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*Proceedings of the National Academy of Sciences of the United States of America*, Vol. 84, No. 21 (Nov. 1, 1987), 7629-7633.

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## B-cell-stimulatory factor 2 ( $\beta_2$ interferon) functions as a second signal for interleukin 2 production by mature murine T cells

(hybridoma growth factor/colony-stimulating factor/26-kDa protein/interleukin 6/anti-T-cell antigen receptor antibody)

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Communicated by Herman N. Eisen, July 6, 1987 (received for review May 21, 1987)

**ABSTRACT** Purified peripheral murine T cells, in the presence of concanavalin A, can be activated to produce interleukin 2 (IL-2) through stimulation either with a previously described murine lymphokine designated T cell-activating factor (TAF) or with a cloned human lymphokine that has been called  $\beta_2$  interferon, B-cell-stimulatory factor 2, hybridoma growth factor, inducible 26-kDa protein, or hematopoietic colony-stimulating factor 309 by different investigators. We and others propose the designation interleukin 6 (IL-6) for the latter molecule. Our experiments demonstrate that either murine TAF or human IL-6 can restore the ability of purified T cells to proliferate in response to Con A or antibodies against the T-cell antigen receptor. Most if not all of the proliferation can be blocked by antibodies against the  $\alpha$  chain of the IL-2 receptor. Furthermore, highly purified CD8<sup>-</sup> T cells can be activated by IL-6 in the presence of Con A to secrete IL-2. We propose that IL-6 and murine TAF are important "second signals" in primary antigen-receptor-dependent T-cell activation. Whether or not murine TAF is a homologue of human IL-6 remains to be determined.

The antigen-dependent pathway of T-cell proliferation appears to require engagement of the T-cell receptor complex by antigen associated with one of the major histocompatibility complex (MHC)-encoded glycoproteins (1) and antigen-nonspecific signals produced by specialized antigen-presenting cells (dendritic cells and macrophages; refs. 2 and 3). If both signals are provided, T cells synthesize and secrete soluble growth factors [interleukin 2 (IL-2) and interleukin 4 (IL-4)] that can act in an autocrine fashion to stimulate T-cell proliferation (4, 5).

Our recent efforts have focused on elucidation of the signal requirements for induction of IL-2 production by populations of purified, peripheral murine T lymphocytes from which antigen-presenting cells have been depleted (6, 7). The experimental system involves provision of the "first signal" with reagents such as Con A or multivalent derivatives of monoclonal antibodies that bind the antigen/major histocompatibility complex receptor on T cells (8, 9). Previous studies (10, 11) have shown that either of these reagents can stimulate purified T cells to respond to exogenous IL-2 but not to produce IL-2. Despite the well-entrenched notion that the cytokine interleukin-1 (IL-1) is a sufficient second signal for IL-2 production (12-14), a variety of recent reports have failed to demonstrate IL-2 production by purified T cells stimulated with IL-1/Con A (6, 7, 15-18).

In a previous communication, we described a murine T cell-activating factor (TAF) that stimulates the production of IL-2 (7). TAF was shown to be biochemically separable and biologically distinct from IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, CSF-1, and  $\gamma$  interferon (IFN- $\gamma$ ). We report here that a

recombinant human lymphokine, variously designated  $\beta_2$  interferon (IFN- $\beta_2$ ) (19), 26-kDa protein (20), B-cell-stimulatory factor 2 (BSF-2) (21), hybridoma growth factor (22), and colony-stimulating factor-309 (CSF-309),<sup>§</sup> will also indirectly support the proliferation of either Con A- or anti-T cell receptor antibody-activated T cells and that this effect is largely mediated by the stimulation of IL-2 production.

### MATERIALS AND METHODS

**Mice.** BALB/c AnN mice were bred and were maintained in the Center for Cancer Research Animal Facility (Massachusetts Institute of Technology).

