Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 824–827, February 1987
Immunology

B-cell stimulatory factor 1 and not interleukin 2 is the autocrine growth factor for some helper T lymphocytes

(T cell growth factors/interleukin 1/T cell clones/lymphokines)

ANDREW H. LICHTMAN*, EVELYN A. KURT-JONES, AND ABUL K. ABBAS

Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Communicated by Herman N. Eisen, October 20, 1986

ABSTRACT Clonal expansion of T lymphocytes of the helper/inducer class is generally thought to be mediated by an interleukin 2 (IL-2)-dependent autocrine mechanism. Thus, T cells stimulated by antigens or mitogenic lectins secrete IL-2 and, under appropriate conditions, express membrane receptors for IL-2, and the specific hormone-receptor interaction induces cellular proliferation. Recent studies indicate that B-cell stimulatory factor 1 (BSF-1) is secreted by T cells and is capable of stimulating T-cell proliferation. We now report that BSF-1 and not IL-2 is the sole autocrine growth factor for certain cloned lines of inducer T lymphocytes. On stimulation by the lectin concanavalin A, anti-receptor antibody, or specific antigen with antigen-presenting cells, such clones secrete a lymphokine that stimulates DNA synthesis by the "IL-2 indicator line," HT2, but is identified as BSF-1 by specific inhibition with monoclonal antibodies. The proliferative response of such BSF-1-secreting clones to receptor-mediated signals is dependent on BSF-1 and not IL-2. These results demonstrate a function of BSF-1 and confirm the existence of a previously unknown autocrine pathway of T-cell activation.

Proliferation and differentiation of lymphocytes are mediated by a variety of lymphokines. Until recently, the major or only growth factor that has been identified for T lymphocytes is interleukin 2 (IL-2). Thus, it has been thought that helper/inducer T lymphocytes, recognizing antigens in association with products of class II major histocompatibility genes (Ia molecules), are stimulated to secrete IL-2 and to express high-affinity surface receptors for IL-2. Subsequent proliferation of these T cells is a consequence of the autocrine IL-2-receptor interaction (1). Several stimuli have been shown to induce and/or enhance the expression of IL-2 receptors (IL-2R). These include the monokine, interleukin 1 (IL-1) (2), and IL-2 itself (3). Recently, it has become apparent that in different situations, T-cell expansion does not correlate with expression of IL-2R (4) and that IL-2-independent pathways of T-cell proliferation exist (5). The nature of such pathways is unknown.

B-cell stimulatory factor 1 (BSF-1; provisionally termed interleukin 4) was originally identified as a helper/inducer T-cell-derived lymphokine that acted, together with anti-immunoglobulin antibodies, as a costimulator for proliferation of resting B lymphocytes (6). More recent studies using highly purified and recombinant DNA-derived BSF-1 have shown that this lymphokine also stimulates the proliferation of T lymphocytes and mast cells, induces IgG1 and IgE antibody production, and enhances the expression of Ia molecules on resting B cells (7–11). During experiments analyzing lymphokine production by murine helper/inducer T-cell clones, we observed that some clones secreted a factor that stimulated DNA synthesis by the prototypical "IL-2 indicator line," HT2 (12), but it was not IL-2. We now report the identification of this factor as BSF-1. More importantly, cloned T cells that secrete BSF-1 in response to receptor-mediated signals exclusively utilize this lymphokine as their autocrine growth factor, whereas clones that secrete IL-2 proliferate via an IL-2-dependent mechanism. Thus, BSF-1 represents a newly defined, IL-2-independent autocrine pathway for T-cell activation.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Lines. The following murine helper/inducer T-cell clones have been used in the experiments described in this paper: D10.G4 (specific for conalbumin and I-A^k determinants) (13), CDC 25 [specific for rabbit immunoglobulin (RGG) and an I-$A^{k/d}$ F_1 hybrid determinant] (14), and 10G11 (specific for ovalbumin and I-A^d), which we derived by limiting dilution cloning of immune lymph node T cells in the presence of antigen, irradiated BALB/c splenocytes, and Con A-induced rat spleen cell supernatant. All clones were maintained by restimulation every 2 weeks with antigen, 1500 R irradiated splenocytes, and Con A supernatant. For lymphokine assays we used the HT2 line, which synthesizes DNA in response to IL-2 and is, therefore, considered to be an IL-2 indicator cell.

