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Research Article

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Responder Cells in the Human Autologous Mixed Lymphocyte Reaction

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ABSTRACT Isolated human $T4^+$ cells proliferate in the autologous mixed lymphocyte reaction (AMLR), whereas isolated $T8^+$ cells do not. However, in the presence of Interleukin 2 or $T4^+$ cells, the $T8^+$ cells demonstrated substantial proliferation. These studies suggest that $T8^+$ cells recognize signals from autologous non-T cells, but require an additional factor for the subsequent proliferative response. Since this stimulus can be provided by $T4^+$ cells, the AMLR appears to constitute an inducer circuit. Different defects in this circuit may be responsible for the common abnormality of the AMLR in different diseases.

INTRODUCTION

The T cell-proliferative response to autologous non-T cells is termed the autologous mixed lymphocyte reaction (AMLR)[1] (1, 2). The AMLR has been shown to bear the characteristics of a specific immune response (3), and to generate immunoregulatory cells or factors (4–7). Such observations have led to the assumption that the AMLR may be an important pathway for the activation of regulatory and effector mechanisms. This assumption has been further strengthened by observations of an abnormal AMLR in diseases with associated dysfunction of the immune system (8–10).

In this study we have investigated the human T cell subsets previously defined by monoclonal antibodies (11, 12), OKT4 (inducer) and OKT8 (cytotoxic/suppressor), with regard to their ability to proliferate in response to stimulation by autologous non-T cells. We found that a purified $T4^+$ population, but not a purified $T8^+$ population, can respond in the AMLR; however, the $T8^+$ population can proliferate if a source of help is provided.

METHODS

Peripheral blood mononuclear cell (PBMC) subpopulations. PBMC were isolated and fractionated as described previously (13). Briefly, PBMC were isolated from buffy coats obtained from healthy blood donors by gradient centrifugation on lymphocyte separation medium (LSM, Litton Bionetics, Kensington, Md.). The cells were then incubated on precoated plastic petri dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) in complete medium (13). The nonadherent cells were harvested, adjusted to $10–15 \times 10^6$ cells/ml in RPMI 1640 medium, and mixed with an equal volume of 5% sheep erythrocytes. This mixture was layered onto lymphocyte separation medium and centrifuged for 30 min at room temperature for separation of T and (B + null) cells (13). After lysis of sheep erythrocytes with ammonium chloride buffer (National Institutes of Health Media Unit), the cells were washed twice with Hanks' balanced salt solution. Adherent cells, Mϕ, were harvested from the plastic petri dishes with the aid of a rubber policeman and vigorous pipetting.

Purification of T cell subsets was performed using the monoclonal antibodies OKT4 and OKT8 (Ortho Pharmaceutical Corp., Raritan, N. J.). OKT4 binds to the inducer cell subset, OKT8 to the suppressor/cytotoxic cell subset (11, 12). The method was analogous to one described by Thomas et al. (14). Briefly, 10×10^6 T cells/ml RPMI were incubated with an equal volume of a 1:40 dilution of OKT4 or OKT8 antibody (previously dialyzed against Hanks' balanced salt solution) for 1 h at room temperature. Selected, prescreened rabbit complement (N. L. Cappel Laboratories Inc., Cochranville, Pa.) was added and the incubation continued for an additional hour at 37°C. The cells were washed, resuspended in RPMI, and the procedure repeated. To assess the completeness of killing, the residual cells were analyzed using the anti-leu 2a and anti-leu 3a reagents (Becton, Dickinson & Co., Mountain View, Calif.), which identify the same T cell subsets as OKT8 and OKT4, respectively (12, 15). As analyzed by flow cytometry (FACS II, Becton, Dickinson & Co.), the OKT8 + C pretreated population yielded >90% Leu 3a-positive

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[1] *Abbreviations used in this paper*: AMLR, autologous mixed lymphocyte reaction; IL-2, Interleukin 2; Mϕ, adherent cells; PBMC, peripheral blood mononuclear cells.

(inducer) cells and <5% Leu 2a-positive (suppressor/cytotoxic) cells; treatment with OKT4 + C yielded >90% Leu $2a^+$ cells and <5% Leu $3a^+$ cells. In all functional experiments, T cells pretreated with C alone, were used as controls; these cells contained normal percentages of both Leu 2a- and Leu 3a-positive cells. For simplicity, control T cells will be referred to as T cells, OKT8 + C treated cells as $T4^+$ cells, and OKT4 + C treated cells as $T8^+$ cells.

