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SUMMARY ARTICLE

Induction of T Cell Proliferation and Cytotoxicity by Lymphokines

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INTRODUCTION

Induction of cytotoxic T lymphocyte (CTL) responses in vitro requires a series of discrete activation signals. CTL-precursors (CTL-p) express receptors for the T cell growth factor interleukin 2 (IL-2) after an initial antigen or mitogen stimulus. The presence of IL-2 is necessary for sustained T cell growth, i.e., IL-2 receptor-bearing T cells expand clonally by consuming IL-2 (1-3). Requirement for other lymphokines to convert CTL-p into functional CTL-effector cells has been described previously (4-8).

In mixed lymphocyte cultures, all necessary helper signals are provided by cells within the cultures. Culture conditions with limited helper potential were established in order to study the required helper mediators. When limited numbers (10^5) of unfractionated thymocytes were cultured in the presence of metabolically inactive stimulator cells, even very high concentrations of purified mouse or recombinant human IL-2 were unable to induce either proliferative or cytotoxic responses (8).

A T CELL-DERIVED CYTOTOXICITY-INDUCING FACTOR

Coculture of 10^5 thymocytes with metabolically active allogeneic stimulator cells in the presence of sufficient amounts of IL-2 results in antigen-specific cytotoxic responses. The level of cytotoxic activity after 5 days of culture was dependent on the number of metabolically active stimulator cells but was not dependent on the amount of antigen that was presented. Therefore, all required helper signals were generated within these cultures. In the absence of metabolically active stimulator cells, however, no cyto-

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toxicity was found even in IL-2 excess. It was possible to replace the metabolic activity of the stimulator cells by adding a T cell cytotoxicity-inducing factor (TCF1) preparation to these deficient cultures(8).

This soluble helper mediator was obtained from supernatants of several T cell sources after stimulation but not from cells of the macrophage lineage. Good activity was found in supernatants of several subclones of the EL-4 cell line after PMA-stimulation or in Con A-pulsed spleen cell supernatants (9). In culture supernatants from cells of other origin even after a variety of stimulation procedures, no such activity was detected. Contaminating mitogen was not responsible for the cytotoxicity-inducing effect of TCF1. Con A at the highest possible contaminating dose in the TCF1 preparations was unable to generate cytotoxic responses even in the presence of IL-2. The activity was H-2 nonspecific, sensitive to pronase, trypsin, and chymotrypsin, resistant to neuraminidase, relatively heat labile ($T\frac{1}{2}$ at 56°C=4 minutes), and stable within a pH range from 3 to 10. Preliminary results from gel filtration revealed a molecular weight of about 40 KD for the EL-4-derived TCF1.

TCF1-INDUCED CYTOTOXIC RESPONSES

Cytotoxic responses of thymocytes measured in the presence of excess amounts of IL-2 in lectin-free cultures were TCF1 dose-dependent (Table 1).

Table 1. TCF1-Dependent Cytotoxic Responses

Sample*	% Specific [^{51}Cr] Release †
Medium	2.5 ± 0.7
IL-2	3.6 ± 2.0
TCF1 20 µl	2.2 ± 1.6
TCF1 40 µl	5.0 ± 1.8
IL-2 + 20 µl TCF1	21.3 ± 5.9
IL-2 + 40 µl TCF1	35.9 ± 14.4

*The cultures contained either purified IL-2 (20 U/ml) or no IL-2 and graded amounts of TCF1 from IL-2-absorbed Con A-pulsed spleen cell supernatants.

†Cytotoxic responses of 10^5 C3H thymic responder cells in the presence of 3 x 10^5 TNP-modified glutaraldehyde-fixed C3H splenic stimulator cells were determined in a 4 hour [^{51}Cr]-release test on day 5 on 5 x 10^3 TNP-modified L929 tumor targets.

The presence of TCF1 for 48 hours was sufficient to induce optimal cytotoxic responses (10). The effect of TCF1 was not due to other known lymphokines, such as interferon γ (IFN-γ), colony-stimulating factor (CSF), or interleukin 1 (IL-1) (11). Cytotoxic responses on day 5 were sensitive to Lyt 2-antibody and complement treatment, and >80% of the viable cells were positive for Thy 1 and Lyt 2 in the fluorescence-activated cell sorting analysis

thus carrying surface markers of CTL. Cold target inhibition studies suggested that the cytotoxic resonses were polyclonal. Experiments in autologous mouse serum showed that the polyclonal effect was not due to the presence of fetal calf serum in the cultures. In this nonspecific action and also in their morphologic appearance, the effector cells resemble lymphokine-activated killer cells or large granular lymphocytes (12, 13).

