Brief Definitive Report

THE FUNCTIONAL RELATIONSHIP OF THE INTERLEUKINS*

By KENDALL A. SMITH, LAWRENCE B. LACHMAN, JOOST J. OPPENHEIM, AND MARGARET F. FAVATA

From the Immunology Program, Dartmouth Medical School, Norris Cotton Cancer Center, Hanover, New Hampshire 03755; the Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710; and the Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205

Recent studies have indicated that the initiation and maintenance of T cell proliferation is mediated by a soluble T cell growth factor [TCGF(IL2)][1] released from lectin- or antigen-activated mononuclear cells (1, 2). Although it was shown that immunocompetent, Thy-1-positive cells were required for the release of TCGF(IL2) (3, 4), it was possible that the adherent cell product, lymphocyte-activating factor [LAF(IL1)][1] (5), was responsible for the T cell growth. It was found, however, that LAF(IL1) had no TCGF(IL2) activity, whereas partially purified TCGF(IL2) was readily detectable in the LAF(IL1) assay (6). One explanation for these observations, was the possibility that LAF(IL1) might promote the production of T cell-derived TCGF(IL2). In this report, we describe the results of experiments that support this hypothesis.

Materials and Methods

Animals. C57BL/6 and C3H/HeJ mice, 5–7 wk of age, were purchased from The Jackson Laboratory, Bar Harbor, Maine. BALB/c female, athymic, nu/nu (nude) mice, 5–7 wk of age were purchased from ARS/Sprague-Dawley Div., GIBCO/Invenex Div., Madison, Wis.

Cell Fractionation Procedures. Adherent cell-depleted, T cell-enriched splenocyte populations, and adherent splenocyte populations were prepared and utilized as described in detail previously (4).

LAF(IL1) Preparations. Murine conditioned medium that contained LAF(IL1) was derived from either unsolicited C57BL/6 peritoneal cells or PU5-1.8 cells (7) that were cultured (2×10^6 cells/ml) in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), and 10 μg/ml lipopolysaccharide (LPS) (Grand Island Biological Co., Grand Island, N. Y.). After 4 d of culture, the supernate was harvested, clarified by centrifugation (1,000 *g* for 10 min), and dialyzed against 1,000 volumes of RPMI-1640 medium for 24 h. The conditioned medium was then filtered through a 0.2-μm filter (Millipore Corp., Bedford, Mass.) and stored at 4°C until used.

Human LAF(IL1) was obtained from LPS (20 μg/ml)-stimulated peripheral blood mononuclear cells and partially purified by ultrafiltration using a hollow fiber filtration device, followed by isoelectric focusing with a sucrose gradient as previously described (8). Based upon the specific activity of LAF(IL1) present in the starting material, there was a >25,000-fold purification after these steps.

Before use in experiments designed to test the effect of LAF(IL1) on TCGF(IL2) production,

* Supported in part by National Cancer Institute grants CA-17643, CA-17323, and CA-23108; and National Cancer Institute contract NO1-CB-74141.

[1] At the Second International Lymphokine Workshop (Ermatigen, Switzerland, May 1979) a standard nomenclature was agreed upon for lymphocyte-activating factor (LAF), and T cell growth factor (TCGF). LAF was renamed Interleukin 1 (IL1) and TCGF was termed Interleukin 2 (IL2). To avoid confusion, in this report, both acronyms will be used as follows: LAF(IL1); TCGF(IL2). For a complete description of the nomenclature, see: letter to the editor, *J. Immunol.* **123**:2928 (1979).

the samples were titered in a LAF(IL1) assay (see below) and the concentrations which yielded 50% maximal activity were selected (usually a 1:10 dilution). All LAF(IL1) preparations were screened for TCGF(IL2) activity in the presence and absence of lectin using the TCGF(IL2) microassay (see below) and were found to be negative.

TCGF(IL2) Production. TCGF(IL2) for use in experiments to determine the effect of dexamethasone (DEX) on tritiated thymidine ([^{3}H]Tdr) incorporation by thymocytes was produced by the 24-h stimulation of C57BL/6 splenocytes (1×10^7 cells/ml) with concanavalin A (Con-A; 2.5 μg/ml; Miles-Yeda Laboratories, Rehoveth, Israel] as previously described (4). In experimentation designed to monitor TCGF(IL2) produced by different murine cell populations, responding cells (1×10^7 cells/ml) were stimulated with Con-A (2.5 μg/ml) for various time periods (Results) as previously described (9). At the conclusion of the culture periods, the cells were removed by centrifugation (1,000 *g* for 10 min) and the supernates assayed for TCGF(IL2) activity.

