Lymphocyte activating factor promotes T-cell growth factor production by cloned murine lymphoma cells

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Evidence has indicated that two soluble factors are essential for a T-cell proliferative response to antigen or lectin. One such factor, thought to be of T-cell origin, is distinguished by its ability to initiate and maintain continuous T-cell growth (T-cell growth factor, TCGF) 

A second activity of macrophage origin augments lectin-initiated proliferative responses of thymocytes or adherent cell-depleted splenocytes (lymphocyte activating factor, LAF) 

Although it has been suggested that LAF promotes T-cell proliferation by facilitating the production of TCGF by T-cells 

direct evidence supporting this hypothesis has been lacking. We describe here experiments with a murine thymic lymphoma which releases TCGF in response to lectin. 

In serum-free, defined medium, highly purified LAF promotes a concentration-dependent increase in TCGF release. 

Because the proliferation of T cells depends on the concentration of TCGF available, these results indicate that LAF and TCGF constitute an essential bimodal amplification system which ultimately determines the extent of T-cell clonal expansion.

To approach the functional relationship of LAF and TCGF, we sought LAF-responsive cells which could be cultured in a lymphokine-free medium. WEHI-7, an irradiation-induced Thy 1 lymphoma cell line of BALB/c origin 

(Salk Institute Cell Distribution Center), was placed into culture with Iscove's modification of Dulbecco's modified Eagle's medium (IMDM) supplemented with bovine serum albumin, transferrin, cholesterol and soybean lipid. 

The WEHI-7 cells were screened for TCGF production at multiple cell concentrations in the presence of concanavalin A (Con A) or phytohaemagglutinin (PHA) and LAF. No detectable TCGF activity was found in the culture medium in the absence of lectin stimulation, although both PHA- and Con A-elicited TCGF activity were found. LAF obtained from lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells did not cause detectable TCGF production, although LAF increased lectin-dependent TCGF production three- to fivefold. A typical experiment is shown in Fig. 1, where TCGF activity elicited by Con A in the absence and presence of LAF was assayed by the dose-dependent titrinated thymidine (3H-TdR) incorporation of a TCGF-dependent cytolytic T-lymphocyte line (CTLL) 

To investigate whether WEHI-7 was heterogeneous with respect to TCGF production, the cell line was cloned by limiting dilution in microtiter plates (0.3 cells per well, Poisson-calculated probability of co-fertile wells < 0.05). Forty-one clones were obtained, and when tested for LAF, 50% of the clones did not produce detectable TCGF activity regardless of whether lectin or LAF was present. In contrast, all of those clones which released TCGF on lectin stimulation also responded to LAF by at least a twofold increment in TCGF production. Thus, the WEHI-7 cells behaved similarly to normal T-cells: both lectin and LAF were required for optimal TCGF production 

In previous experiments it was observed that the extent of T-cell proliferation was TCGF-concentration dependent. Therefore, if LAF augmented TCGF production, it seemed likely that LAF availability might be rate limiting in the ultimate T-cell proliferative response, especially if the effect of LAF on TCGF production was also concentration dependent. To examine this possibility, LAF partially purified by molecular gel chromatography and isoelectric focusing was added to TCGF-producing and nonproducing WEHI-7 clones in the presence of an optimal stimulatory concentration of Con A. As shown in Fig. 2, LAF clearly promoted a dose-dependent increase in TCGF release from one clone but not from the other clone.