Lymphokines, Monokines, Antigens, and Antibodies. BSF-1 was purified from the culture supernatant of the phorbol ester-stimulated EL4 thymoma cell line by high pressure liquid chromatography (15) and was kindly provided by J. Ohara and W. Paul (National Institutes of Health). Units of BSF-1 are expressed as described (15). Recombinant murine IL-2 was obtained from Genzyme (Boston), and purified human monocyte IL-1 was from Cistron (Pine Brook, NJ); concentrations of both are expressed in units used by the suppliers.

The rat monoclonal antibody specific for murine BSF-1, 11B11, was produced and provided as ascitic fluid by J. Ohara and W. Paul (16). For inhibiting the effects of IL-2, we used a combination of two rat anti-IL-2R monoclonal antibodies, 7D4 (17) and PC61 (18). Globulin fractions (concentrated 20 times) of anti-IL-2R hybridoma culture supernatants were prepared by precipitation in 45% ammonium sulfate, mixed in equal proportions, and used at the final dilutions indicated. The mouse monoclonal antibody specific for the clonotypic receptors of D10.G4, 3D3, was produced by Kaye *et al.* (13) and was generously provided as ascitic fluid by C. Janeway (Yale University School of Medicine).

The antigens, conalbumin and ovalbumin, and the lectin Con A, were purchased from Sigma. 2,4,6-Trinitrophenyl (TNP) conjugates of conalbumin were prepared as described

Abbreviations: APC, antigen-presenting cells; BSF-1, B-cell stimulatory factor 1; IL-1, interleukin 1; IL-2, interleukin 2; IL-2R, interleukin 2 receptors; RAMG, rabbit anti-mouse immunoglobulin; RGG, rabbit immunoglobulin; TNP, 2,4,6-trinitrophenyl.

*To whom reprint requests should be addressed.

(19). RGG (Sigma) and affinity-purified rabbit anti-mouse immunoglobulin (RAMG) were digested with pepsin and $F(ab')_2$ fragments purified by protein A-Sepharose chromatography, and are referred to as RGG and RAMG, respectively.

Cell Cultures and Assays. Viable cells of different cloned T-cell lines were purified by centrifugation over Ficoll-Isopaque and cultured with various stimulants in flat-bottomed microculture plates in duplicate or triplicate. Each well contained 2×10^4 T cells in a total volume of 0.2 ml of RPMI 1640 medium supplemented with 2 mM L-glutamine, penicillin, streptomycin, nonessential amino acids, 1 mM sodium pyruvate, 10% heat-inactivated fetal calf serum, and 50 μM 2-mercaptoethanol. Cells were stimulated with Con A, antireceptor antibody, various lymphokines, or antigen and antigen-presenting cells (APC). The APC used were 1500 R irradiated splenocytes (5×10^5 per well) from AKR mice (for D10.G4), (C3H × DBA/2)F_1(C3D2F1) mice (for CDC 25) or BALB/c mice (for 10G11). In some experiments, we used 5×10^4 900 R irradiated TNP-specific splenic B cells from normal mice purified by binding to and eluting from TNP gelatin-coated dishes as described (20). This was done to exclude the possibility of T-cell lymphokines being derived from the APC populations. We have shown that such hapten-specific B lymphocytes are highly efficient at presenting hapten-proteins to T–T hybridomas (20, 21), as well as cloned lines such as D10.G4 (19). TNP-binding B cells also present low concentrations of TNP–RGG and RAMG to the CDC25 clone (A.H.L., H. P. Tony, D. C. Parker, and A.K.A., unpublished data). Although purified B cells and antigen are sufficient for stimulating lymphokine secretion, maximal T-cell proliferation requires the presence of IL-1 (19).