Autologous Mixed Lymphocyte Reaction. Standard AMLR (13) contained 1×10^5 responder cells (T cells, $T4^+$, $T8^+$ cells or mixtures of these cells as indicated) and 1×10^5 mitomycin C-treated stimulator cells, (B + null) cells or Mϕ as indicated, in 200 μl of complete medium. Cultures were performed for 7 d at 37°C in a humidified atmosphere of 5% CO_2 in air. 20 h before termination of the cultures, 1 μCi [^{3}H]thymidine (New England Nuclear, Boston, Mass.) was added to the cultures. Results were expressed as means±SEM of triplicate or quadruplicate cultures minus mean counts per minute of cultures containing T cells alone (Δ cpm). In some experiments AMLR were performed in the presence of Interleukin 2 (IL-2).

Preparation of and assay for IL-2. PBMC obtained from human buffy coats were cultured in RPMI 1640 under serum-free conditions in the presence of 5 ng/ml phorbol myristic acetate (Sigma Chemical Co., St. Louis, Mo.) and 10 μg/ml of concanavalin A (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.). After incubation for 48 h at 37°C in a 5% CO_2 atmosphere, the supernatants were harvested and assayed for IL-2 activity as described previously (16). In the present experiments, IL-2 was used at a final concentration of 10 U/ml. At this concentration, no mitogenic activity of the supernatant was detected. AMLR supernatants were assayed for IL-2 as described (16).

RESULTS

Purified $T4^+$ but not $T8^+$ cells proliferate in the AMLR. When $T4^+$ cells were incubated with autologous (B + null) cells, their AMLR-proliferative response was comparable to that observed with unseparated T cells. In contrast to the $T4^+$ cells, purified $T8^+$ cells proliferated only minimally in the AMLR (Fig. 1). The differential reactivity of $T4^+$ and $T8^+$ cells was similarly observed when the stimulator cell population consisted of Mϕ (data not shown).

The low responsiveness of $T8^+$ cells was not due to different proliferation kinetics, since analogous results

