cells to exogenous lymphokines, and the roles of accessory factors in T cell

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<sup>1</sup>Abbreviations used in this paper: BSF-1, B cell stimulatory factor 1; RCS, Con A-stimulated rat spleen cell supernatant.

proliferation. Our results demonstrate striking differences between such clones in responses to exogenous IL-2 and IL-4, and in their dependence on IL-1 as an accessory signal. The implications of these results for our current understanding of T cell activation and the regulation of cellular immunity are discussed.

### Materials and Methods

**T Cell Clones.** The following T cell clones, whose properties are described in Results, were used for this study: D10.G4, obtained from Dr. C. Janeway, Yale University School of Medicine, New Haven, CT (1); CDC25 and CDC35, from Dr. D. Parker, University of Massachusetts Medical School, Worcester, MA (12); B8 and G11, from Dr. W. H. Boom, Harvard School of Public Health, Boston, MA (13); and D1.1, D1.5 and D1.6, generated in our laboratory from lymph nodes of BALB/c mice immunized with rabbit IgG in CFA. These cells were subjected to multiple cycles of restimulation and rest, and cloned by limiting dilution (14). All clones were maintained by weekly stimulation with irradiated splenocytes and antigen. Splenocytes were isolated from AKR/J mice (for D10.G4), BALB/cJ (for CDC35, D1.1, D1.5, and D1.6), C57BL/6 (for B8 and G11), and (C3H  $\times$  DBA/2)F<sub>1</sub> (for CDC25). Mice, 8–12 wk old, were purchased from The Jackson Laboratory, Bar Harbor, ME, and maintained in accordance with guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23). Clones were maintained by stimulating  $10^5$  viable T cells with  $5 \times 10^6$  1,500 rad irradiated splenocytes and 100  $\mu$ g/ml of antigen in 2 ml of RPMI 1640 supplemented with 2 mM L-glutamine, penicillin, streptomycin, nonessential amino acids,  $5 \times 10^{-5}$  M 2-ME and 10% heat-inactivated FCS. Cultures also contained 5% vol/vol Con A-induced supernatant of rat spleen cells (RCS), from which the Con A was removed by absorption with Sephadex G10. Residual Con A was neutralized with an excess of  $\alpha$ -methyl mannoside. Cells were incubated in 16-mm wells at 37°C in 5% CO<sub>2</sub>. For some experiments, D10.G4 cells were restimulated without RCS for 7–8 d, at which time RCS was added to a final concentration of 5% vol/vol. The specificity and Ia restrictions of clones were defined by blocking proliferative responses to antigen plus stimulator cells with mAbs specific for I-A and I-E.

**Cytokines, Antibodies, and Chemicals.** Human and murine rIL2 were purchased from Genzyme Corp., Boston, MA, and used according to units as defined by the supplier. Recombinant murine BSF-1/IL-4 (expressed in yeast) was generously provided by Dr. S. Gillis, Immunex Corp., Seattle, WA (15). The activity of this preparation was determined at Immunex Corp.; 1 U is equivalent to 0.1–0.2 U we have used in earlier studies using T cell-derived HPLC- and affinity-purified BSF-1 (5, 10, 16). For some experiments, we also used the unfractionated supernatant of D10.G4 cells stimulated for 18 h with 2  $\mu$ g/ml Con A as a crude source of BSF-1/IL-4. Residual Con A was neutralized with 20 mg/ml of  $\alpha$ -methyl mannoside. By antibody inhibition (see below), the only T cell-stimulating activity in this supernatant is BSF-1/IL-4, i.e., no detectable IL-2 is present. Human rIL-1 $\beta$  was purchased from Cistron Technologies, Pine Brook, NJ; units are defined by the supplier on the basis of thymocyte proliferation assays. Con A and the antigens, conalbumin and rabbit IgG, were purchased from Sigma Chemical Co., St. Louis, MO. The neutralizing mAb for BSF-1/IL-4, 11B11, has been described previously (17) and was used as hybridoma culture supernatant at a final dilution of 1:40. Anti-IL-2 antibody, S4B6, was a gift of Dr. T. Mosmann, DNAX Corp., Palo Alto, CA, and was used at a 1:20 dilution of an (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-precipitated 20 $\times$  concentrate of hybridoma supernatant. Anti-IL-2-R antibodies used were 7D4 (18) and PC61 (19); for antibody blocking, we used hybridoma supernatants concentrated 20 times by (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation, mixed in equal proportions, and added to cultures at a final dilution of 1:100 or 1:200, as described previously (10). Other mAbs used as hybridoma supernatants for immunofluorescence, were anti-L3T4 (GK1.5) and rat anti-mouse Lyt-2 (No. TIB105; American Type Culture Collection, Rockville, MD).