Lymphokine secretion was measured in cultures incubated for 16–20 hr at 37°C in a humidified atmosphere of 5% CO_2/95% air. Aliquots of supernatants (0.1 ml) were transferred to fresh microculture plates, and 10^4 HT2 cells were added with and without anti-BSF-1 or anti-IL-2R, to a final vol of 0.2 ml. These cultures were incubated for 16 hr at 37°C, pulsed for 5–6 hr with 1 μCi of [^{3}H]thymidine (Amersham) per well and samples were harvested in a PHD cell harvester (Cambridge Technology, Cambridge, MA). [^{3}H]Thymidine incorporation was measured by scintillation counting and was used as an index of DNA synthesis; all results are expressed as mean cpm per culture.

T-cell proliferation was measured in cultures incubated for 48 or 60 hr at 37°C, pulsed with [^{3}H]thymidine for the final 6–8 hr, and harvested as described above. Controls included in all experiments showed that the irradiated APC populations did not secrete IL-2 or BSF-1 or proliferate when cultured alone, and that neither anti-BSF-1 nor anti-IL-2R antibodies alone stimulated DNA synthesis by HT2 cells or by any of the T-cell clones studied.

RESULTS

The studies described below evolved from our initial observation that antigen and APC-induced supernatants of the D10.G4 clone stimulated DNA synthesis by HT2 cells, but this stimulation could not be inhibited by concentrations of anti-IL-2R antibodies that completely abrogated the response of HT2 cells to recombinant murine or human IL-2 (200 units/ml). This finding suggested that another HT2-stimulating lymphokine, possibly BSF-1, was present in these supernatants. We, therefore, developed an assay for distinguishing IL-2 from BSF-1 using HT2 cells as the test system. As shown in Fig. 1, both recombinant IL-2 and HPLC-purified murine BSF-1 stimulate the proliferation of HT2 cells. At maximal concentrations, the response to IL-2 is 2–5 times the response to BSF-1; similar results have recently been reported with purified recombinant BSF-1 (11). More importantly, the response to BSF-1 is specifically blocked by a monoclonal antibody, 11B11, reactive with this lymphokine (15), while the response to IL-2 is inhibited by antibodies specific for IL-2R (Fig. 1 *C* and *D*). Neither antibody shows any cross-inhibition, and control rat monoclonal antibodies specific for mouse Lytl or LFA1 are not inhibitory (data not shown).