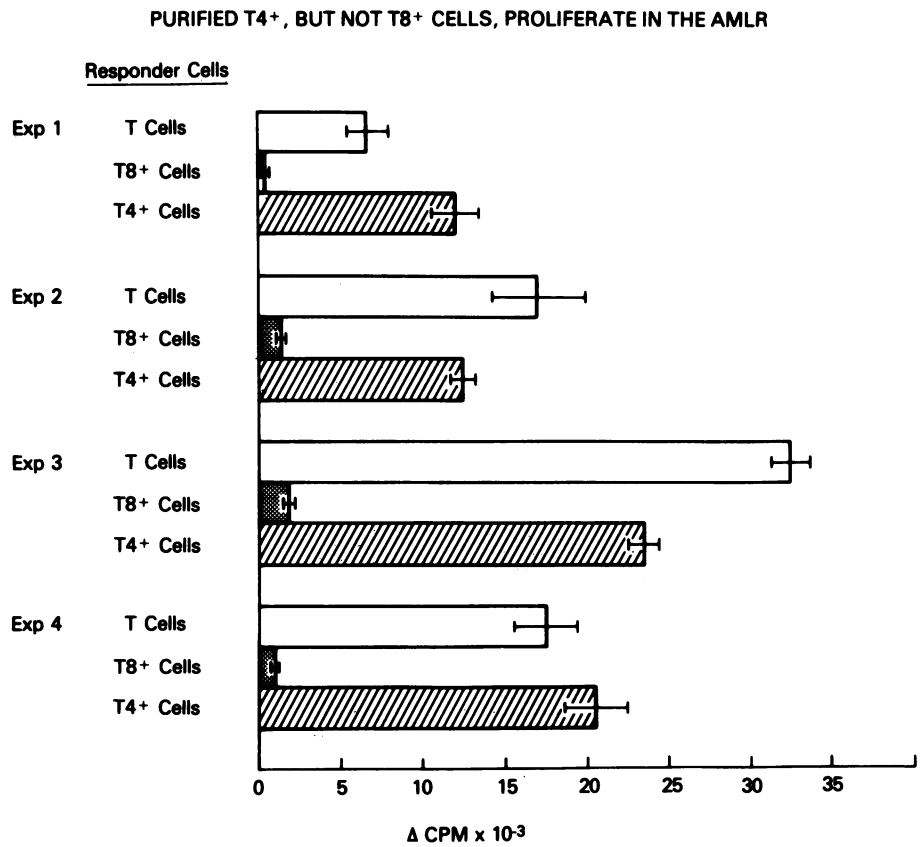

FIGURE 1 Response of purified $T4^+$ but not purified $T8^+$ cells to autologous non-T cells in a 7-d AMLR. The unseparated T cells (open bars) and the $T4^+$ cells (cross hatched bars) responded to mitomycin C-treated autologous (B + null) cells. In contrast, the $T8^+$ cells (shaded bars) failed to respond. Similar results were obtained when Mϕ were used as the stimulation cells (data not shown). In all experiments, 1×10^5 T cells were cultured with 1×10^5 non-T cells.

were observed during the time course of the AMLR (data not shown). Moreover, fluorescence studies have shown that both $T4^+$ and $T8^+$ cells proliferate in the AMLR when unseparated T cells are responders (manuscript in preparation).

$T8^+$ cells do not suppress the AMLR, but rather proliferate in the presence of $T4^+$ cells. Because in some systems $T8^+$ cells act as suppressor cells (11, 12), we investigated whether or not they suppressed the AMLR response of $T4^+$ cells. In five separate experiments, we found that the addition of $T8^+$ cells did not suppress the AMLR reactivity of $T4^+$ cells (Fig. 2); rather, the response of the mixture of $T4^+$ cells and $T8^+$ cells was significantly greater than the sum of responses of $T4^+$ and $T8^+$ cells cultured separately, suggesting that $T8^+$ cells proliferated in the presence of $T4^+$ cells. In additional experiments, increasing numbers of $T4^+$ cells and decreasing numbers of $T8^+$ cells gave a linear increase in proliferation (data not shown) indicating that $T4^+$ cells do not proliferate better in the presence of $T8^+$ cells.

$T8^+$ cells proliferate in the presence of IL-2. The different responder T cell populations were cultured with autologous (B + null) cells in the absence or presence of IL-2. Control cultures containing T cells, $T4^+$ cells or $T8^+$ cells plus IL-2, in the absence of autologous non-T cells, demonstrated little proliferation (<800 cpm). Moreover, the optimal proliferation in AMLR cultures containing IL-2 occurred on day 7 (with minimal proliferation on day 3). The IL-2 had no effect when added to AMLR cultures containing either T cells or $T4^+$ cells; however, when $T8^+$ cells were cultured with autologous (B + null) cells in the presence of IL-2, a highly significant increase in proliferation was observed (Fig. 3). This experiment was repeated twice with the same results. Moreover, similar results were seen when Mϕ were used as stimulator cells (data not shown). Thus, $T8^+$ cells are not unresponsive to autologous non-T cells, but they require the presence of "helper" factors in order to proliferate in the AMLR.

IL-2 production by T cell subsets. IL-2 production after 5 d of culture was assessed for unseparated T cells, $T4^+$ cells, and $T8^+$ cells stimulated with mitomycin C-treated (B + null) cells. The IL-2 production U/ml was 6±1, 15±2, and 1±1 for upseparated T cells, $T4^+$ cells, and $T8^+$ cells, respectively.

DISCUSSION

In this study, we have demonstrated, that the $T4^+$ (inducer) cell subset proliferates to autologous non-T