**Cultures and Assays.** The effects of lymphokines on the proliferation of T cell clones

were measured using cells harvested 8–10 d after stimulation with antigen. Viable cells were purified by centrifugation over Ficoll-Isopaque, and  $2 \times 10^4$  cells were cultured in duplicate with various stimuli in 0.2 ml of RPMI 1640, supplemented as described above, in flat-bottomed microculture plates. Cultures were incubated for 2–5 d at 37°C in 5% CO<sub>2</sub>, pulsed for the final 6–8 h with 1  $\mu$ Ci [<sup>3</sup>H]thymidine (Amersham Corp., Arlington Heights, IL) per well and harvested in a PHD cell harvester (Cambridge Technology, Inc., Cambridge, MA). Radioactivity incorporated into DNA was measured by liquid scintillation counting, and data were expressed as mean cpm [<sup>3</sup>H]thymidine incorporation per culture.

For assays of lymphokine secretion by T cells,  $2 \times 10^4$  viable cells were cultured in 0.2 ml with 2  $\mu$ g/ml of Con A or  $5 \times 10^5$  1,500 rad irradiated splenocytes from the appropriate strain and 50 or 100  $\mu$ g/ml antigen. Supernatants were collected on day 1 or 2, and assayed at 50% vol/vol for stimulation of HT2 cells in the presence and absence of anti-BSF-1/IL-4 or anti-IL-2-R antibodies, as described previously (10).

*Measurement of BSF-1/IL-4-R Expression.* Affinity-purified BSF-1/IL-4 was iodinated as previously described. Biologic activity of <sup>125</sup>I-IL-4 was comparable to that of noniodinated IL-4 as measured in a T cell costimulation assay (5). BSF-1/IL-4-R expression was measured as described (20). Briefly, receptor number was determined by incubating  $1-2 \times 10^6$  T cells for 2 h at 0°C with <sup>125</sup>I-labeled affinity-purified BSF-1/IL-4, with and without a 200-fold excess of unlabeled lymphokine. Cells were centrifuged through silicon oil, and cell-associated radioactivity was measured. Numbers of binding sites per cell were calculated from Scatchard plots (20), or by using an amount of <sup>125</sup>I-BSF-1/IL-4 sufficient to occupy >90% of available receptors.

## Results

*Characterization of T Cell Clones.* The T cell clones used in this study are listed in Table I. In response to stimulation by Con A or antigen and irradiated splenocytes, all cloned lines secrete a lymphokine that stimulates DNA synthesis by HT2 indicator cells and is identified as either IL-2 or BSF-1/IL-4 by specific antibody inhibition (Table I). On the basis of this assay, clones D1.1, D1.5, D1.6, B8, and G11 belong to the IL-2-producing Th1 subset, whereas D10.G4, CDC25, and CDC35 are prototypical BSF-1/IL-4-producing Th2 lines. In agreement with previous results (10), the autocrine growth factor used by each subset is also IL-2 or BSF-1/IL-4, as determined by antibody blocking of proliferative responses to antigen plus APC (not shown).

As expected from the class II MHC restriction of these lines, all are phenotypically L3T4<sup>+</sup>, Lyt-2<sup>-</sup> and all the clones express IL-2-R (data not shown). The level of IL-2-R expression varies among clones and in the same clone assayed at different times. The basis for this variation is unclear, but, at least with D10.G4, it is not attributable to the presence or absence of exogenous IL-2 (in the form of RCS) in the culture medium during stimulation with antigen and APC.

*Proliferative Responses to Exogenous Lymphokine.* Viable cells from different T cell clones were cultured with Con A, recombinant murine BSF-1/IL-4, a Con A-induced supernatant of D10.G4 (as a crude source of BSF-1/IL-4) or human rIL-2, in the presence or absence of rIL-1 $\beta$  (0.5 U/ml). The pattern of proliferation detected after 2 d is strikingly different for Th1 and Th2 cells. All three Th2 clones respond to Con A, BSF-1/IL-4, and IL-2; their response to Con A (which induces secretion of BSF-1/IL-4) and to exogenous BSF-1/IL-4 is entirely dependent on or markedly enhanced by IL-1 (Fig. 1). These lines do proliferate when cultured with IL-2 alone, and this is generally enhanced by IL-1. In contrast, the IL-2-producing Th1 lines respond maximally to IL-2 but fail to

TABLE I  
T Cell Clones: Specificity and Lymphokine Secretion

Clone	Specificity	Stimulation of HT2, [ <sup>3</sup> H]TdR incorporation (cpm/culture) by T cell SN, in presence of:		
		No antibody	Anti-BSF-1/IL-4	Anti-IL-2-R
D10.G4	Conalbumin + I-A <sup>k</sup>	55,619	<u>942</u>	43,550
CDC25	RGG + I-A <sup>(kxd)F1</sup>	65,610	<u>3,003</u>	43,175
CDC35	RGG + I-A <sup>d</sup>	42,609	<u>5,170</u>	30,983
B8	PPD + I-A <sup>b</sup>	66,052	<u>46,426</u>	<u>771</u>
G11	PPD + I-A <sup>b</sup>	101,754	101,983	<u>31,076*</u>
D1.1	RGG + I-A <sup>d</sup>	132,006	122,375	<u>13,764*</u>
D1.5	RGG + I-A <sup>d</sup>	21,014	20,564	<u>977</u>
D1.6	RGG + I-A <sup>d</sup>	24,865	16,536	<u>329</u>
Controls: BSF1/IL-4		25,650	<u>653</u>	16,722
IL-2		126,225	135,990	<u>409</u>

Antigen + Ia specificities of T cell clones used in this study were established by inhibition of proliferative responses to antigen + APC by mAbs specific for I-A and I-E determinants (not shown).