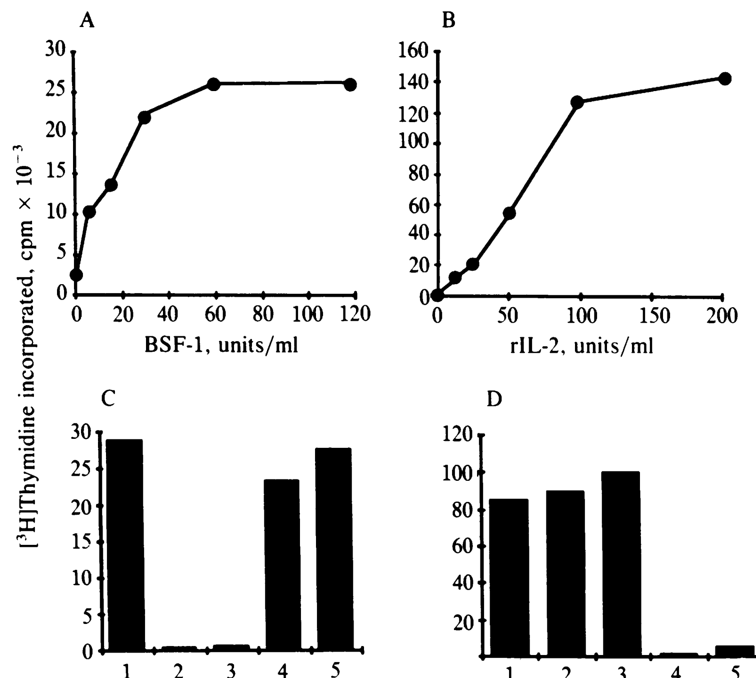

FIG. 1. Stimulation of HT2 cells by BSF-1 and IL-2. Duplicate cultures of 10^4 HT2 cells were stimulated with HPLC-purified BSF-1 (*A*, as shown; *C*, 30 units/ml) or recombinant murine IL-2 (rIL-2) (*B*, as shown; *D*, 100 units/ml). In *C* and *D*, cultures also contained anti-BSF-1 antibody or anti-IL-2 receptor antibodies. Data shown are means of radioactivity incorporated (cpm) per culture. Both antibodies selectively and completely inhibit the activity of the respective lymphokine. Bars: 1, no antibody; 2, anti-BSF-1 diluted 1:1000; 3, anti-BSF-1 diluted 1:5000; 4, anti-IL-2R diluted 1:100; 5, anti-IL-2R diluted 1:500.

Table 1. T-cell clones D10.G4 and CDC 25 secrete BSF-1

Supernatant†		Proliferation of HT2 cells*, [^{3}H]thymidine incorporation in the presence of		
T-cell clone	Stimulus	No antibody	Anti-BSF-1	Anti-IL-2R
D10.G4	Con A	55,691	942	43,550
D10.G4	3D3	26,649	2,269	20,960
D10.G4	Spleen + conalbumin	28,326	1,447	21,491
D10.G4	TNP-B + TNP-conalbumin	30,868	746	23,489
CDC 25	Con A	65,610	3,003	43,175
CDC 25	Spleen + RGG	26,426	2,282	21,689
CDC 25	TNP-B + RAMG	50,984	4,227	37,327
10G11	Con A	159,042	164,375	6,448

Results are expressed as mean cpm of duplicate cultures. Standard deviations were <10% of the mean. [^{3}H]Thymidine incorporation into HT2 cells obtained with supernatants of unstimulated T cells or T cells cultured with APC alone was <3000 cpm. Each T-cell supernatant has been tested for HT2 stimulating activity in three to five experiments; representative data are shown.

*Supernatants from 18-hr stimulated T-cell clones were assayed at 50% vol/vol in 0.2-ml microcultures containing 10^4 HT2 cells in the presence or absence of anti-BSF-1 antibody (11B11, 1:1000 dilution of ascites) or anti-IL-2R antibody (7D4 + PC61, 1:100 dilution).

†Supernatants were prepared by stimulating 2×10^4 cloned T cells with Con A (2 μg/ml), 3D3 antireceptor antibody (1:1000 dilution of ascites); 5×10^5 spleen cells + 100 μg/ml of conalbumin or RGG, or 5×10^4 TNP-B cells + TNP-conalbumin (10 μg/ml) or RAMG (0.1 μg/ml), as shown and detailed in *Materials and Methods*.

Thus, differential inhibition by these monoclonal antibodies provides a reliable means of distinguishing two lymphokines, BSF-1 and IL-2, both of which stimulate T-cell proliferation.

