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Role of interleukin 1 in the activation of T lymphocytes

(lymphokines)

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ABSTRACT **The activation of T lymphocytes requires their stimulation via clonotypic antigen receptors as well as nonantigen-specific costimulators, the best defined of which is the cytokine interleukin 1 (IL-1). Recent studies have shown that murine CD4$^+$ helper T lymphocytes consist of two nonoverlapping subsets that selectively utilize interleukin 2 (IL-2) or interleukin 4 as their autocrine growth factors and are called Th1 and Th2 cells, respectively. We now show that IL-1 functions as a costimulator for the proliferation of Th2 but not of Th1 clones and only Th2 cells express high-affinity receptors for IL-1. Secretion of autocrine growth-promoting lymphokines by Th1 and Th2 cells occurs after stimulation via the antigen receptor–CD3 complex and is neither dependent on nor affected by IL-1. These findings suggest that the activation of T lymphocytes can be divided into two stages, lymphokine secretion and proliferation, and only proliferation requires costimulators such as IL-1. Moreover, the prevailing view that IL-1 functions as a costimulator by inducing secretion of IL-2 or expression of IL-2 receptors may not be generally applicable, because IL-2-producing Th1 clones do not express receptors for IL-1 and are insensitive to this cytokine.**

Helper/inducer thymus-derived (T) lymphocytes, which are characterized by the expression of the CD4 marker, are pivotal in inducing and regulating both humoral and cell-mediated immune responses. T cells recognize and respond to antigens presented by specialized antigen-presenting cells (APC). Activation causes lymphokines to be liberated and the cells to proliferate, usually as a result of autocrine stimulation. The secretory and proliferative responses of helper T lymphocytes require two signals—one is provided by interaction of foreign antigen bound to histocompatibility gene products with the antigen-specific clonotypic receptors on T cells and the second signal is provided by costimulators. The best-defined costimulator for CD4$^+$ T lymphocytes is the cytokine interleukin 1 (IL-1). IL-1 is a 17-kDa protein now known to be produced in secreted and/or membrane-associated forms by a variety of cell types, including macrophages and B lymphocytes, which can also function as APC for T-lymphocyte stimulation (1, 2). IL-1 is a mediator of the acute inflammatory response and has been shown to influence growth and differentiation of immunocompetent lymphocytes. Its role as a costimulator for T cells is attributed to two complementary effects. (*i*) IL-1 can enhance transcription and secretion of the autocrine T-cell growth factor interleukin 2 (IL-2) (3–6). (*ii*) IL-1 stimulates the expression of membrane receptors for IL-2 (7, 8). Thus, the combination of enhanced secretion of growth factor(s) and receptor expression can account for the T-cell stimulating function of IL-1.

Recent studies with cloned lines have shown that murine CD4$^+$ T lymphocytes can be divided into two nonoverlapping subsets based on their profiles of lymphokine secretion and autocrine growth factor use. One subset, Th1, secretes IL-2 and interferon γ and uses IL-2 as its growth factor, whereas the other, Th2, secretes interleukins 4 (IL-4) and 5 and uses IL-4 as its autocrine growth factor (9–12). Cloned lines of Th1 and Th2 cells differ in their proliferative responses to cytokines (13) and in their ability to stimulate the growth and differentiation of resting B lymphocytes (14, 15).

In this study, we analyzed the role of IL-1 as a costimulator for murine Th1 and Th2 clones. Our results show that only Th2 cells express high-affinity receptors for IL-1 and require this cytokine for their proliferation. Moreover, stimulation via antigen receptors is sufficient to induce lymphokine secretion by T cells, and IL-1 has no influence on this response. These findings indicate that the events leading to lymphokine secretion and cellular proliferation differ in their requirements for costimulators and that conventional views of the effects of IL-1 on the IL-2-mediated autocrine pathway may not be generally applicable.