To measure lymphokine secretion, SN were collected after 1 d culture of  $2 \times 10^4$  T cells with 2  $\mu\text{g/ml}$  of Con A, or 2 d culture with 100  $\mu\text{g/ml}$  antigen and  $10^6$  1,500 rad irradiated splenocytes. SN were tested at 50% vol/vol for stimulation of HT2 cells in the presence and absence of antibodies to BSF-1/IL-4 or to IL-2-R, as described in Materials and Methods. Controls included recombinant BSF-1/IL-4 (250 U/ml) and recombinant human IL-2 (10 U/ml). Groups showing significant inhibition of HT2 stimulation are underlined.

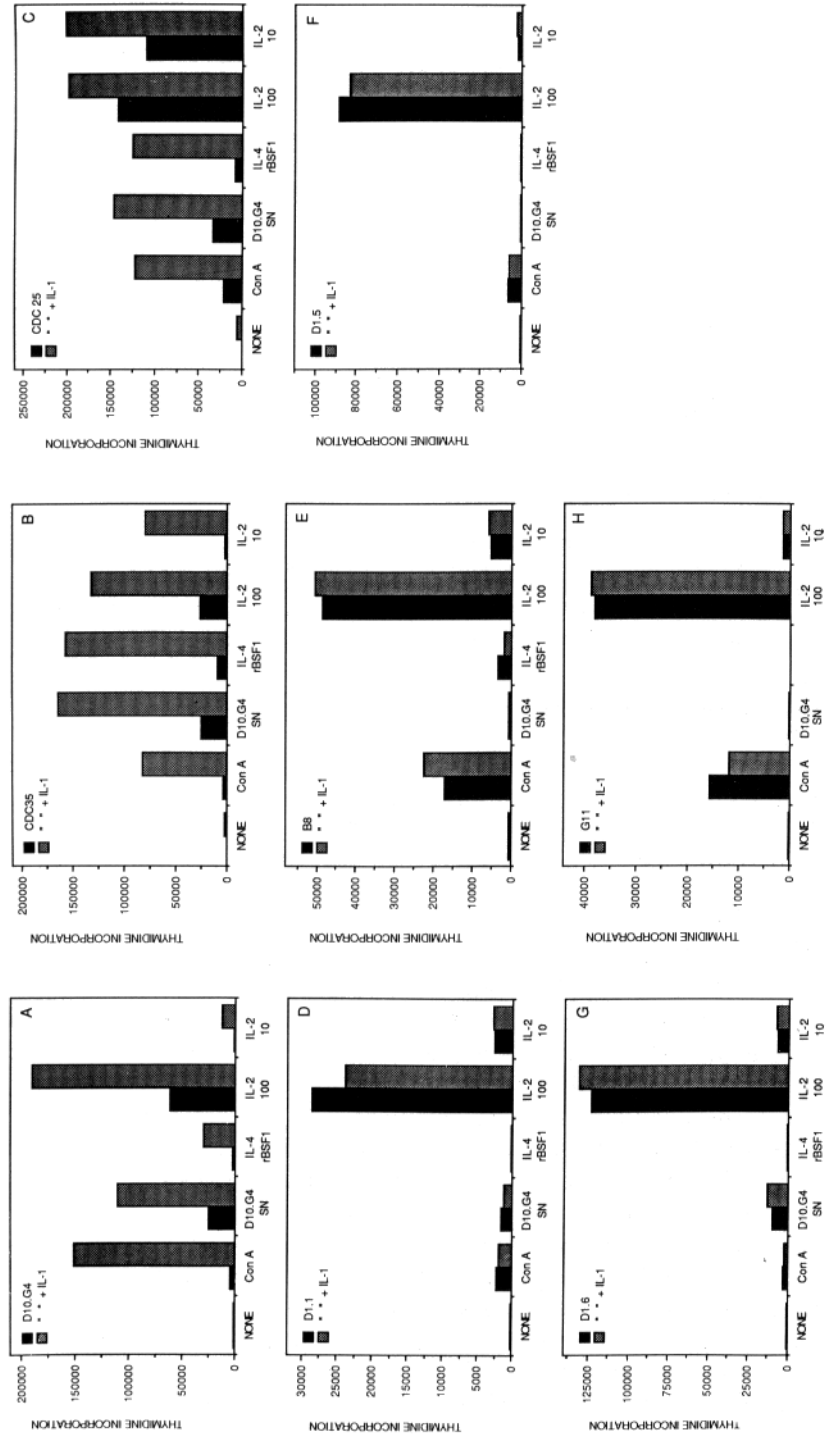
\* Incomplete inhibition is due to the high concentrations of IL-2 produced.