Using this approach, we sought to define the type of lymphokine secreted by D10.G4 cells in response to Con A, the anti-receptor antibody, 3D3, or antigen and AKR(H-2^k) APC. As shown in Table 1, all of these stimuli induce the secretion of BSF-1 but not IL-2 from D10.G4 cells. [Anti-IL-2R antibodies at the dilution used in these assays (1:100) frequently cause ≈20% inhibition of T-cell proliferation, even in response to purified BSF-1; this is presumably a nonspecific effect due to antibody binding to responding cells.] Another T-cell clone we have analyzed in detail is CDC 25. When stimulated with Con A or antigen and C3D2F1APC, CDC 25, like D10.G4, secretes BSF-1 and not IL-2 (Table 1). Moreover, the addition of IL-1 does not alter the pattern or quantity of lymphokine secretion by these clones (data not shown). In a survey of 17 additional cloned lines of different antigen specificities and Ia restrictions, we have identified 12 that secrete only IL-2 and 5 that secrete only BSF-1 in response to stimulation by Con A or antigen and APC. Results with a prototypical IL-2-producing clone, 10G11, are shown in Table 1.

These results raised the unexpected possibility that the proliferation of T-cell clones such as D10.G4 and CDC 25 is mediated by BSF-1 and not IL-2. To test this hypothesis, we assessed the ability of antibodies to BSF-1 or IL-2R to block the proliferative responses of these clones to Con A, antireceptor antibody, or antigen and APC, in the presence of exogenous IL-1 (since IL-1 is necessary for maximal proliferation) (13, 19). As shown in Table 2, the responses to lectin or receptor-mediated stimulation are markedly inhibited by anti-BSF-1 but are not significantly affected by anti-IL-2R antibodies. Several important controls are also illustrated in Table 2. D10.G4 cells proliferate in response to IL-2 or to BSF-1 and IL-1 (but not BSF-1 alone) and the antibodies show a completely specific pattern of inhibition, as demonstrated with HT2 cells (Fig. 1). Moreover, in the absence of IL-1, the D10.G4 cells respond weakly or not at all to any stimuli except IL-2, confirming earlier observations (13, 22). Thus, these results establish that the proliferative responses of two T-cell clones to lectins or to signals delivered via antigen receptors are mediated largely or solely by BSF-1, even though these clones are capable of responding to exogenous IL-2. Finally, the proliferative response of the prototypical IL-2-secreting clone, 10G11, to antigen and APC is selectively inhibited by anti-IL-2R but not by anti-BSF-1 antibodies (Table 2). This confirms the specificity of the

Table 2. Inhibition of D10.G4 and CDC 25 T-cell proliferation with anti-BSF-1 antibody

			[^{3}H]Thymidine incorporation in the presence of				
				Anti-BSF-1		Anti-IL-2R	
T-cell clone	APC/factor	Antigen/mitogen	No antibody	1:250	1:1000	1:100	1:500
D10.G4	IL-1	Con A (2 μg/ml)	37,630	3,169	3,301	33,463	30,727
D10.G4	IL-1	3D3 (1:5000)	52,966	ND	4,070	58,474*	ND
D10.G4	TNP-B + IL-1	TNP-conalbumin (10 μg/ml)	63,298	8,526	12,100	64,247	57,079
D10.G4	Spleen	Conalbumin (100 μg/ml)	107,438	12,658	17,496	85,051	88,779
D10.G4	—	IL-2	25,285	34,297	32,079	5,521	ND
D10.G4	—	BSF-1	4,784	ND	ND	ND	ND
D10.G4	IL-1	BSF-1	25,049	4,382	1,108	18,681	ND
CDC 25	TNP-B + IL-1	RAMG (0.1 μg/ml)	74,995	8,184	13,313	64,232	79,240
10G11	Spleen	Ovalbumin (100 μg/ml)	34,064	ND	29,334	1,522	ND

Cultures containing 2×10^4 cloned T cells were incubated with the indicated stimulants, with and without antibodies, for 48 or 60 hr, and pulsed with 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) per well for the final 6–8 hr. Purified human IL-1 was used at 1 unit/ml, recombinant murine IL-2 was used at 100 units/ml and HPLC-purified BSF-1 was used at 30 units/ml. Results are expressed as mean cpm of duplicate determinations. Standard deviations were <10% of the mean. [^{3}H]Thymidine incorporation of T cells incubated with antigen or APC alone was <3000 cpm. ND, not done.

