Human Lymphocytes with Either the OKT4 or OKT8 Phenotype Produce Interleukin 2 in Culture

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ABSTRACT In this study, we demonstrate that both highly purified $T4^+$ and $T8^+$ lymphocytes can produce substantial amounts of Interleukin 2(IL 2) when stimulated with the combination of concanavalin A (Con A) and phorbol myristate acetate. Furthermore, addition of IL 1 to macrophage-depleted T lymphocytes significantly increased IL 2 production by lymphocytes of either the $T4^+$ or $T8^+$ phenotype. These findings provide a basis for further studies of the molecular mechanisms involved in human immune cell interactions.

INTRODUCTION

Activation and proliferation of lymphocytes by antigens or mitogens is the result of complex cellular interactions. These interactions are, in fact, mediated by secretory products of immunocompetent cells (1). Interleukin 2 (IL 2),[1] a factor secreted by T cells, plays a central role in inducing lymphocyte proliferation and differentiation (2). Furthermore, IL 2 production is augmented by a secretory product of macrophages, namely Interleukin 1 (IL 1) (3, 4). Human T cells have been separated into two major subsets. One subset bears the differentiation antigens OKT4 and Leu 3a, whereas the other have the surface antigens OKT8 and Leu 2a (5). Secretion of mitogenic factors has been attributed to the $T4^+$ subset only (6). However, in the present study, using highly purified human T cell subpopulations, we demonstrate that $T8^+$ as well as $T4^+$ cells equally are able to produce significant levels of IL 2.

METHODS

Peripheral blood mononuclear cell (PBMC) subpopulations. PBMC were isolated and fractionated from buffy coats obtained from healthy blood donors as described previously (7).

Purification of T cell subsets was performed both by killing and by sorting. For the killing experiments the monoclonal antibodies OKT4 and OKT8 (Ortho Pharmaceutical, Raritan, NJ) were used, as described previously (8). The residual cells were analyzed by flow cytometry (FACS II, Becton, Dickinson & Co., Mountain View, CA) using fluorescinated anti-Leu 2a and anti-Leu 3a reagents (Becton, Dickinson & Co.), which identify the same T cell subsets as OKT8 and OKT4, respectively (9). The OKT8 + C pretreated population yielded >90% Leu 3a-positive cells and <5% Leu 2a-positive cells; treatment with OKT4 + C yielded >90% Leu $2a^+$ cells and <5% Leu $3a^+$ cells.

Flow cytometric separation of T cell subsets was performed as follows: The isolated T cells were incubated with fluorescein conjugated anti Leu 2a or anti Leu 3a, washed, and sorted on a Becton, Dickinson FACS-II. The sorted positive and negative populations were then reanalyzed for purity on the FACS. This yielded 99.1% Leu $3a^+$ cells and 98.9% Leu $3a^-$ cells when the cells were sorted with anti-Leu 3a. There were 98.8% Leu $2a^+$ and 99.6% Leu $2a^-$ cells when anti-Leu 2a was used.

Autologous mixed lymphocyte reaction (AMLR). As an

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[1] *Abbreviations used in this paper*: AMLR, autologous mixed lymphocyte reaction; IL 1, Interleukin 1; IL 2, Interleukin 2; PBMC, purified blood mononuclear cells; PMA, phorbol myristate acetate.

additional control for the purity of the T cell populations, standard AMLR cultures were performed (7).

IL 2 induction and bioassay for IL 2. T cells, as well as subsets of T lymphocytes, were incubated in microtiter plates at 1×10^6 cells/ml in the presence of 5 ng/ml phorbol myristate acetate (PMA) (Sigma Chemical Co., St. Louis, MO) and 5 μg/ml concanavalin A (Con A) (Calbiochem-Behring Corp., La Jolla, CA) in RPMI 1640 containing 5% FCS in a total volume of 200 μl/well. After incubation for 48 h at 37°C in a 5% CO_2 atmosphere, the supernatants were harvested and assayed for IL 2 activity (10). Cell viability at the end of the culture period was always >75%.