**Cytokines.** Recombinant human IL-1 $\alpha$  and IL-1 $\beta$  were purchased from Genzyme (Boston). Recombinant human IL-2 was provided by Biogen Research (Cambridge, MA). Recombinant human IL-6 was prepared by transfecting monkey COS-1 cells with an IL-6 expression plasmid (pCSF-309) that was identified through functional mammalian cell expression cloning to be described elsewhere.<sup>§</sup> The conditioned medium from these transfections contained high levels of IL-6 activity (10,000 half-maximal units per ml, as measured in a murine bone-marrow colony-forming assay) and was used directly as the source of recombinant factor. Murine IL-4, immunoaffinity-purified from Con A-stimulated CDC25 cells, was provided by D. Parker (University of Massachusetts Medical School, Worcester). Conditioned medium was prepared from Ficoll/Hypaque-enriched human peripheral blood lymphocytes or from human T-cell leukemia type I-transformed human C10-MJ2 cells (23) stimulated for 24 hr in the presence of 10  $\mu$ g of phytohemagglutinin (PHA-P) per ml and 10 ng of phorbol 12-myristate 13-acetate per ml. Partially purified murine TAF was obtained by sequential chromatography of the conditioned medium from Con A-stimulated murine spleen cells as described (7).

**Cytokine Bioassays.** Culture medium used in all experiments was RPMI 1640 supplemented with 5% fetal calf serum, 50  $\mu$ M 2-mercaptoethanol, and antibiotics. IL-2 activity of samples was determined by using a CTLL-2-15H (24) indicator cell subline that responds to IL-2 and does not respond to IL-4 (R.D.G. and D.H.R., unpublished data). IL-2 activity is expressed in half-maximal units: one half-maximal unit is  $\approx$ 820 pg of IL-2. TAF activity was measured by using accessory cell-depleted murine lymph node T cells as detailed (7). In some experiments we used highly purified neuraminidase-treated helper phenotype CD8<sup>-</sup> T cells (>98% CD4<sup>+</sup>). Conditioned medium from the microcultures was removed 17

Abbreviations: IL-1, -2, -4, and -6, interleukins 1, 2, 4, and 6; IFN- $\beta_2$  and IFN- $\gamma$ ,  $\gamma$  and  $\beta_2$  interferons; TAF, T cell-activating factor; BSF, B-cell-stimulatory factor; CSF, hematopoietic colony-stimulating factor.

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§G. G. Wong, J. S. Witek-Giannotti, P. A. Temple, R. Kriz, C. Ferenz, R. M. Hewick, S.C.C., K. Ikebuchi, and M. Ogawa, unpublished data.

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hr after initiation of the cultures for the measurement of IL-2 produced by the cells. For assessment of the purity of T-cell populations, microcytotoxicity assays were performed as described (7).

**Monoclonal Antibodies.** Purified anti-IL-2 receptor rat IgG monoclonal antibodies 3C7 and 2E4 (25) were provided by A. Krusbeek (National Institutes of Health, Bethesda, MD). Purified F23.1 IgG2a mouse antibodies [anti-mouse T-cell receptor  $\beta$  chain variable (V) region segments 8.1, 8.2, and 8.3, ref. 26] were coupled to agarose beads as described (27) and referred to as F23.1 beads. Purified monoclonal rat IgG2a (187.1; anti-mouse  $\kappa$  light chain) and mouse IgG2a (COB6, anti-Con A) for use as class-matched controls were provided by T. Imanishi-Kari and F. Owen (Tufts Medical School, Boston).

**Reverse-Phase HPLC.** Reverse-phase HPLC was carried out as described (7) except that a 10 mm  $\times$  25 cm RPSC C<sub>3</sub> column (Beckman Instruments; Berkeley, CA) was used.