Assays for LAF(IL1) and TCGF(IL2). LAF(IL1) activity was determined by the dose-dependent augmentation of phytohemagglutinin (PHA)-initiated thymocyte proliferation as described by Lachman et al. (8), with the exception that serial twofold dilutions of each sample were performed.

In experiments where the effect of DEX on LAF(IL1) and TCGF(IL2) activity was tested, DEX (Steraloids, Inc., Hancock, N. H.) was prepared and added to the cultures in concentrations from 0.1 to 100 nM as described previously (10).

TCGF(IL2) activity was assayed as described in detail previously (3) using CTLL-2 cells as the indicator cell population. The results were quantified by probit analysis and expressed as units of activity based on a standard rat TCGF(IL2) preparation.

Results

The requirement for both T cells and adherent cells for the release of TCGF(IL2) upon lectin stimulation is displayed in Fig. 1 A. Although unfractionated splenocytes released easily measurable quantities of TCGF(IL2), the separation of cells into T cell-enriched and adherent cell-enriched fractions resulted in a marked diminution of TCGF(IL2) production. Reconstitution of the T cell-enriched population with only 1–5% adherent cells completely restored the response.

Although these data favored the hypothesis that TCGF(IL2) was T cell derived, they also indicated that adherent cells played an essential role. Because LAF(IL1) was known to markedly augment lectin-initiated proliferation of thymocytes (which contain <1% macrophages) and adherent cell-depleted splenic T cells, experiments were performed to examine the effect of LAF(IL1) on TCGF(IL2) release. As displayed in Fig. 1 B, in the absence of lectin, LAF(IL1) had no effect on TCGF(IL2) production by nylon column-enriched splenic T cells: both LAF(IL1) and lectin were required for optimal TCGF(IL2) release. Peak levels of TCGF activity were found after 2 d of culture, and detectable activity declined thereafter. The dual effect of LAF(IL1) and lectin on TCGF(IL2) production, and the resultant proliferation of both thymocytes and splenic T-cells is evident from the experiment displayed in Table I. Of note was the direct correlation between the amount of TCGF detectable on the second day of culture and the peak cellular proliferative response as indicated by [^{3}H]Tdr incorporation 1 d later.

In a previously reported series of experiments (10), we found that glucocorticoids suppressed antigen- or lectin-initiated T cell proliferation primarily by inhibition of TCGF(IL2) release, although there was only a slight inhibition (20–30%) of TCGF(IL2)-driven T cell proliferation. If LAF(IL1) functioned to provide a signal necessary for TCGF(IL2) production, and if TCGF(IL2) release was essential for the proliferative cellular response, then it might be expected that glucocorticoids would abrogate LAF(IL1) activity. The glucocorticoid dose-dependent inhibition of