MATERIALS AND METHODS

Cytokines and Antibodies. Recombinant human IL-1α (Leu^{119}–Ser^{271}) (rhIL-1α) was purified to homogeneity from *Escherichia coli* as described (16) and exhibited half-maximal stimulation in the standard murine thymocyte proliferation assay at 25 pM (10^7 half-maximal units per mg for assays conducted in 200 × 10^{-6} liter). rhIL1-α was labeled with $Na^{125}I$ to a specific radioactivity of 1600–2700 Ci/mmol (1 Ci = 37 GBq) using chloramine T and purified by HPLC size-exclusion chromatography. The radioligand preparations were >98% precipitable with cold 10% trichloroacetic acid; >70% of the radioligand could specifically bind to IL-1 receptor-bearing cells (e.g., MRC-5 human lung fibroblasts). A single 17.5-kDa-labeled species was seen on SDS/PAGE. Recombinant murine IL-4 was produced in yeast and provided by S. Gillis (Immunex Corp., Seattle). Recombinant human IL-2 was a gift from Biogen (Cambridge, MA). Monoclonal antibody specific for murine CD3 (hybridoma 145.2C11) was provided by J. Bluestone (University of Chicago). The antibody was purified from hybridoma culture supernatant by affinity chromatography on Protein A-Sepharose and used at 50 μg/ml to coat microculture wells overnight at 4°C.

T-Lymphocyte Clones. The established cloned lines we used have been described (13). The IL-2-producing Th1 clones were D1.1 and D1.6 (specific for rabbit IgG plus I-A^d), B8 and G11 (specific for purified protein derivative of *Mycobacterium tuberculosis* plus I-A^b), and 03 (specific for ovalbumin plus I-A^d). IL-4-producing Th2 clones were as follows: D10.G4 (conalbumin plus I-A^k), CDC25 (rabbit IgG plus I-A$^{(kxd)F_1}$) and C4 (hen egg lysozyme plus I-A^k) obtained from C. Weaver and E. Unanue (Washington University

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Abbreviations: APC, antigen-presenting cells; IL-1, IL-2, and IL-4, interleukin 1, 2, and 4, respectively; rhIL-1α, recombinant human interleukin 1α.

School of Medicine, Saint Louis). All clones were maintained by biweekly stimulation with antigen and 1500-roentgen (1 R = 0.258 mC/kg)-irradiated splenocytes as APC, as described (13).

Activation of T Cells: Lymphokine Secretion, Proliferation. Viable cloned T cells were purified by centrifugation over Ficoll-Diatrizoate (Organon Teknika). Approximately 2×10^4 cells were cultured in duplicate or triplicate in 0.2 ml of RPMI 1640 supplemented with penicillin, streptomycin, 2 mM L-glutamine, nonessential amino acids, 10% heat-inactivated fetal calf serum and 5×10^{-5} M 2-mercaptoethanol in flat-bottom 96-well microtiter plates. The cells were stimulated with immobilized (plate-bound) anti-CD3 antibody or with a range of concentrations of recombinant IL-2 or IL-4 in the presence and absence of IL-1α. Cultures were incubated at 37°C in humidified 5% CO_2. For measuring lymphokine secretion, cells were stimulated for 16–18 hr; 50-μl aliquots of supernatants were removed and assayed for IL-2 or IL-4 by their ability to stimulate DNA synthesis in the indicator line HT2 as described (10, 13). For measuring proliferation of T cells, cultures were incubated for 2 days, pulsed for the final 6–8 hr with 1 μCi of [^{3}H]thymidine per well, harvested in an automated sample harvester, and then the incorporated radioactivity was assayed by scintillation counting (13). In all experiments, controls included T cells cultured without any stimulus.

IL-1-Binding Experiments. Competitive binding experiments were performed by a modification of the method of Chin *et al.* (17). IL-1 was radioiodinated as described above. Purified, viable cloned T cells were washed in phosphate-buffered saline and resuspended at 1×10^7 cells per ml in binding buffer (phosphate-buffered saline, pH 7.4, with 0.05% gelatin and 0.1% sodium azide) containing 10 pM ^{125}I-labeled IL-1α and varying concentrations of unlabeled IL-1α. The cell suspensions were incubated in 1.5-ml tubes at room temperature for 2 hr with constant shaking and were then centrifuged at 10,000 *g* for 30 sec over a cushion of *N*-butylpthalate/corn oil (10:3 vol/vol). The tubes were frozen on dry ice, and the tips containing the cell pellets were severed and analyzed for γ-radioactivity. Data analysis, including Scatchard-curve fitting, was performed using the EBDA and LIGAND programs (Elsevier–BIOSOFT, Cambridge, U.K.).