## RESULTS

**Identification of a Human TAF Activity.** We have described (6, 7) a system for the detection of soluble factors capable of activating purified murine lymph node T cells to produce IL-2 in the presence of Con A. Such T cells have been depleted of accessory cells by passage over nylon-wool columns and treatment with anti-Ia (I region-associated) monoclonal antibodies and complement, rendering them nonresponsive to Con A stimulation in the absence of other factors. Of the cytokines previously tested for the ability to support the proliferation of these purified T cells in the presence of Con A, only IL-2 and partially purified murine TAF were found to be effective; in the presence of Con A, murine TAF activated the T cells to produce IL-2 and to proliferate (7). More recently, we have determined that such T cells will also proliferate in response to exogenous IL-4 (see Fig. 2D; R.D.G. and D.H.R., unpublished results).

To identify human factors with TAF activity, we fractionated lectin-stimulated human peripheral blood lymphocyte-conditioned medium using reverse-phase HPLC. Two peaks that were eluted from the column with 50% (peak 1) and 59% (peak 2) acetonitrile were active in the TAF assay, whereas only the second peak contained high levels of IL-2 (Fig. 1A). A similar analysis of conditioned medium from activated human T-cell leukemia virus type I-transformed C10-MJ2 cells revealed a single non-IL-2 species that was active in the TAF assay and migrated similarly to peak 1 (Fig. 1C). A sample of murine TAF, run in a parallel experiment, was eluted from the HPLC column at a position identical to the non-IL-2 TAF-like species produced by both peripheral blood lymphocytes and C10-MJ2 cells (Fig. 1B).

To identify human cDNA clones encoding the human TAF-like activity, an expression library constructed from C10-MJ2 mRNA was screened by testing the supernatants from COS-1 cells transfected with pools of plasmid DNAs from this library. We observed that pools that scored positive in the TAF assay correlated well with pools previously shown to contain cDNAs encoding a hematopoietic colony-stimulating factor (CSF). One of these cDNAs designated CSF-309<sup>8</sup> (data not shown) proved to be identical to a cytokine with multiple biological activities known as BSF-2 (21) and IFN- $\beta_2$  (19). pCSF-309-transfected COS-1 cell supernatant was active in the murine TAF assay and displayed hydrophobic chromatographic properties identical to those of the TAF activity from C10-MJ2 cells and the TAF activity from Con A-stimulated murine splenocytes (Fig. 1D). Supernatants from COS-1 cells transfected with irrelevant cDNA clones had no activity in the assay (data not shown). From these results, we conclude that BSF-2/IFN- $\beta_2$  has TAF activity and is most likely the factor responsible for this