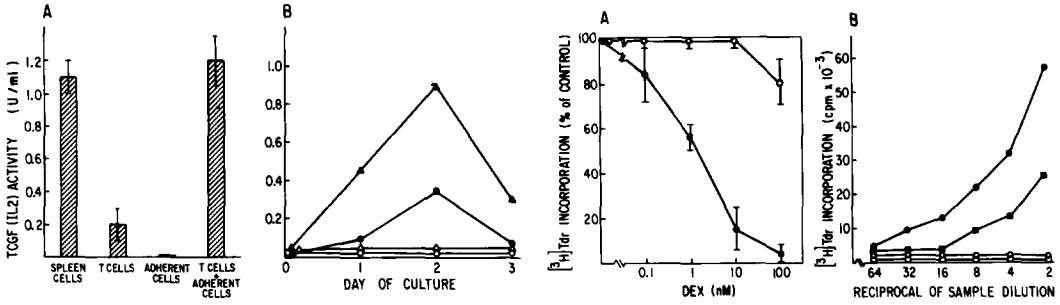

Fig. 1 Fig. 2

FIG. 1. (A) the production of TCGF(IL2) by different cell populations. Cells (1×10^7 cells/ml) were cultured for 24 h in the presence of Con-A (2.5 μg/ml) before the assay of supernates for TCGF(IL2) activity. Vertical brackets depict 1 SD about the mean of three experiments. (B) The effect of LAF(IL1) on TCGF(IL2) production by splenic T cells. LAF(IL1) was obtained from LPS-stimulated human mononuclear cells and partially purified by diafiltration and isoelectric focusing. Cells (1×10^7 cells/ml) were cultured with: (○) medium; (△) LAF(IL1); (●) 1% PHA-M (Difco Laboratories, Detroit, Mich.); (▲) 1% PHA-M + LAF(IL1). Supernates were removed from the cultures on the days indicated and tested for TCGF(IL2) activity.

FIG. 2. (A) The effect of DEX on: (●) murine LAF(IL1)-induced and (○) TCGF(IL2)-induced thymocyte [^{3}H]Tdr incorporation. Vertical brackets depict 1 SD about the mean of three experiments. (B) The effect of LAF(IL1) on lectin-initiated [^{3}H]Tdr incorporation of normal thymocytes (closed symbols) and athymic (nu/nu) splenocytes (open symbols). LAF(IL1) was derived from LPS-stimulated murine peritoneal cells (circles), and LPS-stimulated human mononuclear cells (squares).

TABLE I

LAF(IL1)-induced TCGF Release and the T Cell Response*

Stimuli	Thymocytes		Splenic T cells	
	TCGF(IL2)‡	[^{3}H]Tdr incorporation§	TCGF(IL2)‡	[^{3}H]Tdr incorporation§
	μml	*cpm*	*μml*	*cpm*
Medium	0.00	186 ± 10	0.00	345 ± 32
Con-A	0.04	5,805 ± 861	0.16	22,190 ± 968
LAF(IL1)	0.00	516 ± 5	0.00	402 ± 39
LAF(IL1) + Con-A	1.30	177,920 ± 4,940	0.88	120,517 ± 2,923

* LAF(IL1) was derived from LPS (20 μg/ml)-stimulated PU5-1.8 cells.
‡ TCGF(IL2) activity was assayed on culture fluid samples harvested 2 d after the initiation of the culture.
§ [^{3}H]Tdr incorporation was determined 3 d after the initiation of the culture.

LAF(IL1) activity as determined by [^{3}H]Tdr incorporation of thymocytes is shown in Fig. 2A. One-half maximal suppression occurred at a concentration of 1.5 nM DEX, and complete inhibition occurred at 100 nM DEX, a concentration which we had previously found almost completely saturated thymocyte glucocorticoid receptors (10). Also shown in Fig. 2A is the effect of DEX on TCGF(IL2)-mediated thymocyte proliferation. There was no inhibition observed at 0.1–10 nM DEX, and only 20% suppression of the proliferative response occurred at 100 nM DEX. These results are consistent with our previous data (10), and they support the hypothesis that glucocorticoids inhibit TCGF(IL2) release by a direct interference with LAF(IL1) stimulation of TCGF(IL2) production by T cells, rather than by suppressing the proliferative response to TCGF(IL2).

As a final approach to revealing the mechanism by which LAF(IL1) potentiates lymphocyte proliferation, we examined the effect of LAF(IL1) on cells from nude mice. In previously reported experiments (9), we observed that lymphoid cells from

nude mice were incapable of releasing detectable TCGF(IL2) activity, yet were capable of a vigorous proliferative response in the presence of lectin or alloantigen, provided TCGF(IL2) was supplied exogenously. If LAF(IL1) promoted T cell proliferation via the facilitation of TCGF(IL2) release from thymic-dependent cells, then one would expect LAF(IL1) to have no effect on nude mouse splenocytes. As shown in Fig. 2B, neither LAF(IL1)-containing medium, nor partially purified human LAF(IL1) promoted a detectable response from nude mouse splenocytes, whereas these same LAF(IL1) preparations clearly mediated dose-dependent stimulation of thymocyte [^{3}H]Tdr incorporation.