RNA Analysis. For analysis of IL-4 gene transcription, viable cloned T cells were purified 7–10 days after antigen restimulation, washed, and cultured at 2×10^6 cells per ml in tissue culture medium without added stimulatory factors for 8 hr and then cultured in the presence of medium alone, IL-1α (2 pM), or concanavalin A (Con A, 2 μg/ml) for 4 hr. Total cellular RNA was then isolated by a guanidinium thiocyanate/phenol/chloroform extraction method (18). For Northern (RNA) blot analysis, 10 μg of RNA per lane was electrophoresed on formaldehyde-agarose gels and transferred to Nytran filters (Schleicher & Schuell). Filters were baked at 80°C for 2 hr and then hybridized with a murine IL-4 cDNA probe radiolabeled with [^{32}P]dCTP by a random-primer-extension method. The IL-4 probe [a 400 base-pair (bp) *Eco*RI/*Hin*dIII fragment from pCB1-6) was obtained from W. Paul (National Institutes of Health). The highest stringency washes of the blots were 0.2× SSC at 50°C. Autoradiography was carried out at −80°C for 18 hr with enhancer screens.

RESULTS

Role of IL-1 in Proliferation of Cloned T Cells. To assess the function of IL-1 as a costimulator in T-cell activation, clones representing Th1 and Th2 cells were stimulated with immobilized anti-CD3 antibody, which mimics stimulation via antigen receptors, with and without graded concentrations of recombinant human IL-1α. The proliferation of two representative Th2 clones in response to anti-CD3 was absolutely dependent on or significantly enhanced by IL-1, with maximal effects being seen at 0.1–0.5 pM (Fig. 1). Similar results were seen with three other Th2 clones (data not shown). In contrast, five of five Th1 clones tested showed no effect of IL-1 even to 10 pM (Fig. 2). It is noteworthy that different Th1 clones vary markedly in the peak response observed with anti-CD3. Some, such as clones D1.1 and D1.6, responded relatively weakly, and in parallel experiments the same lines showed 5- to 10-fold greater DNA synthesis when stimulated with antigen and APC (data not shown). This suggests a requirement for costimulator(s) that may be provided by the APC. Our experiments, however, demonstrate that IL-1 cannot function as a costimulator for IL-2-producing (Th1) cells.

This differential effect of IL-1 was also seen when T cells were stimulated with exogenous lymphokines instead of anti-CD3. Thus, Th2 clones cultured with their physiologic growth factor, IL-4, proliferated maximally only when IL-1 was also added (Fig. 3*A*). In contrast, DNA synthesis by Th1 clones in response to their growth factor, IL-2, was independent of an unaffected by IL-1 (Fig. 3*B*). Similar findings using a limited range of concentrations of IL-1β have been reported (13).

Effects of IL-1 on Lymphokine Production by T Cells. The requirement for IL-1 in the proliferation of T-cell clones may be due to IL-1 enhancement of gene transcription and secretion of growth-promoting lymphokines in response to receptor-mediated stimulation. To test this hypothesis,