respond to BSF-1/IL-4, and their DNA synthesis is unaffected by IL1 (Fig. 1). The same pattern of proliferation is observed after 5 d of culture, indicating that the differences cannot be attributed to different response kinetics (not shown). Similar results have been observed with four other Th1 and two other Th2 clones (data not shown).

The unresponsiveness of Th1 clones to BSF-1/IL-4 and to IL-1 was investigated in more detail. Over a range of concentrations of recombinant BSF-1/IL-4 from 100–1,000 U/ml, two prototypical Th2 lines, D10.G4 and CDC25, show increasing DNA synthesis (in the presence of IL-1) with a plateau at 100–200 U/ml. Under the same conditions, three IL-2-secreting Th1 clones, D1.1, D1.5, and D1.6, fail to incorporate [<sup>3</sup>H]thymidine (Fig. 2).

Both T cell subsets respond to IL-2, but here, too, the differences in IL-1 effects are observed. Thus, DNA synthesis by Th1 clones is unaffected by IL-1. In contrast, D10.G4 and CDC25, two representative Th2 clones, clearly show an enhancing effect of IL-1 on IL-2-induced proliferation (Fig. 3).

Dose-response studies were also done with IL-1. As shown above (Fig. 1) and described previously (1), D10.G4 proliferates in response to Con A or receptor-mediated stimulation only in the presence of IL-1. The maximal effect of IL-1 in this assay using human IL-1 is observed at a concentration of 0.1–0.5 U/ml. Similar dose responses are seen in the stimulation of Th2 clones by BSF-1/IL-4, i.e., maximal costimulator effects of IL-1 at 0.1–0.5 U/ml (Fig. 4). In contrast, Th1 clones do not respond to BSF-1/IL-4 even with IL-1 concentrations up to 2 U/ml (Fig. 4). At supramaximal concentrations of BSF-1/IL-4, Th2 cells



**FIGURE 1.** Proliferative responses of T cell clones to Con A and lymphokines (10%,) or IL-2 (10 or 100 U/ml), in the absence (solid bars) or presence (stippled bars) of IL-1 (0.5 U/ml). Proliferation was measured by [<sup>3</sup>H]-thymidine incorporation on day 2.

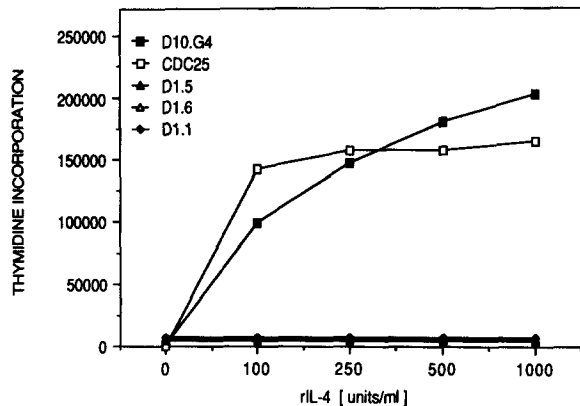


FIGURE 2. Dose response of BSF-1/IL-4-induced stimulation of T cell proliferation.  $2 \times 10^4$  T cells were cultured for 2 d with increasing concentrations of recombinant BSF-1/IL-4, in the presence of IL-1 (0.5 U/ml). Proliferation was measured as in Fig. 1.