Discussion

The results of this study indicate that one mechanism by which activated macrophages participate in the promulgation of a T cell proliferative response is via the release of LAF(IL1), which is essential for the production of TCGF(IL2) by T cells. Thus, among the cells involved in the reaction, there are at least two hormone-like soluble cell products, which function to regulate the magnitude of the final T cell proliferative response. That LAF(IL1) promotes T cell proliferation through the stimulation of TCGF(IL2) production was suggested by several experimental approaches: small numbers of adherent cells were required to maximize TCGF(IL2) release from thymocytes or adherent cell-depleted splenocytes; LAF(IL1) effectively replaced the requirement for adherent cells; glucocorticoids, which abrogate TCGF(IL2) production, completely inhibited the LAF(IL1) effect on lectin-initiated thymocyte proliferation; LAF(IL1) had no proliferative effect on nude mouse splenocytes, which are incapable of TCGF(IL2) production.

In addition to the requirement for the presence of LAF(IL1) and TCGF(IL2), the quantity of each of these soluble factors dictates the extent of T cell clonal expansion. The dose-dependency of the LAF(IL1) effect on T cell proliferation is evident from Fig. 2B, and the dose-dependency of the TCGF(IL2) effect has been noted previously (3). It is this dose effect which serves as the basis for the quantitation of each of these factors in their respective assays. Because TCGF(IL2) ultimately mediates T cell proliferation (1–4), and because the magnitude of TCGF(IL2) production is dependent upon the quantity of LAF(IL1) available to TCGF(IL2)-producer cells, the data indicate that LAF(IL1) and TCGF(IL2) function in a bimodal amplification system which finally determines the extent of antigen- or lectin-initiated T cell clonal expansion.

The LAF(IL1) preparations used for the experiments reported here were derived from LPS-stimulated murine or human adherent cells. Mizel et al. have shown that LPS caused the release of LAF(IL1) from adherent cells in the absence of T cells, whereas T cells were required for the release of LAF(IL1) from macrophages in response to T cell lectins or antigens (11). Because we had previously determined that LPS did not cause the release of detectable TCGF(IL2) (3), we could be confident that the LAF(IL1) preparations used for these experiments were free of any contaminating TCGF(IL2). Aside from considerations of experimental design, the effect of LPS on LAF(IL1) release, and the realization that T cell clonal expansion is indirectly [through TCGF(IL2)] dependent upon the quantity of LAF(IL1) available, suggests that much of the adjuvant effect of LPS may be mediated by the stimulation of LAF(IL1) release. Thus, as has been suggested (5), one could explain the LPS-

augmentation of both the cell-mediated and humoral immune response at least in part by the effect on adherent cells: enhanced LAF(IL1) release would amplify TCGF(IL2) release. In that both cytotoxic and helper T cell proliferation is TCGF(IL2) dose-dependent (12, 13), the final result would be a marked expansion of these functional T cell populations. The implication of these observations is that because the production and actions of both LAF(IL1) and TCGF(IL2) can be assayed separately, it may be possible for the first time to identify the target cells of immunological adjuvants and to define their mode of action.

A similar approach could also be applied to the study of immunosuppressive agents. For example, the consequences of the interruption of the LAF(IL1)-TCGF(IL2) amplification network is evident from the experiments detailed on the effects of glucocorticoids (Fig. 2 A). Because glucocorticoids primarily functioned to inhibit the effect of LAF(IL1) in promoting production of TCGF(IL2), rather than preventing the T cell proliferative response to TCGF(IL2), saturating concentrations of glucocorticoid impeded the initial steps in the reaction, resulting in a complete suppression of T cell proliferation. That the addition of TCGF(IL2) circumvented glucocortocoid suppression suggests that an increased understanding of the mechanisms of action of glucocorticoids and other immunosuppressive agents may allow for the design of new, more specific immunosuppressive therapeutic approaches.

A final implication of this study may be drawn from the observations made with nude mouse lymphoid cells. In this instance, LAF(IL1)-responsive TCGF(IL2)-producer cells appear to be either nonfunctional or lacking. In previously reported studies (14), adherent cells from nude mice were found to release even greater quantities of LAF(IL1) than cells from normal mice, and in other studies (9), TCGF(IL2)-responsive cells were demonstrated to be present in nude mouse lymphoid cell populations. Because TCGF(IL2) was capable of promoting the in vitro proliferation and generation of cytolytic nude mouse T cells, we questioned whether TCGF(IL2) replacement therapy would by-pass the defect in TCGF(IL2) production and restore T cell function to nude mice. Indeed, we have recently demonstrated that the administration of TCGF(IL2) to nude mice, followed by alloimmunization, results in the appearance of splenocytes capable of proliferation and differentiation to alloantigen-specific cytolytic effector cells (15).