Preparation of partially purified IL 1. Partially purified IL 1 was prepared as described and used at a concentration of 25 U/ml (11).

RESULTS

IL 2 production by T cell subpopulations purified with specific monoclonal antibodies + C mediated killing. Human peripheral blood leukocytes were cultured with various doses of Con A + PMA for 48 h in the presence or absence of partially purified IL 1. IL 1 by itself, did not have any IL 2 activity. However, addition of IL 1 increased supernatant IL 2 activity in a dose-dependent manner (data not shown).

Purified T cell subpopulations, as well as unseparated T cells, were tested for IL 1-induced IL 2 production. In the presence of optimal doses of PMA + Con A, the IL 2 production of fractionated T cells was significantly enhanced by addition of IL 1 (Fig. 1). $T4^+$ cells as well as $T8^+$ cells produced IL 2 and IL 2 activity was enhanced by the addition of IL 1. In the absence of mitogens, IL 1 failed to enhance IL 2 production by unseparated or purified T cell subpopulations (Fig. 1).

In order to ascertain the functional capabilities of these T cell subsets, the subpopulations were tested in an AMLR assay in which only $T4^+$ cells are known to be reactive (8). Untreated T cells, as well as $T4^+$ cells, proliferated most actively in a 7-d AMLR-stimulated with autologous (B + null) cells (Table I). In contrast, the purified $T8^+$ cells failed to respond in the AMLR (Table I). Analogous results were obtained during the entire time course of the AMLR. Aliquots of the T cell subsets studied in the AMLR were stimulated with Con A + PMA and tested for their IL 2 production. Supernatants of unseparated T cells, $T4^+$ cells, and $T8^+$ cells all contained significant levels of IL 2 activity (Table I).

IL 2 production by T cells separated by flow cytometry. When T cell subpopulations, highly purified by flow cytometry, were analyzed for their IL 2 production, once again both $T4^+$ and $T8^+$ cells produced IL 2 (Table II).

T cells were sequentially treated with cytotoxic antiserum + C followed by selection using flow cytometry to further ensure the purity of the subpopulations. The resultant subsets, when stimulated with Con A + PMA and IL 1 both still produced substantial levels of IL 2 activity (data not shown). Moreover, restaining the cells at the end of the culture period and reanalysis by flow cytometry showed that none of the $T8^+$ and Leu $2a^+$ thymocyte changed the T4 or Leu 3a phenotype by the end of the culture period (data not shown).

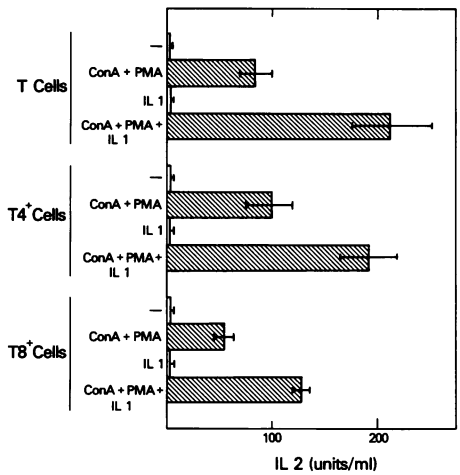

FIGURE 1 IL 2 production by T cell subpopulations. 1×10^5 unseparated T cells or $T4^+$ or $T8^+$ lymphocytes separated by treatment with specific monoclonal antibodies + C were cultured in the presence of Con A (5 μg/ml) and PMA (5 ng/ml) with or without IL 1 (25 U/ml). The IL 2 supernatant activity was measured after 48 h of incubation. Unstimulated T cells failed to produce IL 2. The results are expressed as mean±SE units of IL 2 activity of five different experiments.