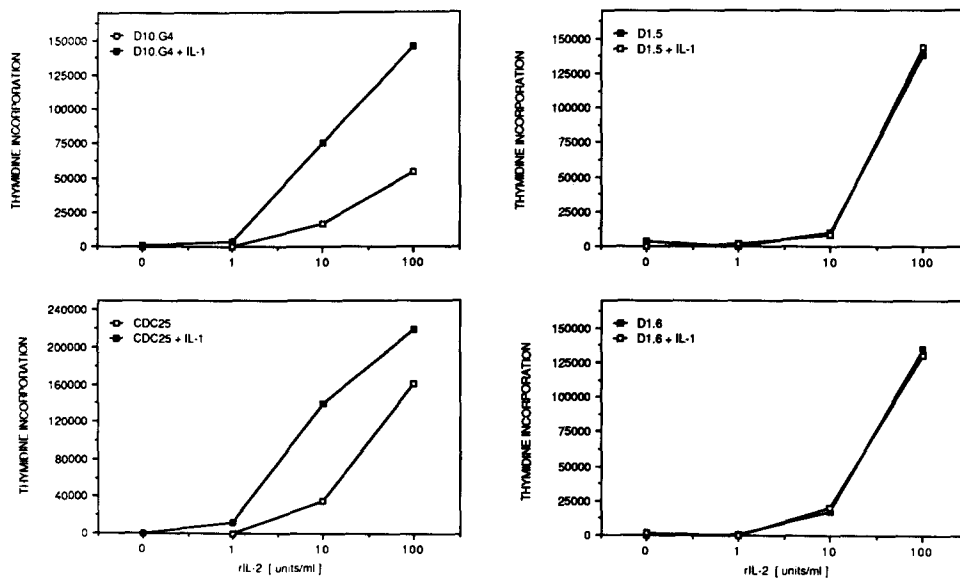


FIGURE 3. Dose response of IL-2-induced stimulation of T cell proliferation. Cloned T cells were cultured for 2 d with increasing concentrations of human rIL-2, in the absence (□) or presence (■) of IL-1 $\beta$  (0.5 U/ml). Proliferation was measured as in Fig. 1.

occasionally proliferate in the absence of exogenously added IL-1 but the response is invariably enhanced by IL-1. Similar results have been obtained in limited experiments with human rIL-1 $\alpha$  and with a partially purified murine macrophage-derived IL-1 (not shown).

*Influence of Culture Conditions on Lymphokine Responses.* Both IL-2 (21) and BSF-1/IL-4 (Ohara, J., and W. E. Paul, unpublished observations) are known to enhance expression of their own receptors. It is, therefore, possible that the lack of BSF-1/IL-4 responsiveness of Th1 clones is solely because the cells never encounter this lymphokine during growth in vitro. Thus, when stimulated with antigen and antigen-presenting cells they secrete IL-2, and the only lymphokine added during passage in vitro is a Con A-induced RCS, which may not contain any BSF-1/IL-4 reactive with murine cells. Two approaches were taken to address

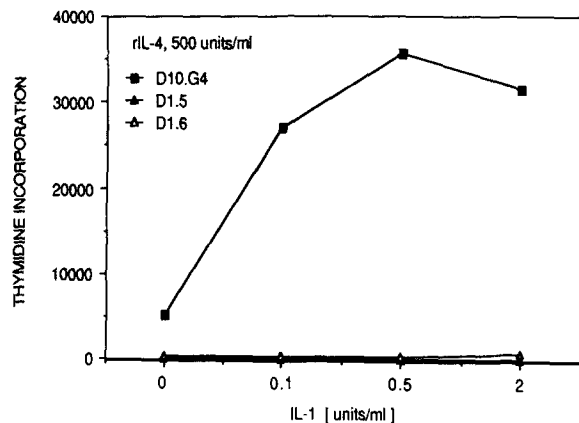


FIGURE 4. Dose response of IL-1 effect on T cells: stimulation with BSF-1/IL-4.  $2 \times 10^4$  T cells were cultured with 500 U/ml of BSF-1/IL-4, and increasing concentrations of IL-1. Proliferation was measured as in Fig. 1.

TABLE II  
*Influence of Culture Conditions on Lymphokine Responsiveness of T Cells*

T cell clone	Cultured with antigen + APC in the presence of:	Response to lymphokine (U/ml)	$[^3\text{H}]$ Thymidine incorporation (mean cpm/culture)	
			-IL-1	+IL-1
D1.5	RCS	—	120	144
		BSF-1/IL-4 (500)	513	327
		IL-2 (10)	70,606	78,890
D1.5	RCS + D10.G4 SN	—	285,053	302,743
		BSF-1/IL-4 (500)	329	104
		IL-2 (10)	500	570
D1.5	RCS + D10.G4 SN	—	127,943	124,699
		BSF-1/IL-4 (500)	255,895	238,998
		IL-2 (10)	332	115
D1.6	RCS	—	236	220
		BSF-1/IL-4 (500)	137,481	161,435
		IL-2 (10)	204,437	196,463
D1.6	RCS + D10.G4 SN	—	209	92
		BSF-1/IL-4 (500)	217	137
		IL-2 (10)	65,793	62,047
D1.6	RCS + D10.G4 SN	—	101,375	92,045
		BSF-1/IL-4 (500)	217	137
		IL-2 (10)	65,793	62,047

D1.5 and D1.6 cells were cultured for 8 d with antigen + APC with and without Con A-induced D10.G4 SN (10% vol/vol). Viable cells were purified and tested for proliferative responses to lymphokines, in the presence and absence of IL-1 (0.5 U/ml).

this possibility. First, D1.5 and D1.6, two IL-2-producing clones, were restimulated according to the standard protocol, but Con A-induced D10.G4 supernatant (a rich source of BSF-1/IL-4) was added to some wells. These paired cultures were then assayed for responses to lymphokines. As shown in Table II, the addition of D10.G4 supernatant during cell passage does not alter the pattern of response of two Th1 clones, i.e., they remain insensitive to BSF-1/IL-4 and to IL-1. Moreover, the pattern of proliferation remains unchanged from 2 to 5 d of culture, although responses were considerably lower at 5 d (data not shown).