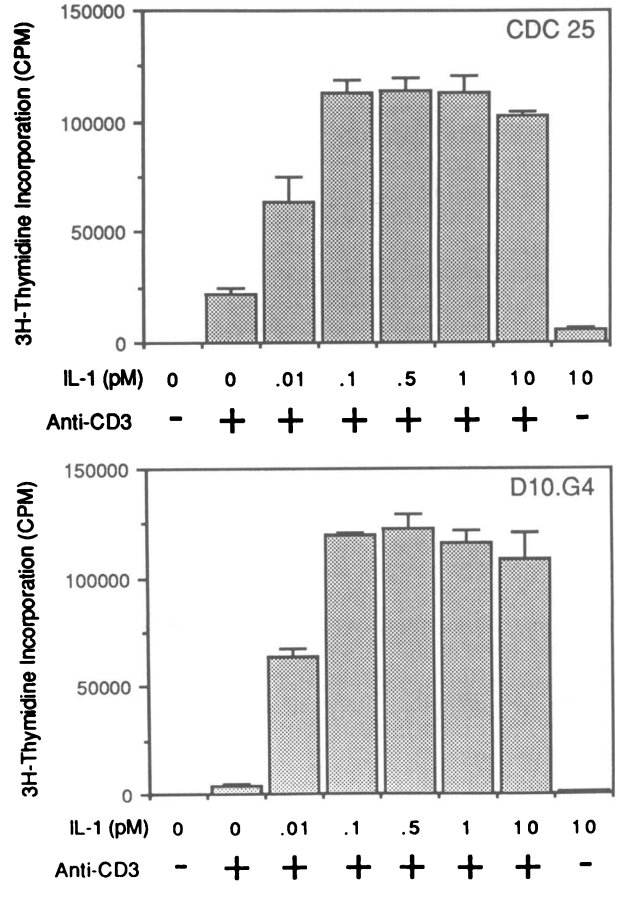

FIG. 1. Effects of IL-1 on the proliferative response of Th2 clones to anti-CD3 antibody. Approximately 2×10^4 viable Th2 cells were cultured for 48 hr in untreated microwells or microwells coated with anti-CD3 antibody (50 μg/ml) plus various concentrations of soluble IL-1α. DNA synthesis was measured in cultures pulsed for 6 hr with [^{3}H]thymidine.