The identification of a murine T-cell lymphoma that produces TCGF in response to lectin stimulation supports the impression that TCGF is T-cell derived. It was necessary to utilize cloned cells to provide such evidence as it was known that T-cell-enriched, normal cell populations required the presence of adherent cells or optimal TCGF production 

Therefore, the small amount of TCGF released by T-cell-enriched splenocytes could have been derived from residual adherent cells. As cloned thymic lymphoma cells were capable of producing TCGF in the absence of adherent cells and as LAF augments TCGF production, the data indicate that adherent cells contribute an early amplifying signal to T cells capable of TCGF production. Thus, the availability of a clonal population of cells, which can be studied in the absence of serum, provides the cellular system for detailed studies of the mechanism of LAF action and the molecular mechanism of TCGF production. Because both LAF and TCGF are essential for the T-cell proliferative response, such experiments will be important not only to investigate the nature of these lymphokines but also because information gained at the molecular level may increase insight into possible defects of lymphokine function which could underlie disease states.

![Fig. 1 TCGF activity elicited from WEHI-7 cells by Con A in the absence (○) and presence (●) of LAF. WEHI-7 cells (2 × 10⁶ cells ml⁻¹) were cultured for 24 h in IMDM (10.2, with Con A (2.5 μg ml⁻¹), Miles-Yeda). LAF was obtained from LPS (20 μg ml⁻¹, Escherichia coli, Gibco-stimulated human peripheral blood mononuclear cells cultured in serum-free RPMI 1640 medium (Gibco) for 48 h. Before use, the LAF-containing conditioned medium was concentrated 60-fold by passage through a YM-10 Amicon filter (Amicon), dialysed against 7,500 vol of saline, at 500 vol of RPMI 1640, and assayed for LAF and TCGF activity as previously described. No TCGF activity was detectable in the LAF preparation. The LAF preparation was added to the WEHI-7 cell cultures at a final dilution of 1:1,000. WEHI-7 conditioned medium was assayed for TCGF activity using a TCGF-dependent cytolytic T-lymphocyte line (CTLL-2). The T cell response to TCGF was quantified and displayed as the reciprocal of the sample dilution which resulted in 50% of the maximum TCGF. TCGF production was elicited by a standard rat TCGF preparation (3H-TdR incorporation was determined by the addition of 2 μCi ml⁻¹ 3H-TdR (specific activity 1.0 Ci mmol⁻¹ Schwarz–Mann) during the last 4 h of a 24-h culture period as described previously). In the experiment shown, maximum 3H-TdR incorporation yielded 19,500 c.p.m. in the absence of TCGF the CTLL incorporated 75 c.p.m.](image-url)
Fig. 2 LAF-concentration-dependent production of TCGF activity from WEHI-7 clone 49 (Ⅲ) and clone 8 (Ⅱ). Clones were tested for TCGF production as described in Fig. 1 legend. The LAF preparation was partially purified by Sephadex G-75 chromatography (active fractions migrated with an apparent molecular weight of 20,000) followed by isoelectric focusing over a pH gradient of 3-10. LAF used for the experiment displayed was derived from the isoelectric focusing gradient, pH 6.85. Both the Sephadex G-75 fractions and the isoelectric focusing fractions which contained LAF activity displayed no detectable TCGF activity before addition to the WEHI-7 clones.

Because TCGF ultimately mediates T-cell proliferation and because the magnitude of TCGF elaboration is LAF-concentration dependent (Fig. 2), the data indicate that LAF and TCGF function in a bimodal amplification system which finally determines the extent of antigen- or lectin-initiated T-cell clonal expansion. Thus, it may be anticipated that physiological and pharmacological agents known to influence the immune response may do so by altering lymphokine function. Immunosuppressive agents which inhibit LAF-mediated TCGF production would have profound effects on the humoral and cellular immune response, as both cytolytic and helper T-cell proliferation is TCGF-concentration dependent. For example, we have recently found that glucocorticoid hormones inhibit both the production and action of LAF which in turn results in a complete abrogation of TCGF production. Similarly, immunoenhancing agents may function through the LAF-TCGF amplification network. As pointed out by Oppenheim et al., many of the agents used as immunological adjuvants are also potent LAF inducers. As one can study separately the production and action of both LAF and TCGF, the mode of action of immunosuppressive and immunoenhancing agents may be amenable to experimental analysis.

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