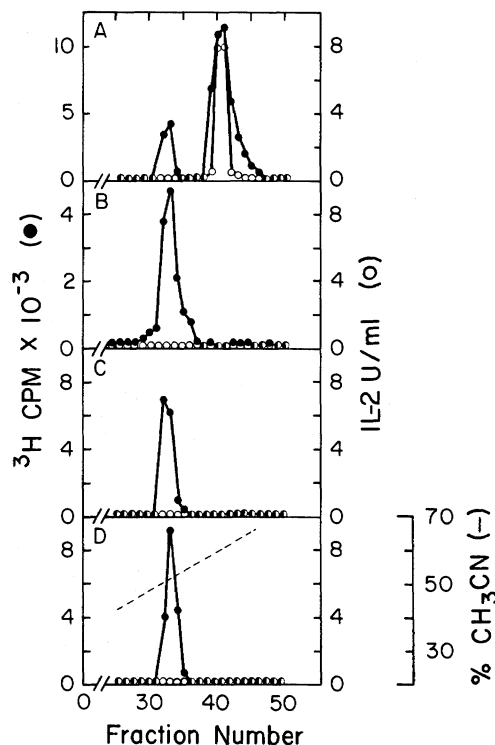


Fig. 1. Reverse-phase HPLC analysis of molecules active in the TAF assay. Conditioned medium (20 ml) from phytohemagglutinin- and phorbol 13-myristate 12-acetate-stimulated peripheral blood lymphocytes (A), partially-purified murine TAF (B), phytohemagglutinin-, and phorbol 13-myristate 12-acetate-stimulated C10-MJ2 cells (C), or 10  $\mu$ l of conditioned medium from COS-1 cells transfected with pCSF-309 (human IL-6) (D) were applied to a 10 mm  $\times$  25 cm C<sub>3</sub> reverse-phase HPLC column equilibrated with 0.1% CF<sub>3</sub>COOH, which was eluted with a linear gradient of CH<sub>3</sub>CN in 0.1% CF<sub>3</sub>COOH. Fractions (3 ml) were collected and dialyzed against culture medium, and fractions 25–50 were tested at 1:6 in the TAF assay (●) and in the IL-2 assay (○).

activity found in the C10-MJ2 cell-conditioned medium. To simplify the nomenclature and because BSF-2/IFN- $\beta_2$  is now known to display a wide range of biological effects with different target cells, we propose that this lymphokine be designated interleukin-6 (IL-6).

**IL-6-Induced T-Cell Proliferation Is Largely IL-2-Mediated.** To ascertain whether T-cell proliferation induced by IL-6 or TAF is mediated via stimulation of IL-2 production by T cells, we added anti-IL-2 receptor monoclonal antibodies to the cultures. These antibodies completely blocked the T-cell proliferation stimulated by IL-2 with Con A (Fig. 2A) and dramatically inhibited proliferation caused by IL-6 with Con A (Fig. 2B) or murine TAF with Con A (Fig. 2C). In control experiments, class-matched irrelevant antibodies added to the cultures did not inhibit proliferation of the T cells (Fig. 2A–D) and the anti-IL-2 receptor antibodies did not inhibit the IL-4-supported proliferation of purified Con A-activated T cells (Fig. 2D) or of an IL-4-dependent T-cell line (data not shown). These results suggest that most of the T-cell proliferation induced by IL-6 or murine TAF is mediated by IL-2.

As further confirmation of this conclusion, we tested the culture medium from purified Con A-activated T cells stimulated with recombinant IL-6 for IL-2 by using a subline of the IL-2-dependent cell line CTLL-2-15H, which does not respond to IL-4 (data not shown). IL-6 by itself did not support the proliferation of the CTLL-2-15H cells, but in the presence of Con A, IL-6 induced the dose-dependent release of IL-2 by the purified murine T cells (Table 1). Considering our previous finding that murine TAF induces the production