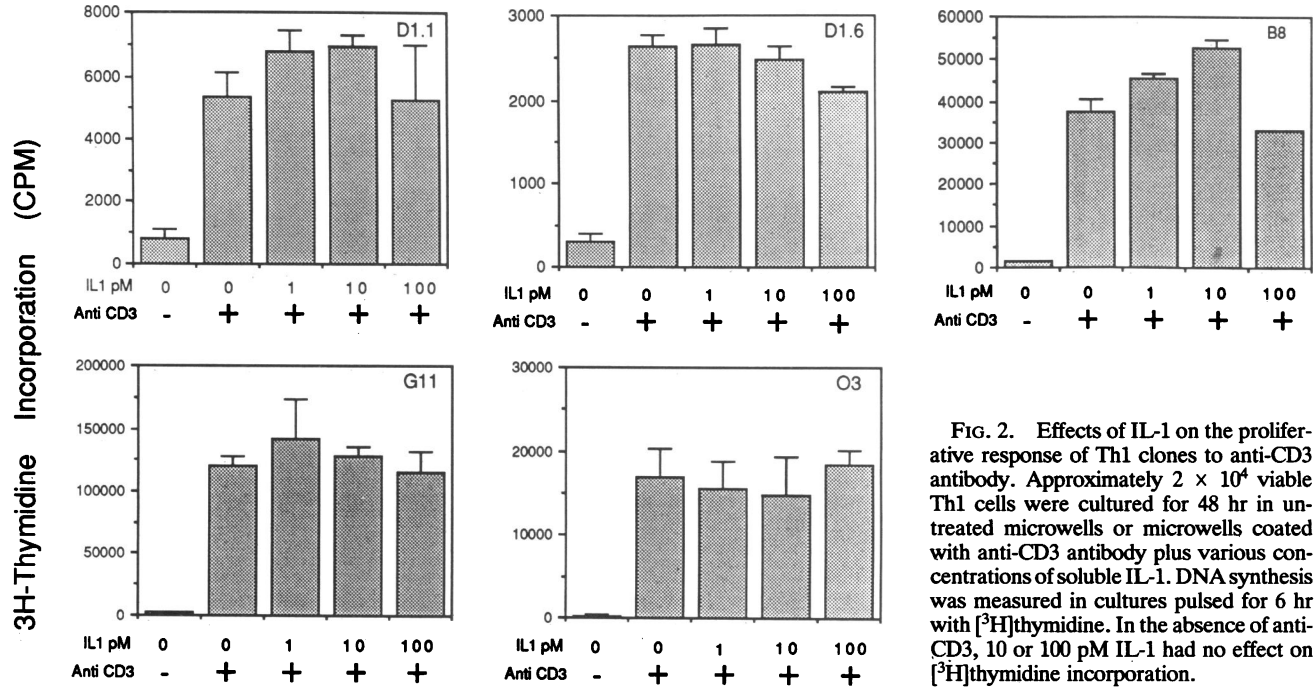

FIG. 2. Effects of IL-1 on the proliferative response of Th1 clones to anti-CD3 antibody. Approximately 2 × 10^4 viable Th1 cells were cultured for 48 hr in untreated microwells or microwells coated with anti-CD3 antibody plus various concentrations of soluble IL-1. DNA synthesis was measured in cultures pulsed for 6 hr with [^{3}H]thymidine. In the absence of anti-CD3, 10 or 100 pM IL-1 had no effect on [^{3}H]thymidine incorporation.

cloned T cells were cultured with anti-CD3 antibody with and without IL-1, and culture supernatants were assayed for the secretion of IL-2 (for Th1 cells) or IL-4 (for Th2 cells). As shown in Table 1, both sets of clones secreted their appropriate lymphokines in response to anti-CD3 antibody alone, and up to 10 pM IL-1α had no effect. The same result was seen with a range of suboptimal concentrations of anti-CD3—i.e., IL-1 did not enhance the secretion of IL-4 by cloned T cells (data not shown). Moreover, time-course studies showed that IL-1 did not alter the kinetics of IL-4 secretion in response to anti-CD3 (data not shown). Finally, the lack of effect of IL-1 on lymphokine production was also seen when lymphokine RNA levels were examined. Thus, Con A induced IL-4 RNA in two Th2 clones but IL-1 did not (Fig. 4).

IL-1 Receptors on Cloned T Cells. The differential sensitivity of Th1 and Th2 clones to IL-1 can be explained most simply by differences in the expression of functional high-affinity receptors for this cytokine. Cellular receptors for IL-1 were measured on three Th2 and five Th1 clones using ^{125}I-labeled IL-1α. The three Th2 clones (D10.G4, CDC25, C4) tested expressed saturable, specific receptors for IL-1. By Scatchard analysis of one to five independent binding experiments for each clone, the mean K_d on D10.G4 cells was 166 ± 114 pM, on CDC25 cells, 93 ± 69 pM, and on C4 cells, 220 pM, and receptor numbers were 4986 ± 362 per cell on D10.G4 clone, 1401 ± 282 on CDC25 clone, and 334 on C4 clone. Representative data for two of these clones are shown in Fig. 5. Moreover, only a single class of receptors was detected on these cells, although we cannot formally exclude the presence of higher affinity binding sites at densities in the range of 10–100 per cell. In contrast, none of five Th1 lines showed detectable saturable and specific binding of IL-1 (Fig. 6).

DISCUSSION

With antigen-specific cloned lines as models for analyzing the activation of T lymphocytes, we demonstrated that IL-1 acts together with receptor-mediated stimuli to induce maximal proliferative responses of IL-4-producing Th2 cells. Thus, IL-1 fulfills the definition of a costimulator for these lymphocytes. Essentially identical results are obtained when the receptor-mediated stimulus is provided by anti-CD3 antibody, lectin, anticlonotypic antibody (7, 10) or antigens

Table 1. Effects of IL-1 on T-cell growth factor secretion by Th1 and Th2 clones

	HT-2 cell proliferation in supernatants from treated T cells, cpm						
Clone	Control	αCD3	αCD3 IL-1 (0.1)	αCD3 IL-1 (1.0)	αCD3 IL-1 (10)	αCD3 IL-1 (100)	IL-1 (10)
Th2							
CDC25	1145	73,078	71,072	54,831	60,266	ND	998
D10	1301	64,602	57,809	59,932	56,408	ND	1136
Th1							
D1.1	992	103,958	ND	126,189	120,627	111,158	ND
D1.6	1052	100,902	ND	98,633	123,291	111,945	ND
G11	596	51,890	ND	53,297	45,836	49,593	ND
O3	1243	15,817	ND	15,902	18,216	17,785	ND
B8	1043	145,201	ND	145,511	159,011	128,581	ND