INDUCTION OF IL-2 RESPONSIVENESS BY TCF1 OR IL-1

To determine the mechanism of TCF1 action, proliferative responses of thymocytes were examined in the presence of IL-2. When 10^5 thymocytes of lipopolysaccharide-low responder C3H/HeJ mice were incubated with large amounts of IL-2 (20 U/ml), only marginal proliferation was measured in the absence of stimulator cells or mitogen. Addition of graded amounts of TCF1 preparations led to a dose-dependent proliferative response (Fig. 1A). Pulse kinetics

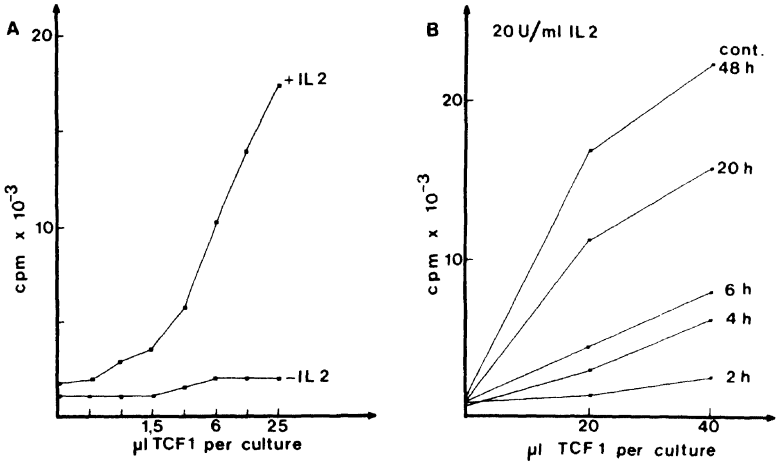

FIG. 1. Proliferative responses of 2 x 10^5 C3H/HeJ thymocytes were determined after 48 hours by a 5 hour pulse with tritiated thymidine. **A.** The cultures contained either purified IL-2 (20 U/ml) or no IL-2 and graded amounts of TCF1 from IL-2-absorbed Con A-pulsed spleen cell supernatants. **B.** All cultures contained purified IL-2 (20 U/ml) for the 72-hour culture period and graded amounts of TCF1 from IL-2-absorbed Con A-pulsed supernatants for the indicated time period.

with TCF1 showed that the maximal response was obtained after a 48 hour TCF1 pulse (Fig. 1B). IFN-γ preparations were unable to substitute for TCF1. However, pure IL-1 preparations that did not cause CTL responses in thymocyte cultures were found to also induce IL-2 responsiveness in thymocytes (Fig. 2). Using antibodies to IL-1, it was possible to distinguish between IL-1-induced and TCF1-induced proliferation. Only the IL-1-induced proliferative response of thymocytes was inhibited with anti-IL-1 antibodies but not the TCF1-induced IL-2-dependent proliferation. A second parameter to differentiate between the two mediators in this system was the sensitivity to α-methylmannoside (αMM). TCF1-induced thymocyte proliferation was abolished in the presence of 10 mM α MM, whereas the IL-1-induced proliferation was not affected at all. It must again be mentioned that TCF1 is not a lectin and that the trace amounts of Con A in some TCF1 preparations did not induce any proliferative response together with IL-2.

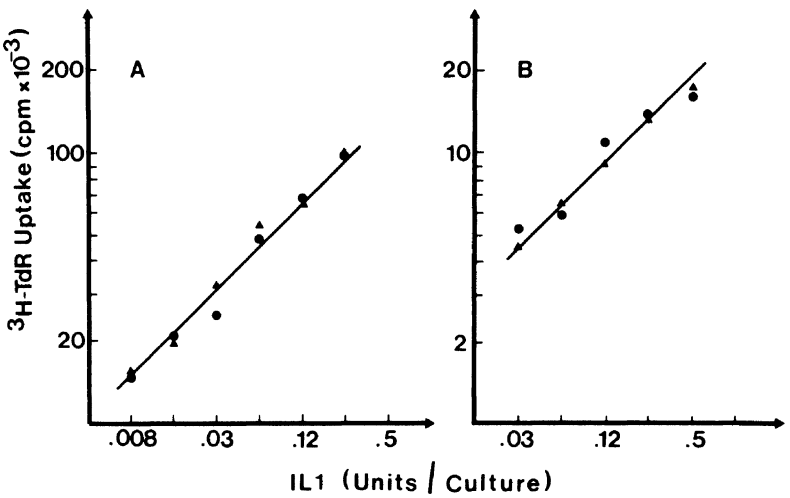

FIG. 2. Induction of proliferative responses of A 3 x 10^5 and B 1 x 10^5 C3H/HeJ thymocytes with twofold serial dilution of purified IL-1. In A, 10 U of purified murine IL-2 (Δ) or 5 U of recombinant human IL-2 (o) and in B, 5 U (Δ) or 20 U (o) of recombinant human IL-2 were present in the cultures.