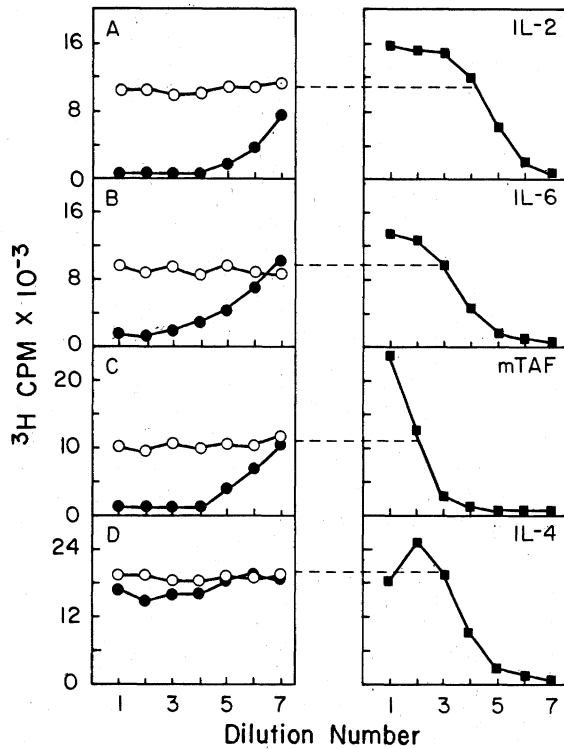


Fig. 2. Antibodies against the  $\alpha$  chain of the IL-2 receptor inhibit T-cell proliferation induced by Con A with IL-2, IL-6, or murine TAF. (Right) Recombinant human IL-2 (A), recombinant human IL-6 (B), partially-purified murine TAF (C), or purified murine IL-4 (D) were titrated in the TAF assay. Serial dilutions (1:2) of IL-2, IL-6, murine TAF (mTAF), and IL-4 were begun at 1:2, 1:200, 1:2, and 1:20 dilutions, respectively. (Left) The same preparations were added to the TAF assay at the levels indicated by the dashed lines in the presence of various dilutions of control rat IgG (o) or anti-IL-2 receptor rat IgG 3C7 and 2E4 (●). Serial dilutions (1:2) of the antibodies were begun at 100  $\mu$ g/ml.

of IL-2 by activated T cells (7), the present results support the conclusion that IL-6 and murine TAF support T-cell proliferation through a similar mechanism.

We have further characterized the response to IL-6 of CD8<sup>-</sup> T cells (98% CD4<sup>+</sup>), which were rigorously depleted of

Table 1. IL-6 induces Con A-activated purified T cells to secrete IL-2

Cells	Con A, 2 $\mu$ g/ml	Sample*	<sup>3</sup> H, cpm <sup>†</sup>	IL-2, <sup>‡</sup> unit(s)/ml
T cells <sup>§</sup>	-	Medium	186	0.00
	+	Medium	354	0.00
	+	1:600 IL-6	11,834	0.22
	+	1:1,800 IL-6	6,321	0.11
	+	1:5,400 IL-6	2,641	0.04
CD8 <sup>-</sup> T cells <sup>¶</sup>	+	1:16,200 IL-6	767	0.02
	+	Medium	9,497	0.05
	+	1:100 IL-6	61,184	2.18
	-	1:100 IL-6	-	0.00

\*At the dilutions shown, recombinant IL-6 (culture medium from COS-1 cells transfected with pCSF-309) was added.

<sup>†</sup>Tritiated thymidine incorporation of T cells during a 4-hr pulse at 40 hr of culture.

<sup>‡</sup>IL-2 measured in conditioned medium from T-cells cultured for 17 hr.

<sup>§</sup>Nylon wool-passaged and anti-Ia/complement-treated lymph node cells (2  $\times$  10<sup>5</sup>).

<sup>¶</sup>Twice nylon wool-passaged and twice anti-Ia/anti-CD8/complement-treated lymph node cells (2.5  $\times$  10<sup>5</sup>) treated with neuraminidase.

accessory cells by two rounds of nylon-wool column passage and by two rounds of anti-Ia and complement treatment. These cells were treated with neuraminidase before culture because untreated highly purified T cells do not respond to Con A and lymphokines such as IL-2 and murine TAF, apparently because of a deficiency of cells capable of presenting Con A to T cells; neuraminidase-treated T cells are rendered capable of presenting Con A to other T cells (10). The addition of IL-6 to the cultures resulted in a 40-fold increase in the level of IL-2 production by the helper T cells compared to the Con A-only controls, and the highly purified helper-phenotype T cells proliferated vigorously after activation with Con A and IL-6, largely retaining their helper phenotype (97% Thy-1<sup>+</sup>, 93% CD4<sup>+</sup>) (Table 1). From these results, we conclude that IL-6 in the presence of Con A stimulates CD8<sup>-</sup> T cells to release IL-2 that in turn supports the proliferation of the activated T cells.

