Brief Definitive Report

B CELL STIMULATING FACTOR 2/INTERLEUKIN 6 IS A COSTIMULANT FOR HUMAN THYMOCYTES AND T LYMPHOCYTES

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Growth and differentiation of thymocytes and T lymphocytes is in part regulated by soluble factors that are released by lymphocytes themselves or by nonlymphoid cells in the thymus, lymph nodes, or at sites of inflammation (1, 2). B cell stimulating factor (BSF-2), which is now known to be identical to IFN-β2, hybridoma plasmacytoma growth factor (HPGF), or 26-kD protein and recently named IL-6, was initially thought to be a lymphokine of T cell origin whose major role was in the induction of differentiation of activated B lymphocytes into antibody-secreting cells (reviewed in references 3, 4). Recently, however, multiple nonlymphoid cells have been found to be capable of either producing this cytokine or of responding to it. Thus, monocytes (5), fibroblasts (6, 7), hepatocytes (Lotz, M., B. Zuraw, F. Jirik, and D. A. Carson, submitted for publication), cardiac myxoma (8), glial (9), and vascular endothelial cells (Jirik, F. R., T. J. Podor, T. Hirano, T. Kishimoto, D. J. Loskutoff, D. A. Carson, and M. Lotz, submitted for publication) all appear capable of IL-6 production. In addition to the effects on B lymphocytes, IL-6 has other important functions, such as the regulation of fibroblast proliferation (6, 7) and acute phase protein production by hepatocytes (9, 10, 11). This broad spectrum of IL-6 sources and actions has significantly altered the character of IL-6 from that of a T cell–derived lymphokine acting on B cells, to that of a pleiotropic mediator that is similar to IL-1 in the diversity of its effects on cell function. The findings reported here support this notion and document a role for IL-6 in T cell activation.

Materials and Methods

Thymocyte Isolation. Human thymocytes were isolated from glands obtained from pediatric patients (3 mo to 2 yr old) undergoing corrective cardiac surgery. The tissue

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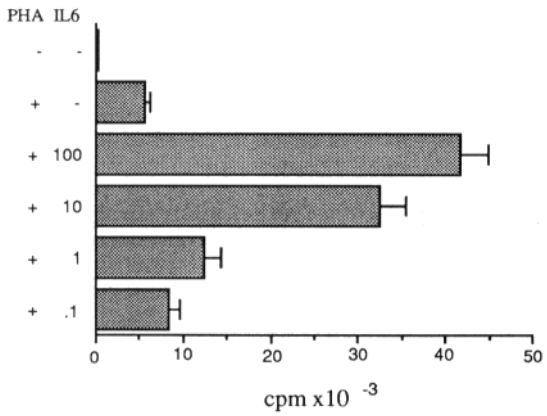

FIGURE 1. BSF-2/IL-6 augments proliferation of PHA-activated human thymocytes. Cells were stimulated with the indicated combinations of BSF-2/IL-6 and PHA (0.5%). Proliferation was measured after 4 d. Data are shown as cpm ± SEM.

was finely minced and single cell suspensions were fractionated over Ficoll-Hypaque gradients. Cells isolated by this technique usually were 99% positive for the CD2 antigen, as analyzed by flow cytometry.

Thymocyte Cultures. Cells were resuspended in RPMI 1640, supplemented with 2% heat-inactivated FCS, 2 mM L-glutamine, 50 μM 2-ME and antibiotics. 1–2 × 10^5 cells in 200 μl medium were added per well in 96-well flat-bottomed plates. During the last 4 h of the 96-h culture, [^{3}H]thymidine (sp act 60 Ci/mmol, 1 μCi/well) was added. The cells were harvested by filtration and radioactivity was counted by scintillation spectrophotometry. Peripheral blood T lymphocytes were purified as described previously (12).

Reagents. Antibody to the IL-2-R was purchased from Becton Dickinson & Co., Mountain View, CA. This antibody inhibits the binding of IL-2 and has similar reactivity as anti-Tac. Human rIL-2 was kindly provided by Cetus Corp., Palo Alto, CA.