In a second set of experiments, cells of the IL-2-producing Th1 clone, D1.5, were cultured for 3 d with rIL-2 or with recombinant BSF-1/IL-4, and then

TABLE III  
*Lymphokine Responsiveness of D1.5 Cells in Two-Stage Cultures*

T cell clone	First culture	Second culture: lymphokine (U/ml)	<sup>3</sup> H]Thymidine incorporation (mean cpm/culture)	
			-IL-1	+IL-1
D1.5	Medium	—	527	178
		BSF-1/IL-4 (500)	135	336
		IL-2 (100)	12,109	12,658
D1.5	D10.G4 SN 10%	—	170	168
		BSF-1/IL-4 (500)	246	266
		IL-2 (100)	36,718	35,834
D1.5	BSF1/IL4 500 U/ml	—	171	313
		BSF-1/IL-4 (500)	135	264
		IL-2 (100)	24,556	19,164
D1.5	IL2 50 U/ml	—	493	463
		BSF-1/IL-4 (500)	750	486
		IL-2 (100)	40,042	36,291
D1.5	None	—	324	354
		BSF-1/IL-4 (500)	573	616
		IL-2 (100)	90,389	89,523
D10.G4	None	—	582	3,196
		BSF-1/IL-4 (500)	3,100	34,193
		IL-2 (100)	64,159	125,549

D1.5 cells were isolated from culture with antigen + APC, and incubated for 3 d with medium, D10.G4 SN, BSF-1/IL-4, or IL-2. Cells were washed and  $2 \times 10^4$  viable cells were stimulated with BSF-1/IL-4 and IL-2 with and without IL-1 (0.5 U/ml). Also shown are D1.5 and D10.G4 cells freshly harvested from maintenance cultures with antigen + APC and stimulated without preculture. Proliferation was measured on day 2.

assayed for responses to these lymphokines in the presence and absence of IL-1. Again, prior exposure to either lymphokine has no effect on the pattern of response (Table III). The lower responses of cells cultured in medium alone may be due to decreasing viability or metabolic activity. Taken together, these experiments demonstrate that the failure of Th1 clones to proliferate in response to BSF-1/IL-4 with or without IL-1 is presumably an intrinsic property of this T cell subset and is not a reflection of culture conditions.

*Correlation of BSF-1/IL-4 Responsiveness and Receptor Expression.* The simplest explanation for the unresponsiveness of some T cells to BSF-1/IL-4 is that they do not express receptors for this lymphokine. We have examined BSF-1/IL-4 receptors on a representative panel of T cell clones by binding of <sup>125</sup>I-labeled lymphokine. In these experiments, receptor numbers on normal BALB/c spleen cells were calculated to be 1,266 per cell with a  $K$  of  $0.87 \times 10^{10} \text{ M}^{-1}$  (Fig. 5). The value for receptor number is somewhat in excess of the receptor number observed in a previous study (450/cell) and the  $K$  was somewhat lower than the previously measured value ( $3.3 \times 10^{10} \text{ M}^{-1}$ ) (20).

Our calculation of receptor expression on Th2 clones indicates that they express ~2–10 times as many receptors per cell as normal splenocytes. Th1 clones generally express fewer receptors than Th2 clones, but the groups clearly overlap in receptor expression (Table IV). The calculated  $K$  values for two Th2 clones (D10.G4 and CDC 25) and one Th1 clone (G11) are also not significantly



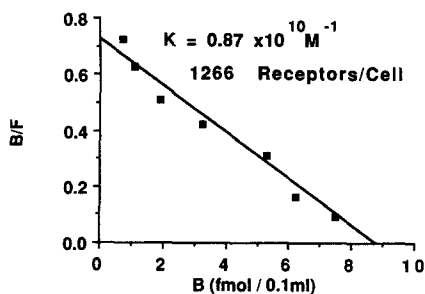


FIGURE 5. Binding of  $^{125}\text{I}$ -IL-4 to spleen cells. Purified T cell-derived BSF-1/IL-4 was radioiodinated as described (20). Specific activity was determined by a competition assay in which  $^{125}\text{I}$ -IL-4 was compared with known quantities of nonradioactive IL-4 (20). 60% of the  $^{125}\text{I}$ -IL-4 preparation used in these experiments was capable of binding to spleen cells. Binding data were corrected for this factor. The specific activity of this preparation at the time of initial assay was  $1.70 \times 10^{-17}$  mol IL-4 per cpm. All binding experiments were carried out at  $4^\circ\text{C}$  for 2 h, conditions that achieve equilibrium. In the experiment illustrated, cell density was  $3.98 \times 10^6$  cells in 0.1 ml.