**Proliferation of Purified T Cells Activated Through the T-Cell Receptor in Response to IL-2, IL-6, or Murine TAF.** Because Con A with various lymphokines could activate T cells through a pathway other than the T-cell antigen receptor (28-30), we tested the effects of IL-2, IL-6, or murine TAF on purified T cells activated by a monoclonal antibody (F23.1) that recognizes a variable region segment of the  $\beta$  chain of the T-cell antigen receptor (anti-V $\beta$ 8). IL-2, IL-6, and murine TAF all supported significant proliferation of the purified T cells in the presence of either Con A or F23.1 beads (Table 2). T cells cultured with medium with or without addition of control beads (an irrelevant, class-matched antibody bound to agarose beads) in the presence of IL-2

Table 2. Anti-T cell receptor-conjugated agarose serves as a first signal for the proliferation of purified T cells in the presence of lymphokines

Sample	Sample dilution	Medium alone	<sup>3</sup> H incorporation during culture,* cpm		
			Additions to medium		Con A at 2 $\mu$ g/ml
			Beads, 1% (vol/vol)		
IL-2 <sup>†</sup>	1:10	1397	46,450	1273	21,217
	1:30	1394	42,452	1191	20,522
	1:90	1039	26,193	880	15,017
	1:270	308	4,951	1135	5,610
	1:810	85	1,562	95	1,548
	1:2,430	57	1,447	71	580
IL-6 <sup>‡</sup>	1:100	71	19,435	110	10,021
	1:300	50	20,033	143	9,402
	1:900	71	15,036	43	10,421
	1:2,700	54	9,555	48	5,896
	1:8,100	48	4,964	63	3,029
TAF <sup>§</sup>	1:24,300	61	2,627	103	989
	1:2	143	34,107	59	16,488
	1:6	57	21,379	41	12,204
	1:18	43	3,969	44	4,400
	1:54	61	1,246	73	1,221
	1:162	113	1,137	47	446
1:486	83	700	48	360	
Medium	-	136	982	127	443

\*Tritiated thymidine incorporation of 2  $\times$  10<sup>5</sup> purified lymph node T cells during a 4-hr pulse at 40 hr of culture in medium alone or with addition of 1% control beads (an irrelevant antibody bound to agarose beads) or 1% F23.1 antibody-conjugated agarose beads or 2  $\mu$ g of Con A per ml.

<sup>†</sup>Recombinant human IL-2 at 100 units per ml.

<sup>‡</sup>Culture medium from COS-1 cells transfected with pCSF-309.

<sup>§</sup>Murine TAF partially purified by sequential gel filtration, DEAE-cellulose chromatography, phenyl-Sepharose chromatography, and reverse-phase HPLC.

displayed only a weak proliferative response. In the absence of Con A or F23.1 beads, neither IL-6 nor murine TAF elicited a detectable proliferative response, indicating that a first signal is required for the activation of purified resting T cells. Finally, we tested the activity of exogenous IL-1 on the proliferation of purified T cells. The results (Table 3) demonstrate that IL-1 failed to support significant proliferation of either the Con A- or F23.1-bead-activated T cells, confirming previous studies (7, 15, 16). Thus, IL-6 and murine TAF, unlike IL-1, appear to be potent second signals for the activation of resting T cells, resulting in IL-2 production when purified T cells are stimulated through the T-cell antigen receptor.

## DISCUSSION

In this report, we have demonstrated that human BSF-2/IFN- $\beta_2$  is capable of activating murine T cells in the presence of either Con A or antibody directed against the T-cell antigen receptor. These studies further extend the range of known activities of this molecule, which now includes enhancement of immunoglobulin production by B cells (21), support of the proliferation of certain murine hybridomas (22), induction of an antiviral state in fibroblasts (19), enhancement of the expression of class I HLA antigens on fibroblasts (31), and support of the growth of murine granulocyte/macrophage progenitors.<sup>§</sup> The broad range of

Table 3. IL-1 does not stimulate the proliferation of purified T cells in the presence of Con A or anti-T cell receptor-conjugated agarose beads