Results

Initially we found that human rBSF-2/IL-6 had growth-promoting activity on PHA-activated thymocytes from (C3H/HeJ) mice, which are commonly used in the lymphocyte-activating factor assay to detect IL-1 activity. Subsequently we prepared human thymocytes (≥99% $CD2^+$) from normal thymus to determine whether they also responded to BSF-2/IL-6. rBSF-2/IL-6 increased proliferation of these cells approximately sevenfold above the response induced by PHA (0.2–0.5% wt/vol) alone. This effect was dose dependent and low doses of BSF-2/IL-6 between 0.1 and 1 U/ml still gave significant stimulation (Fig. 1). In all of the thymocyte preparations tested, BSF-2/IL-6 consistently stimulated proliferation significantly. The magnitude of the BSF-2/IL-6 effect showed some variability, which may reflect the contamination of thymocytes with a low proportion (<1%) of adherent cells that are capable of producing BSF-2/IL-6 (Lotz, M., et al., unpublished observations). The addition of BSF-2/IL-6 alone in the absence of PHA did not result in a detectable increase in proliferation (not shown).

The effects of BSF-2/IL-6 on mature T lymphocytes isolated from peripheral blood were then examined. In an initial series of experiments, unfractionated PBMC were stimulated with a broad range of PHA (0.001–1%) concentrations. Under these conditions, even the use of high doses of BSF-2/IL-6 resulted in only minor, nonsignificant increases in proliferation. However, if the PBMC populations were depleted of monocytes (≤0.5% esterase-positive) by the use of

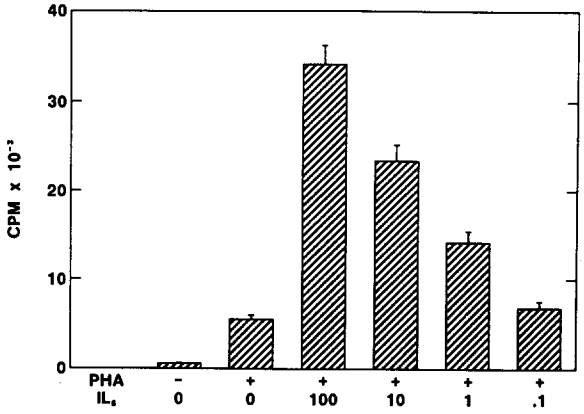

FIGURE 2. BSF-2/IL-6 effect on proliferation of purified T lymphocytes from peripheral blood. T lymphocytes that were essentially free of monocytes were cultured for 3 d in the presence of PHA (0.5%) and the indicated doses of BSF-2/IL-6. Proliferation was assayed by [^{3}H]thymidine incorporation. Data are shown as cpm ± SEM.

E-rosetting and carbonyl-iron treatment (12), a response to exogenous BSF-2/IL-6 was evident. Similar to the results obtained with thymocytes, at suboptimal doses of PHA (0.1–0.5%), BSF-2/IL-6 gave a dose-dependent increase in proliferation (Fig. 2).

The following series of experiments examined whether the proliferative action of BSF-2/IL-6 on thymocytes and T lymphocytes was mediated via the IL-2/IL-2-R pathway. To test for BSF-2/IL-6 effects on IL-2-R expression, thymocytes were cultured for 48 h in medium alone, PHA (0.5%) alone, or in the presence of both PHA and BSF-2/IL-6 (100 U/ml). The cells were stained with directly fluoresceinated antibody to the IL-2-R (P55 component) and analyzed by flow cytometry (13). The relative proportions of cells expressing IL-2-R were 3.6% in the control medium, 28.2% in the PHA-stimulated cells, and 33.8% in the presence of PHA and BSF-2/IL-6. Similar results were obtained using monocyte-depleted PBMC where the relative numbers were 4.2%, 19.6%, 19%, respectively. Cultures prepared in parallel, using identical cell populations, and supplemented with PHA and BSF-2/IL-6, showed the expected augmentation of cell proliferation. These results suggested that the BSF-2/IL-6 stimulation of T cells was not associated with an increase in IL-2-R expression. A separate approach was then used to strengthen this observation. Thymocytes were stimulated with PHA, or with combinations of PHA and IL-2 or BSF-2/IL-6. mAb to the IL-2-R was added to these cultures and proliferation was measured after 72 h. Table I shows that antibody to the IL-2-R at 1 μg/ml markedly inhibited the response of the cells to the combination of PHA and IL-2, whereas the proliferative response to PHA and BSF-2/IL-6 was not significantly depressed. At higher doses of antibody (10 μg/ml), which reduced the response to PHA alone, the IL-2 effect and also most of the IL-6 effect were inhibited. Leu-12 (an anti–B cell mAb of the same subclass [IgG1] as the anti-IL-2-R antibody) used as control, did not interfere with the proliferative effect of either cytokine. Together, these observations indicate that the effect of BSF-2/IL-6 on T cell proliferation is probably not exclusively dependent on the IL-2/IL-2-R pathway.