TABLE IV  
*BSF-1/IL-4-R Expression on Cloned T Cells*

Cells	Type	IL-4-R per cell
BALB/c spleen	Normal spleen	1,266
D10.G4	Th2	10,900
CDC25	Th2	2,400
CD35	Th2	5,200
D1.1	Th1	540
D1.6	Th1	3,300
B8	Th1	1,600
G11	Th1	2,200

BALB/c spleen receptor is derived from data in Fig. 5. The results for Th1 and Th2 cloned T cell lines were obtained in the same experiment. Receptor numbers for D10.G4, CDC25, and G11 cells are based on Scatchard analysis. For other cell types, single point determinations were carried out at near saturation conditions.

TABLE V  
*IL-1 Does Not Increase Expression of IL-4-R on D10.G4 Cells*

D10.G4 cells cultured in	Receptors/cell	
	Exp. 1	Exp. 2
Medium	3,080	4,110
IL-1	3,210	2,810

D10.G4 cells were cultured for 18 h in medium alone or with IL-1 (1 U/ml). IL-4-R were measured in triplicate, as described. Receptor number was calculated from a single point measurement under near saturation conditions.

different from normal splenocytes. Moreover, BSF-1/IL-4-R density on D10.G4 cells is unaffected by culture with IL-1 (Table V). Therefore, it is unlikely that IL-1 functions simply by inducing or stimulating the expression of receptors for this autocrine growth-promoting lymphokine.

*Specificity of Lymphokine-mediated Stimulation.* To confirm the specificity of lymphokine-mediated stimulation, we cultured a Th2 clone that responds to both IL-2 and BSF-1/IL-4 (+IL-1), D10.G4, with these lymphokines in the presence and absence of neutralizing mAbs specific for IL-2 or BSF-1/IL-4. As

TABLE VI  
*Specificity of Lymphokine-induced Stimulation of D10.G4*

Stimuli (U/ml)	<sup>3</sup> H]Thymidine incorporation (mean cpm/culture) with:		
	No anti- body	Anti-BSF- 1/IL-4	Anti-IL-2
—	333	ND	ND
IL-1 (0.1)	3,515	ND	ND
BSF-1/IL-4 (500) + IL-1 (0.1)	22,361	<u>1,370</u>	<u>26,632</u>
IL-2 (50) + IL-1 (0.1)	25,102	<u>15,949</u>	<u>3,942</u>
D10.G4 SN (25%) + IL-1 (0.1)	56,365	<u>4,347</u>	<u>56,315</u>

$2 \times 10^4$  D10.G4 cells were cultured for 2 d as shown, with no antibody, anti-BSF-1/IL-4 (11B11 hybridoma SN, 1:40), or anti-IL-2 (S4B6, 1:20). The IL-2 used in this experiment was recombinant murine IL-2. Proliferation was measured on day 2. Groups showing significant inhibition are underlined.

shown in Table VI, the proliferation of D10.G4 induced by each lymphokine is significantly inhibited only by the specific antibody. In addition to demonstrating specificity, this result indicates that the major portion of the response to an exogenous lymphokine is due to the direct action of that lymphokine, and not due to secondary stimulation of autocrine growth factor production. In other words, IL-2 stimulates D10.G4 directly and not by inducing the secretion of BSF-1/IL-4.

### Discussion

The experiments described in this paper were initiated after the discovery that subsets of helper/inducer T lymphocytes can be distinguished by profiles of lymphokine secretion and autocrine growth factor utilization (9–11). Such differences may provide the biologically most meaningful criteria for distinguishing the functional potential of T cell subsets. The results described above establish two striking differences between these subsets. First, both IL-2- and BSF-1/IL-4-producing (Th1 and Th2) clones proliferate in response to exogenous IL-2, but only the Th2 clones respond to BSF-1/IL-4. Second, responses of Th2 clones are dependent on or enhanced by the accessory cell-derived costimulator, IL-1, but Th1 cells are IL-1-insensitive.

The nonreciprocal pattern of proliferative responses of Th1 and Th2 subsets to exogenous lymphokines is not attributable to culture conditions or to prior exposure to these lymphokines. We also believe that unresponsiveness of Th1 cells to BSF-1/IL-4 is not due to a failure to identify a costimulator that must act in concert with this lymphokine, for two reasons: (a) no such costimulator (other than IL-1) is necessary for clones that are responsive to BSF-1/IL-4, and (b) such an accessory function is not provided by PMA, which is the most efficient costimulator (and the only one other than IL-1) that has been shown to act on T cells (data not shown). Initial studies suggest that most Th1 clones express lower levels of BSF-1/IL-4-R than Th2 clones, but there is overlap among the cell lines, suggesting that the failure of the Th1 cells to respond to this lymphokine may not be solely related to receptor expression, but may reflect other events in the activation pathway. Moreover, recent experiments suggest that Th1 clones

may respond to BSF-1/IL-4 for short periods (up to 3–4 d) after receptor-mediated stimulation, but rapidly lose this responsiveness, whereas Th2 clones continue to proliferate in response to IL-4 (+ IL-1) for at least 2 wk after antigenic stimulation.