Sample	Sample dilution	<sup>3</sup> H incorporation during culture,* cpm	
		1% F23.1-agarose	Con A at 2 $\mu$ g/ml
IL-2 <sup>†</sup>	1:2	17,475	7,211
	1:4	17,066	5,722
	1:8	15,955	5,862
	1:16	10,247	4,912
	1:32	2,674	3,787
	1:64	927	1,830
TAF <sup>‡</sup>	1:2	6,504	13,421
	1:4	8,136	12,472
	1:8	7,763	10,595
	1:16	5,871	9,472
	1:32	3,048	5,771
	1:64	2,143	5,113
IL-1 $\alpha$ <sup>§</sup>	1:2	581	1,073
	1:4	515	1,000
	1:8	725	1,444
	1:16	744	904
	1:32	714	791
	1:64	729	594
IL-1 $\beta$ <sup>¶</sup>	1:2	39	52
	1:4	116	950
	1:8	539	770
	1:16	563	981
	1:32	539	605
	1:64	529	849
Medium	—	631	810

\*Tritiated thymidine incorporation of  $2 \times 10^5$  lymph node T cells during a 4-hr pulse at 40 hr of culture in medium (as in Table 2) containing 1% F23.1-conjugated agarose beads or Con A at 2  $\mu$ g/ml.

<sup>†</sup>Recombinant human IL-2 at 25 units per ml.

<sup>‡</sup>Murine TAF partially purified by sequential gel filtration, DEAE-cellulose chromatography, phenyl-Sepharose chromatography and reverse-phase HPLC.

<sup>§</sup>Recombinant human IL-1 $\alpha$  at 200 units per ml.

<sup>¶</sup>Recombinant human IL-1 $\beta$  at 200 units per ml.

biological activities of this lymphokine has led us and others (32) to propose that this molecule be designated IL-6.

We have reported that another molecule, murine TAF, functions in a fashion analogous to IL-6 (7). IL-6 and murine TAF do not appear to be proximal growth factors for T cells but rather to act indirectly by stimulating T cells that have been provided a "first signal" (Con A or anti-T-cell receptor antibodies) to produce IL-2. None of six other cytokines tested (IL-1, IL-2, IL-3, IL-4, IFN- $\gamma$ , and CSF-1) stimulate significant IL-2 production by our purified preparations of T cells (refs. 6 and 7; R.D.G. and D.H.R., unpublished data). Whether murine TAF is a homologue of human IL-6 or is another lymphokine with a similar activity remains to be determined.

We believe that both the responding cell and the proliferating cell in cultures of purified T cells stimulated with IL-6 are T cells because 97% of the cells were Thy-1.2<sup>+</sup> at the time of [<sup>3</sup>H]thymidine addition. Depletion of CD8<sup>+</sup> cells from the cultures did not diminish the proliferative response to IL-6 in the presence of Con A, suggesting that the remaining CD4<sup>+</sup> cells (93–98% of the cells in these cultures) are IL-6 responsive. Although the possibility that IL-6 acts indirectly by stimulating contaminating B cells or other accessory cells has not been ruled out, we have shown that highly purified populations of T cells (prepared by two cycles of depletion of B cells and accessory cells) proliferate in response to IL-6 in the presence of Con A. Experiments are in progress to more rigorously define the responding cell types.

The activation of resting T cells can be achieved using different ligands through several different pathways. For our initial studies, we used Con A, a lectin that binds to the antigen receptor on T cells (8) and also may bind to other T-cell surface structures (e.g., CD2, Thy-1, and Ly6/TAP) that may be involved in alternative pathways of T-cell activation (28–30). We have repeated our studies using bead-bound anti-T-cell receptor monoclonal antibody, a reagent that should selectively activate T cells through the antigen receptor. This stimulus proved to be very effective in supporting T-cell proliferation in the presence of IL-2, IL-6, or murine TAF. These results are consistent with our hypothesis that IL-6 and murine TAF act as second signals in the activation of resting T cells in the presence of a ligand for the T-cell antigen-receptor complex.