DISCUSSION

Using highly purified T cells, stimulation of either $T4^+$ or $T8^+$ cells led to IL 2 release. Analogous results were obtained when T cell subsets were purified either by

TABLE I
IL 2 Production and Proliferative Responses of T Cell Subpopulations in an AMLR

	Response in a 7-d AMLR*	IL 2 activity‡
T cells	49,417±539	131
$T8^+$ cells	2,118±161	142
$T4^+$ cells	30,234±2,731	188

* 1×10^5 T cells were cultured with 1×10^5 non-T cells. Cells were purified by treatment with specific monoclonal antibodies + C which resulted in a purity of 96%. The results are the mean±SEM of triplicate cultures.

‡ IL 2 activity (units per milliliter) in the supernatants of 1×10^6 cells/ml after 48 h incubation in the presence of Con A (5 μg/ml) and PMA (5 ng/ml).

TABLE II

IL 2 Production by Cultured Negatively Selected T Cell Subpopulations

	-IL 1				+IL 1*			
	—	Con A‡	PMA§	Con A + PMA	—	Con A	PMA	Con A + PMA
T cells	0‖	9	17	109	8	34	37	230
$T8^+$ cells¶	0	1	16	43	1	4	28	129
$T4^+$ cells**	0	1	16	96	1	7	21	187

* IL 1 added in a concentration of 25 U/ml.

‡ Con A 5 μg/ml.

§ PMA 5 ng/ml.

‖ Supernatant IL 2 activity (units per milliliter) after 48 h incubation.

¶ 99.1% purified as determined by flow cytometry.

** 98.9% purified as determined by flow cytometry.

two exposures to specific monoclonal antibodies + C or by positive and negative selection with a fluorescence-activated cell sorter. Furthermore, when $T8^+$ subpopulations were sorted a second time after incubation with T cell stimulants + IL 1, still no contaminant $T4^+$ lymphocytes were detected. This indicates that neither polyclonal T cell stimulants nor IL 1 induce the expression of $T4^+$ in a 99% pure $T8^+$ subset. Thus T cells with the $T8^+$ phenotype have the capacity to produce IL 2 in culture.

Reinherz et al. (6) have demonstrated that lymphocyte mitogenic factor was secreted only by $T4^+$ lymphocytes when stimulated by alloantigens. That study suggested that the $T4^+$ subpopulation is the sole source of IL 2 production. In the present study we have taken advantage of the combination of Con A and PMA to stimulate $T8^+$ cells to produce IL 2 (2, 10). The role of PMA is still uncertain. Although usually viewed as a comitogen PMA has been proposed to act as a substitute for IL 1 (12). However, our results showing PMA and IL 1 to have additive effects suggest that they each may act in a distinct manner. The FACS separated T cell subpopulations were 99% purified and contained <1% macrophages. Although, unlikely, we can not formally exclude the possibility that macrophages may have contributed to the activation of lymphocytes by the polyclonal stimuli in this study. The fact that IL 1 can further increase IL 2 secretion by T cells points out the importance of this augmenting signal produced by macrophages. It is of interest that IL 1 enhanced the reactivity not only of the $T4^+$ lymphocytes, but also of the $T8^+$ subset.

It has been observed that only some of the $T4^+$ cells express the TQ_1 phenotype (13). It is, therefore, tempting to speculate that only a subpopulation of the $T8^+$ cells may be able to produce IL 2. The production of a helper factor apparently contradict the current assumption that all $T8^+$ cells function as suppressor/cytotoxic cells (5, 6). However, it is not known whether the IL 2 produced by $T8^+$ cells is biochemically identical to that produced by $T4^+$ cells. Nevertheless, the finding that $T8^+$ cells, when optimally stimulated, also can produce IL 2 may have important implications for cellular interactions involved in an immune response. Although maximal in vitro stimulation of $T8^+$ lymphocytes by polyclonal stimulants shows them to be capable of releasing IL 2, it remains to be established whether this reflects physiological in vivo activities by this subset and whether this contributes to the pathophysiology of immunological disorders (14).

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