*Anti-IL-2R was used at 1:25 dilution in this experiment.

antibody-blocking experiments and further demonstrates that the lymphokine detected in the Con A supernatants of these three T-cell clones is the primary autocrine growth factor involved in the physiologic response of each clone to antigen. The same result has been observed with six other T-cell clones that have been analyzed similarly—i.e., the proliferative response of each clone to antigen and APC is dependent on the lymphokine (IL-2 or BSF-1) that it secretes following receptor-mediated stimulation (data not shown).

DISCUSSION

The data presented in this report establish the role of BSF-1 as an autocrine factor for the activation of a subset of helper/inducer T-lymphocyte clones. Of the panel of clones specific for different protein antigens and I-A/I-E determinants that we have examined, about one-third secrete BSF-1 and the remainder secrete IL-2 after lectin or antigen activation. Other investigators have reported similar data, in that about one-half of helper/inducer lines secrete IL-2 (and γ-interferon), and the remainder secrete a lymphokine now identified as BSF-1 (22). To date, we have detected no phenotypic differences between these T-cell subsets—all are specific for antigen and Ia, and all are L3T4$^+$ Lyt2$^-$. The factors, such as culture conditions or cloning techniques, that promote the growth of one or the other subset and their relative proportions in normal T-cell populations have not been determined.

The most significant finding from our experiments is that the lymphokine secreted by a particular T cell—i.e., IL-2 or BSF-1—is also the lymphokine that mediates the proliferative response of that T cell to antigen receptor-specific signals (Tables 1 and 2). The recognition of BSF-1 as an autocrine T-cell growth factor may resolve unexplained discrepancies between T-cell activation and IL-2R expression (4) and help to explain proliferative responses to T cells that are clearly independent of IL-2 (5). Recently, the gene for the human BSF-1 equivalent has been cloned and the recombinant protein was shown to have biologic effects similar to murine BSF-1 (J. de Vries, personal communication). Whether human helper/inducer T-cell subsets will show similar IL-2- and BSF-1-mediated autocrine pathways is unknown. It should be pointed out that since BSF-1-producing T cells (such as D10.G4 and CDC25) do express receptors for IL-1, they are capable of responding to IL-2 secreted by other cells in their microenvironment. Whether all T cells exhibit autocrine as well as "bystander" responses is unknown.

Our results also raise questions about functional differences between these two subsets of T cells. It is tempting to speculate that the BSF-1-producing and responsive T cells are particularly efficient at helping B lymphocytes, whereas inducer T cells that secrete IL-1 (and γ-interferon) play a role in macrophage activation and cell-mediated immune responses. There is, however, no direct evidence to support such a hypothesis. Finally, our data suggest that the role of IL-1 in T-cell activation should be reevaluated, since the ability of IL-1 to stimulate IL-2 secretion (23) or IL-2 receptor expression (2) may be of no consequence to BSF-1-producing and responsive T cells. Moreover, although IL-2 alone is capable of stimulating IL-1-dependent T cells such as D10.G4, the response to BSF-1 (like the response to lectin, antigen, or antireceptor antibody) requires the presence of exogenous IL-1 (Table 2). This supports the view that receptor-specific stimulation is mediated by BSF-1 in these T cells, and it also suggests that BSF-1 and IL-1 may provide two independent signals that are necessary for commitment to DNA synthesis and proliferation. More precise quantitative assays for BSF-1 secretion, receptor expression, and intracellular biochemical alterations in the presence and absence of IL-1 will undoubtedly resolve such questions.