T cells from the indicated cloned lines were cultured exactly as described in Fig. 1, and 24-hr supernatants were tested for HT-2-stimulating activity as a measure of IL-2 or IL-4. HT-2 cells (10^4) were cultured in 0.1 ml of T-cell clone supernatant (50% vol/vol) for 18 hr, and incorporated cpm were measured after a 6-hr pulse with [^{3}H]thymidine. Data shown are means of triplicate cultures. Specific antibody blocking studies have established that the growth factors detected in this assay are IL-4 (for Th2 clones) or IL-2 (for Th1 clones). αCD3, anti-CD3. IL-1 concentrations (in parentheses) are in pM. ND, not done.

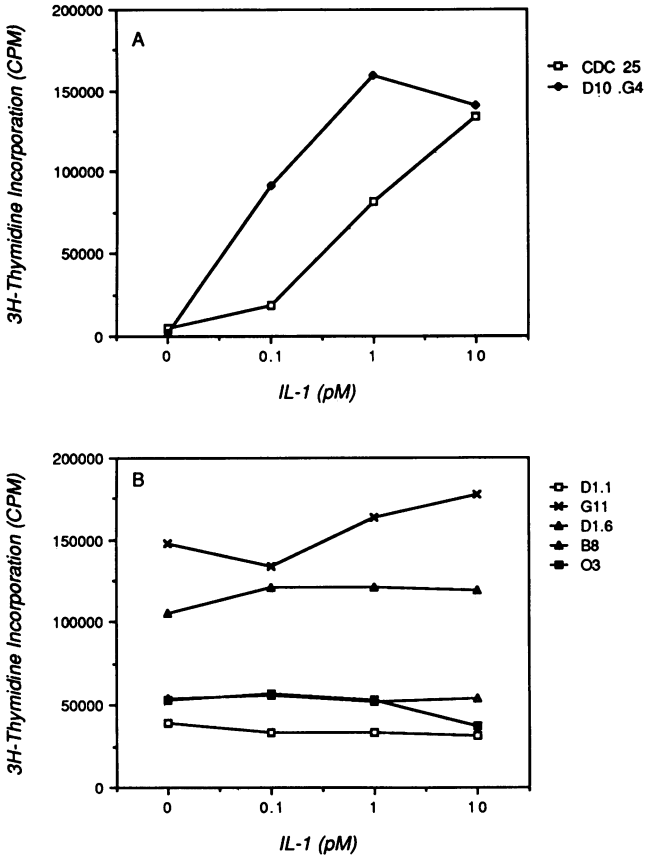

FIG. 3. Effects of IL-1 on the proliferative response of T-cell clones to exogenously added T-cell growth factors. Approximately 2 × 10^4 viable Th2 (*A*) or Th1 (*B*) cells were cultured for 48 hr without or with recombinant murine IL-4 at 50 units/ml (*A*) or recombinant human IL-2 (*B*) plus various concentrations of soluble IL-1α. Proliferation assays were done as described in Fig. 1. Without IL-4 or IL-2, 10 pM IL-1 had no effect on [^{3}H]thymidine incorporation.

presented by APC that produce little or no IL-1 themselves (ref. 19 and W. H. Boom, D. Liano, and A.K.A., unpublished work).

The most striking finding of these experiments is that IL-1 is a costimulator only for Th2 clones, since IL-2-producing

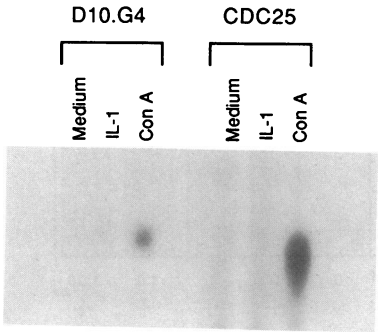

FIG. 4. IL-4 gene expression in Th2 cells. Total cellular RNA was isolated from D10.G4 and CDC25 cells after 4-hr culture with medium alone, 2 pM IL-1, or Con A at 2 μg/ml. Northern (RNA) blot analysis was then done with an IL-4-gene-specific cDNA probe.