TABLE I
Mechanism of BSF-2/IL-6-induced T Cell Proliferation

Antibody	Thymocytes			PBT		
	PHA	PHA IL-2	PHA IL-6	PHA	PHA IL-2	PHA IL-6
No antibody	1,872	19,349	10,420	7,538	42,811	31,682
Anti-IL-2-R (1 μg/ml)	1,022	4,418	8,937	5,132	9,246	28,443
Anti-IL-2-R (10 μg/ml)	376	836	2,131	792	1,233	2,942
Leu-12 (1 μg/ml)	1,632	20,318	11,987	6,044	40,206	30,491
Leu-12 (10 μg/ml)	1,327	18,599	9,983	7,639	43,932	29,935

Human thymocytes and purified blood T lymphocytes (PBT) were stimulated with PHA (0.5% wt/vol) and IL-2 (10 U/ml) or BSF-2/IL-6 (10 U/ml). PHA-activated cells were preincubated for 2 h at 37°C with antibody to IL-2-R or with Leu-12. [^{3}H]Thymidine incorporation was measured after 4 d in culture. Data are shown as cpm, and are the mean of triplicate cultures. Variation between samples was ≤10%.

Discussion

These experiments have identified BSF-2/IL-6 as a novel costimulant for human thymocytes and mature T lymphocytes. IL-6 has comitogenic activity for T lymphocytes preactivated with suboptimal doses of PHA, but does not stimulate proliferation of lymphocytes in the absence of mitogen. This dependence of BSF-2/IL-6 on mitogen prestimulation is consistent with the two-signal requirement for T cell activation. IL-6 effects on T lymphocytes isolated from peripheral blood were only detectable after monocyte depletion, as these cells are capable of producing IL-6 (3). BSF-2/IL-6–mediated increases in proliferation do not appear to be mediated via increases in IL-2-R expression. In addition, doses of antibody to the IL-2-R that inhibited proliferation induced by exogenous IL-2 did not significantly affect the response to BSF-2/IL-6 response. At higher doses of antibody the IL-6 effects were significantly inhibited, although to a lesser degree than IL-2 effects. These results suggest that the IL-6 effect is not exclusively dependent on the IL-2/IL-2-R pathway. The observed effects of BSF-2/IL-6 on T lymphocytes are consistent with the expression of BSF-2/IL-6 receptors on these cells (14) and indicate that this cytokine can potentiate not only humoral but also cellular immune responses.

Summary

Growth and differentiation of thymocytes and mature T lymphocytes is regulated by cellular interactions that are in part mediated by soluble factors. We identify IL-6, formerly called B cell stimulating factor (BSF-2), IFN-β2, or hybridoma-plasmacytoma growth factor (HPGF) as a novel T cell costimulant. rIL-6 induced a six- to seven-fold increase in proliferation of human thymocytes stimulated with suboptimal doses of PHA. A similar effect with added IL-6 could

be observed using peripheral blood T lymphocytes, but only if the cultures were first rigorously depleted of monocytes that release high levels of IL-6. Analysis of the mechanism of the IL-6 effect on thymocytes and T lymphocytes showed that IL-6 did not lead to an increase in IL-2-R expression. Concentrations of antibody to IL-2-R inhibiting IL-2 effects did not block the IL-6-induced proliferation, indicating that the IL-6 effect was relatively IL-2 independent. These results identify IL-6 as a novel costimulant of human thymocytes and mature T lymphocytes, and suggest that IL-6 is also an important regulatory of cellular immunity.

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Note added in proof: After submission of this manuscript similar results on murine cells were reported (15).

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