When the thymocytes were separated according to their peanut agglutinin binding capacity by cell sorting, the TCF1-induced cytotoxicity as well as the TCF1 or IL-1-induced IL-2-dependent proliferation was found in the low binding fraction, i.e., in the more mature medullary thymocytes. In addition, when TCF1 preparations were tested with suboptimal amounts of mitogen in the conventional thymocyte IL-1 assay, they displayed the same activity as the control IL-1 preparations. Again, this TCF1 activity was not inhibited with anti-IL-1 antibodies. Thus, IL-1, a macrophage product, as well as TCF1, a T cell product, were both able to confer IL-2 responsiveness to thymocytes either in the presence of suboptimal amounts of mitogen or in the presence of IL-2.

Since the ability of T cells to react to IL-2 is dependent on the expression of IL-2 is dependent on the expression of IL-2 receptors, the induction of receptor expression on the cell membrane was determined using a monoclonal rat antibody (AMT 13) (14). IL-1, TCF1, and IL-2 by themselves were not able to induce IL-2 receptors on thymocytes. However, 48 hour incubation of either IL-1 or TCF1 together with IL-2 led to the binding of AMT 13 antibodies to thymocytes (Table 2). IFN-γ with or without IL-2 was unable to induce significant IL-2 receptor expression under these conditions.

CONCLUSION

A new function for IL-1 was proposed recently (15). It is suggested that IL-1 acts together with antigen by inducing IL-2 receptors on susceptible T cells rather than by stimulating helper cells for IL-2 production. It is also suggested that T cells might be able to produce such an IL-1-like activity because IL-2 receptor expression and growth of cloned T cells were the outcome of a synergistic action between IL-2-rich supernatants of T cell hybridomas and monoclonal antibodies that bind to the antigen receptors (15).

The data described in this communication show IL-2 receptor-inducing capability of IL-1 in a completely different experimental approach. Moreover, TCF1 seems to be the T cell product counterpart of this IL-2 responsiveness-inducing effect of IL-1. In addition to the induction of IL-2 receptor expression and proliferation of thymocytes, however, TCF1 induces cytotoxic responses in the presence of IL-2. This function was not induced by IL-1 in the described assay system. It can be speculated that IL-1 and TCF1 may act on different subsets of PNA thymocytes or that TCF1 contains an additional differentiation activity for the stimulated T cells.

Table 2. Induction of IL-2 Receptor Expression of Thymocytes

Sample *	Specific AMT 13 Binding (cpm)†
Medium	2,250
IL-2	5,480
IFN-γ	1,580
IL-1	2,230
TCF1	1,820
IL-2 + IFN-γ	4,470
IL-2 + IL-1	29,650
IL-2 + TCF1	37,180
Con A supernatant	145,680

*Thymocytes (5×10^5) after exposure to purified IL-2 (80 U/ml), recombinant IFN-γ (50 U/ml), purified IL-1 (0.50 U/ml) or TCF1 (Con A-pulsed, IL-2-absorbed spleen cell supernatant, 1:5 diluted) for 48 hours were incubated with AMT 13 antibodies.

†Specific binding of AMT 13 antibodies was determined by binding of [^{125}I] sheep antirat Ig by cells exposed to AMT 13 or control cells.

Cytotoxic lymphocyte responses are antigen specific in bulk cultures. When TCF1 is provided exogenously to help deficient cultures, this antigen specificity is lost (16). Assuming that under bulk culture conditions or in vivo TCF1 plays a role for T cell activation, TCF1 activity has to be linked in some way to the antigen on the delivering cell. Otherwise, polyclonal activation of CTL precursors by excess TCF1 could not be prevented, and unspecific cytotoxic responses as measured in the TCF1-supplemented thymocyte cultures would occur. Recently, membrane-bound IL-1 activity was demonstrated by Unanue (17). It is conceivable that the main form of presentation of lymphokine activities is directly from cell to cell and might require membrane contact of the producer cell with the target cell, thus avoiding nonspecific activation.

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