Th1 lines do not respond to or depend on IL-1 for their activation. This difference is readily explained by our observation that only Th2 clones express high-affinity receptors for IL-1, agreeing with the results of Greenbaum *et al.* (12). These findings are surprising in view of the widely held idea that the function of IL-1 is to stimulate the production of IL-2 or the expression of IL-2 receptors. Much of the data supporting these concepts has come from analyses of tumor lines (3–6), which may represent an unusual situation. Moreover, the significance of IL-4 as the exclusive growth factor for a subset of CD4$^+$ T lymphocytes has been recognized only recently. Thus, for many years the proliferative response of the T-cell clone D10.G4 to Con A or antireceptor antibody has been known to depended on the simultaneous presence of IL-1, and the finding that IL-1 stimulated the expression of IL-2 receptors on this line provided a simple explanation for its costimulator function (7). It is now known, in fact, that the autocrine growth factor for D10.G4 is IL-4 and not IL-2 (10, 12), so that IL-2 receptors cannot play a role in the autocrine stimulation of this clone. In any event, our results demonstrating that only IL-4-producing and utilizing T-cell clones respond to IL-1 clearly challenge the conventional view of the role of this cytokine in T-lymphocyte activation.

Experiments aimed at analyzing the mechanisms of action of IL-1 have shown that it induces proliferation of Th2 clones

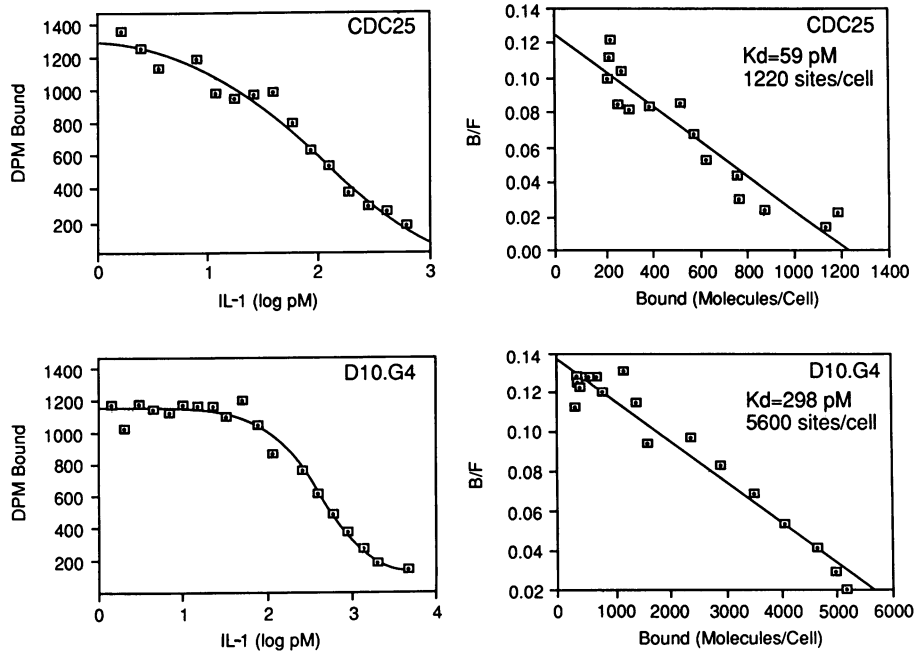

FIG. 5. Competitive binding of ^{125}I-labeled IL-1 to Th2 clones. Ten picomolar ^{125}I-labeled IL-1α was incubated with D10.G4 or CDC25 with the indicated concentrations of unlabeled IL-1, as described. Binding curves (left) and Scatchard plots (right) of the binding data are presented for each clone.