Summary

The mechanism of the lymphoproliferative effect of the macrophage product lymphocyte-activating factor [LAF(IL1)][1] appears to be mediated by the stimulation of the release of T cell growth factor [TCGF(IL2)][1] by T cells. The magnitude of the resultant T cell proliferative clonal expansion is thus dependent upon the quantity of both LAF(IL1) and TCGF(IL2) induced by antigen or lectin stimulation. These observations, coupled with the ability to measure the production and actions of these hormone-like lymphokines, should allow for increased insight into the mode of action of immunoenhancing and immunosuppressive agents, as well as for new therapeutic approaches to disease states involving T lymphocytes.

The authors thank Doctors Francis W. Ruscetti and Clara D. Bloomfield for their excellent critique of the manuscript.

Received for publication 20 February 1980 and in revised form 21 March 1980.

References

1. Morgan, D. A., F. W. Ruscetti, and R. Gallo. 1976. Selective in vitro growth of T-lymphocytes from normal human bone marrows. *Science (Wash. D. C.)* **193:**1007.
2. Smith, K. A., S. Gillis, and P. E. Baker. 1979. The role of soluble factors in the regulation of T-cell immune reactivity. *In* The Molecular Basis of Immune Cell Function. J. G. Kaplan, editor. Elsevier-North Holland, Inc., New York. 223.
3. Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith 1978. T-cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* **120:**2027.
4. Smith, K. A., P. E. Baker, S. Gillis, and F. W. Ruscetti. Functional and molecular characteristics of T-cell growth factor. *Mol. Immunol.* In press.
5. Oppenheim, J. J., S. B. Mizel, and M. S. Meltzer. 1979. Biological effects of lymphocyte and macrophage-derived mitogenic "amplification" factors. *In* Biology of the Lymphokines. S. Cohen, E. Pick, and J. J. Oppenheim, editors. Academic Press, Inc., New York. 291.
6. Oppenheim, J. J., H. Northoff, A. Greenhill, B. J. Mathieson, K. A. Smith, and S. Gillis. Properties of human monocyte derived lymphocyte activating factor (LAF) and lymphocyte derived mitogenic factor (LMF). *In* Biochemistry of Lymphokines. A. de Weck, editor. Academic Press, Inc., New York. In press.
7. Lachman, L., M. P. Hacker, G. T. Blyden, and R. E. Handschumacher. 1977. Preparation of lymphocyte-activating factor from continuous murine macrophage cell lines. *Cell. Immunol.* **34:**416.
8. Lachman, L. B., M. P. Hacker, and R. E. Handschumacher. 1977. Partial purification of human lymphocyte-activating factor (LAF) by ultrafiltration and electrophorectic techniques. *J. Immunol.* **119:**2019.
9. Gillis, S., P. E. Baker, N. A. Union, and K. A. Smith. 1979. The in vitro generation and sustained culture of nude mouse cytolytic T-lymphocytes. *J. Exp. Med.* **149:**1460.
10. Crabtree, G. R., S. Gillis, K. A. Smith, and A. Munck. 1979. Glucocorticoids and immune responses. *Arthritis Rheum.* **22:**1246.
11. Mizel, S. B., J. J. Oppenheim, and D. L. Rosenstreich. 1978. Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, $P388D_1$. I. Enhancement of LAF production by activated T lymphocytes. *J. Immunol.* **120:**1497.
12. Smith, K. A. T-cell growth factor. *Immunol. Rev.* In press.
13. Schreier, M. H., N. N. Iscove, R. Tees, L. Aarden, and H. von Boehmer. Clones of killer and helper T-cells: growth requirements, specificity and retention of function in long-term culture. *Immunol. Rev.* In press.
14. Meltzer, M. S., and J. J. Oppenheim. 1977. Bidirectional amplification of macrophage-lymphocyte interactions: enhanced lymphocyte activation factor production by activated adherent mouse peritoneal cells. *J. Immunol.* **118:**77.
15. Baker, P. E., and K. A. Smith. The potential therapeutic utility of T-cell growth factor. *Fed. Proc.* In press.