The differences in the IL-1 sensitivity of T cell subsets described in this report might account for the fact that in various studies using bulk populations of T cells, proliferative responses have been shown to be either dependent on or unaffected by IL-1. Thus, in many instances the proliferation of naive T cells induced by alloantigens of mitogenic lectins requires accessory cells whose function cannot be replaced by IL-1 (6–8). On the basis of our results, we would predict that these responding T cells belong to the IL-2-producing Th1 subset. In fact, recent experiments have shown that naive murine lymph node T cells stimulated by Con A or alloantigen secrete IL-2 and no BSF-1/IL-4, and the IL-4-secreting subset appears in immunized, antigen-responsive populations, at least within the limits of sensitivity of the assays we have used (Powers, G. D., A. K. Abbas, and R. A. Miller, manuscript in preparation). In contrast to the frequent findings with naive T cells, a critical role for IL-1 in T cell proliferation has been most often observed with cloned lines such as D10.G4 and CDC25 (1, 22, 23), which are now known to belong to the Th2 subset, or with antigen-primed and restimulated bulk populations (24–26), which we have found to contain a significant proportion of BSF-1/IL-4-secreting cells by limiting dilution analysis (Powers, G. D., et al., manuscript in preparation). The mechanism by which IL-1 functions as a costimulator for T cell proliferation is not known. With the cloned lines used in this study, IL-1 has no effect on lymphokine secretion (reference 27 and data not shown) or lymphokine receptor expression. Experiments are in progress to determine if sensitivity to IL-1 is related to the expression of high-affinity IL-1-R.

Based on the results reported above and described previously (10), we can summarize the responses of clones representing Th1 and Th2 subsets of helper/inducer T cells as follows. When stimulated via their antigen receptors, IL-2-producing Th1 cells secrete IL-2 and express receptors for IL-2, and this autocrine pathway is sufficient to induce proliferation. Such cells may express lower levels of receptors for BSF-1/IL-4, and because of this or other reasons they fail to respond to BSF-1/IL-4, regardless of what costimulator or accessory factor is present. Th2 cells also secrete their lymphokines after receptor-mediated stimulation, but their response to the autocrine growth factor requires a costimulator activity that is provided by IL-1. The Th2 cells respond to IL-2, which they themselves do not secrete. Thus, IL-2 produced by Th1 cells is capable of inducing proliferation of adjacent T cells, but BSF-1/IL-4 secretion by Th2 cells will only affect other cells belonging to the same subset. It is, therefore, reasonable to predict that production of IL-2, but not BSF-1/IL-4, may play a role in antigen-independent expansion of “bystander” T cells.

Finally, we do not know to what extent our results with cloned lines are applicable to other helper/inducer clones and unselected, bulk T cell populations. The majority of nonimmune T lymphocytes proliferate in response to exogenous IL-2 (28). This is not surprising given our finding that both Th1 and Th2 cells are responsive to this lymphokine. Recent experiments indicate that resting

peripheral T cells also proliferate in response to BSF-1/IL-4 + PMA (5). The difference between such cells (most of which are IL-2 producers) and established cloned lines is a question that remains to be answered.

### Summary

Antigen-specific, Ia-restricted helper/inducer T lymphocytes consist of subsets that can be distinguished by lymphokine secretion. One, called Th1, secretes IL-2 and the other, termed Th2, produces BSF-1/IL-4 in response to stimulation by lectin or antigen receptor signals, and each uses the respective lymphokine as its autocrine growth factor. Cloned lines representing Th2 cells proliferate in response to both IL-2 and their autocrine lymphokine, BSF-1/IL-4, but this proliferation is dependent on the synergistic costimulator activity of the monokine, IL-1. In contrast, Th1 clones proliferate only in response to IL-2, are unresponsive to BSF-1/IL-4, and their growth is unaffected by IL-1. These response patterns are not attributable to variations in culture conditions but apparently reflect intrinsic properties of the two T cell subsets. Moreover, the unresponsiveness of Th1 cells to BSF-1/IL-4 may be related to lower levels of expression of surface receptors for this lymphokine. These results may explain the observed heterogeneity among bulk populations of T cells in terms of lymphokine responsiveness and requirement for accessory factors (costimulators). In addition, our findings suggest that IL-2, unlike BSF-1/IL-4, is a fully competent growth factor that is potentially involved in antigen-independent expansion of bystander T cells present at sites of immune stimulation.

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*Note added in proof:* Recent experiments have shown that Th2 clones express high-affinity IL-1 receptors, whereas Th1 clones that are insensitive to IL-1 do not express high-affinity receptors for IL-1 (Lichtman, A. H., J. A. Schmidt, and A. K. Abbas, manuscript in preparation).

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