These studies were made possible by generous gifts of HPLC-purified BSF-1 and the 11B11 antibody (Drs. J. Ohara and W. Paul, National Institutes of Health), D10.G4 cells and 3D3 antibody (Dr. C. Janeway, Yale University Medical School), and CDC 25 cells (Dr. David Parker, University of Massachusetts Medical School). We also thank Douglas Liano for outstanding technical help, and Nancy Babine and Robin Lee for excellent secretarial assistance. This work was supported in part by National Institutes of Health Grants AI22560 and AI22802. A.H.L. is the recipient of Public Health Service Training Grant HL07060.

1. Smith, K. A. (1984) *Annu. Rev. Immunol.* **2,** 319–333.
2. Kaye, J., Gillis, S., Mizel, S. B., Shevach, E. M., Malek, T. R., Dinarello, C. A., Lachman, L. B. & Janeway, C. A. (1984) *J. Immunol.* **133,** 1339–1345.
3. Smith, K. A. & Cantrell, D. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 864–868.
4. Malek, T. R., Ortego, G., Jakway, J. P., Chan, C. & Shevach, E. M. (1984) *J. Immunol.* **133,** 1976–1982.
5. Moldwin, R. L., Lancki, D. W., Herold, K. C. & Fitch, F. W. (1986) *J. Exp. Med.* **163,** 1566–1582.
6. Howard, M., Farrar, J., Hilfiker, B., Johnson, K., Takatsu, K., Hamaoka, T. & Paul, W. E. (1982) *J. Exp. Med.* **155,** 914–923.
7. Mosmann, T. R., Bond, M. W., Coffman, R. L., Ohara, J. & Paul, W. E. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5654–5658.
8. Vitetta, E. S., Ohara, J., Myers, C. D., Layton, J. E., Krammer, P. H. & Paul, W. E. (1985) *J. Exp. Med.* **162,** 1726–1731.
9. Coffman, R. L., Ohara, J., Bond, M. W., Carty, J., Zlotnik, A. & Paul, W. E. (1986) *J. Immunol.* **136,** 4538–4541.
10. Noma, Y., Sideras, P., Naito, T., Lindquist, S., Azuma, C., Severinson, E., Tanabe, T., Kinashi, T., Matsuda, F., Yaoita, Y. & Honjo, T. (1986) *Nature (London)* **319,** 640–646.
11. Lee, F., Yokota, T., Otsuka, T., Meyerson, P., Villaret, D., Coffman, R., Mosmann, T., Rennick, D., Roehm, N., Smith, C., Zlotnik, A. & Arai, K.-i. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2061–2065.
12. Watson, J. (1979) *J. Exp. Med.* **150,** 1510–1519.
13. Kaye, J., Porcelli, S., Tite, J., Jones, B. & Janeway, C. A. (1983) *J. Exp. Med.* **158,** 836–856.
14. Tony, H. P., Phillips, N. E. & Parker, D. C. (1985) *J. Exp. Med.* **162,** 1695–1708.
15. Ohara, J., Lahet, S., Inman, J. & Paul, W. E. (1985) *J. Immunol.* **162,** 2518–2523.
16. Ohara, J. & Paul, W. E. (1985) *Nature (London)* **315,** 333–336.
17. Malek, T. R., Robb, R. J. & Shevach, E. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5649–5653.
18. Ceredig, R., Lowenthal, J. W., Nabholz, M. & MacDonald, H. R. (1985) *Nature (London)* **314,** 98–100.
19. Rock, K. L., Haber, S., Liano, D., Benacerraf, B. & Abbas, A. K. (1986) *Eur. J. Immunol.*, in press.
20. Rock, K. L., Benacerraf, B. & Abbas, A. K. (1984) *J. Exp. Med.* **160,** 1102–1113.
21. Abbas, A. K., Haber, S. & Rock, K. L. (1985) *J. Immunol.* **135,** 1661–1667.
22. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. (1986) *J. Immunol.* **136,** 2346–2357.
23. Durum, S. K., Schmidt, J. A. & Oppenheim, J. J. (1985) *Annu. Rev. Immunol.* **3,** 263–288.