Although IL-1 has been reported to be a sufficient second signal for IL-2 production, we and others (7, 15–18) have found that exogenous IL-1 does not restore the response of purified murine T cells to Con A or to anti-T cell receptor antibodies. These results contrast with two recent reports showing that recombinant IL-1 restored the proliferative response of purified human T cells stimulated with immobilized antibodies directed against the T3/T-cell receptor complex (33, 34). However, others have recently reported that IL-1 by itself is incapable of restoring the proliferative response of purified T cells to Con A but instead enhances the ability of purified dendritic cells to restore the Con A responsiveness of the T cells (18). One attractive model to reconcile these differences is that IL-1 activates IL-6 production by small numbers of dendritic or other antigen-presenting cells in the cultures and that at least some of the effects of IL-1 on T cells are mediated by IL-6. While this model has yet to be tested, it is interesting that IL-1 is a potent natural inducer of IL-6 production by fibroblasts (20, 35).

While IL-1 in our hands does not appear to be a sufficient second signal for T-cell activation, we cannot exclude the possibility that it is a necessary signal because the purified T cells may have already been exposed to IL-1 *in vivo* or during their purification. Indeed, T cells express membrane receptors for IL-1 (36), and certain tumors of T-cell phenotype produce IL-2 in response to IL-1 (37)—observations consistent with an important role for IL-1 in the regulation of IL-2

production. The availability of neutralizing antibodies against the individual mediators should allow a better definition of the role played by each factor in regulating T-cell activation.

It has been reported that many different cells including mononuclear blood cells, fibroblasts, endothelial cells, T cells, cardiac myxoma cells, and bladder carcinoma cells produce IL-6 (reviewed in ref. 38); it is noteworthy that IL-6-producing cells (i.e., fibroblasts) are among those nonmacrophage cell types that stimulate primary mixed lymphocyte reactions of at least CD8<sup>+</sup> T cells (39). The production of IL-6 by fibroblasts and T cells is apparently regulated by other signals—IL-1 and tumor necrosis factor (TNF) in the case of fibroblasts (38) (Y. C. Yang, S. Tsai, G. G. Wong, and S.C.C., unpublished data) and mitogenic signals in the case of T cells (38). Our observation that purified T cells require exogenous IL-6 for IL-2 production suggests that IL-6 production by T cells is itself dependent on undefined "second signals" provided by other cells; because IL-1 does not restore the response, it apparently is not a sufficient second signal for either IL-6 production or IL-2 production. In any event, it is clear that IL-6 production by the activated T cell could both serve to recruit additional antigen-responsive T cells to amplify a particular immune response and to serve in an indirect autocrine fashion (that is, IL-2-mediated) to support the clonal expansion of the initial activated T cell.

**Note Added in Proof.** We (R.D.G., D.H.R., and M. Holsti) have found recently that highly purified T cells not treated with neuraminidase fail to respond to either IL-1 or IL-6 with F23.1 beads but do respond to a mixture of IL-1 and IL-6 with F23.1 beads.

We thank Drs. Ada Kruisbeek, David Parker, Thereza Imanishi-Kari, Frances Owen, and Mike Bevan for providing reagents crucial for the experiments outlined; G. Wong and J. Gianotti for providing us supernatants from the C10-MJ2 expression library and recombinant IL-6; Phil Doherty for excellent technical assistance; and Christopher Greco for help in preparing the manuscript. This work was supported by Grant AI21069 from the National Institutes of Health, by a Bristol-Myers Company Cancer Research Grant awarded to the Massachusetts Institute of Technology Center for Cancer Research, and by a Cancer Center Core Grant (CA-14051). R.D.G. was supported by Postdoctoral Fellowship DRG-734 from the Damon Runyon-Walter Winchell Cancer Fund and by an Immunology Training Grant from the National Institutes of Health.

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