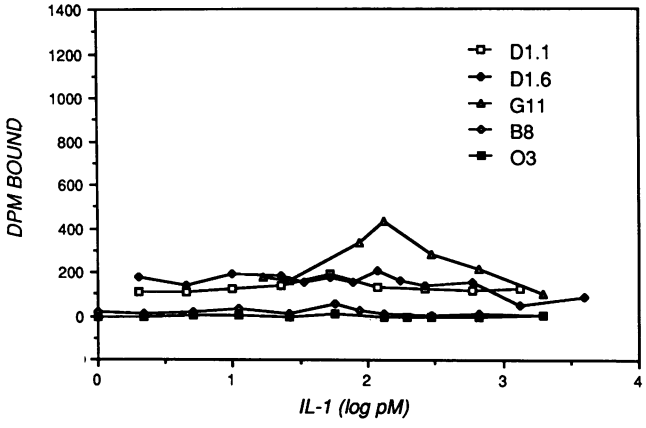

FIG. 6. Competitive binding of ^{125}I-labeled IL-1α to Th1 clones. Binding studies, as described for Fig. 5, were performed with the indicated Th1 clones. Total dpm bound are plotted as a function of the concentration of unlabeled IL-1. The data could not be analyzed by Scatchard plots because of the lack of saturable, specific binding.

(Figs. 1 and 3) but does not influence either production of IL-4 (Table 1) or the expression of receptors for IL-4 (13). The effect of IL-1 is, in fact, independent of IL-4 secretion, because it is necessary for the proliferation of Th2 cells even in response to exogenous lymphokine (Fig. 3). Furthermore, we show that IL-1 does not induce increases in IL-4 mRNA in two representative Th-2 clones, D10.G4 and CDC25 (Fig. 4). Thus for Th2 cells, the autocrine circuit is initiated by ligand binding to the antigen receptor–CD3 complex, which is both necessary and sufficient for secretion of IL-4. This lymphokine subsequently binds to its own receptors but is apparently not capable of inducing DNA synthesis by itself. IL-1 plays its role in commitment to DNA synthesis and cell growth; consistent with this is the finding that IL-1 alone induces transcription of c-*myc* in Th2 clones (ref. 20 and our unpublished work, A.H.L. and A.K.A.). For IL-2-producing Th1 cells, stimulation via the antigen receptor–CD3 complex is again sufficient to stimulate lymphokine secretion. It is likely that costimulator(s), which are as yet undefined, are also involved in optimal growth stimulation for Th1 cells, because several Th1 clones proliferate better in response to antigen and APC than to anti-CD3 antibody (data not shown). The nature of this costimulator is unknown, but our results clearly establish that is cannot be replaced by IL-1. Thus, the findings in this paper suggest that T-cell activation can be divided into two distinct components—lymphokine secretion, which follows antigen binding to specific receptors, and proliferation, which depends on the autocrine growth-promoting lymphokine and a costimulator, now known to be IL-1 only for the Th2 subset of CD4$^+$ T cells.

Finally, these results with T-cell clones have significant implications for current concepts of the activation of unselected T lymphocytes. Historically, the role of IL-1 as a costimulator for T cells was first seen in the proliferative response of thymocytes to phytohemagglutinin (21). With few exceptions, IL-1 has been shown not to have any effect on mature peripheral resting T lymphocytes (22, 23). This is consistent with recent findings that the majority of unimmunized peripheral T cells in the mouse (24) and human (R. Geha, personal communication) secrete IL-2 and IL-4-producing T cells represent only about 1 in 500 of the lymphocytes that express lymphokine RNA in response to polyclonal stimulation (25). Thus, one would predict that most resting peripheral T lymphocytes are the unstimulated analogs of Th1 clones. In agreement with this is our finding that murine lymph node CD4$^+$ lymphocytes and human peripheral blood T lymphocytes do not express high-affinity receptors for IL-1 (data not shown). Our results, however, do not exclude the possibility that, in contrast to cloned lines, resting T cells may require signals in addition to antigen receptor-mediated stimulation for both lymphokine production and proliferation. The requirements for the activation of unselected T lymphocytes at different stages of maturation and the roles of autocrine growth factors and costimulators in T-lymphocyte expansion during ontogeny or after immunization remain as important subjects for investigation.

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