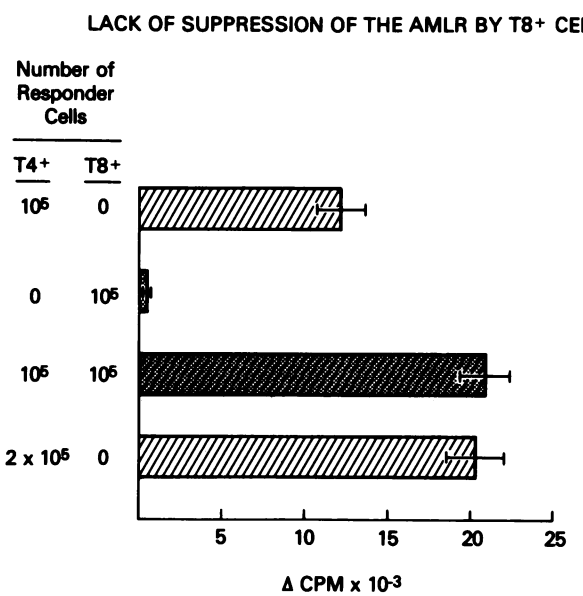

FIGURE 2 Effect on the AMLR of mixing $T4^+$ cells and $T8^+$ cells. 10^5 $T4^+$ cells responded to 1×10^5 mitomycin C-treated (B + null) cells in the 7-d AMLR. The same number of $T8^+$ cells failed to respond. When the two T cell populations were mixed and cultured in the 7-d AMLR, there was no suppression of the response of $T4^+$ cells by the added $T8^+$ cells. In fact, the response of the mixture was similar to that obtained with 2×10^5 $T4^+$ cells in this experiment.

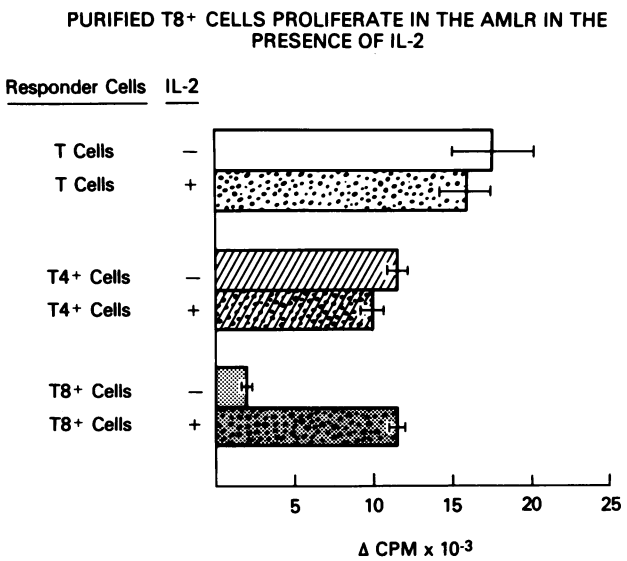

FIGURE 3 Effect of IL-2 on the response of $T8^+$ cells in the AMLR. Unseparated T cells, 1×10^5, or $T4^+$ cells, or $T8^+$ cells (also 1×10^5) were cultured with an equal number of mitomycin C-treated (B + null) cells in the 7-d AMLR. Addition of IL-2 to the AMLR cultures produced insignificant effects when the responder cells were either unseparated T cells or $T4^+$ cells. However, whereas $T8^+$ cells did not respond in the AMLR in the absence of IL-2, the $T8^+$ cells responded well in the presence of IL-2. Control cultures of unseparated T cells, $T4^+$ cells, or $T8^+$ cells did not proliferate in the presence of IL-2 in the absence of stimulatory autologous (B + null) cells (data not shown).

cells in the absence of $T8^+$ cells, whereas the $T8^+$ (suppressor/cytotoxic) cells proliferate only minimally in the absence of $T4^+$ cells. However, the addition of IL-2 markedly increases the proliferation of the $T8^+$ subset to autologous non-T cells. The relatively high proliferative capacity of unseparated T cells, as well as the lack of suppression of the $T4^+$ response by $T8^+$ cells, suggest that $T4^+$ cells may affect the $T8^+$ population in a manner analogous to IL-2. The much greater production of IL-2 by $T4^+$ cells, as compared with $T8^+$ cells, supports this idea. In fact, $T4^+$ but not $T8^+$ cells have been shown to produce helper factors (16), and in the mouse, the Lyt 1^+, 2^-3^- cell, which is the analogue of the human $T4^+$ cell (12, 15) has been shown to be responsible for IL-2 production (17).

In this study we have reported proliferation in terms of tritiated thymidine incorporation into cellular DNA. However, the AMLR studies have been assessed in parallel by cell cycle analysis in collaboration with Dr. Elizabeth Raveche, National Institutes of Health, with a very strong correlation ($P < 0.001$) between the thymidine and cell cycle results (manuscript in preparation).

The data provided herein indicate that measurement of proliferation may not allow full evaluation of recognitive functions of a population following stimulation. Thus, $T8^+$ cells could recognize signals from autologous non-T cells as did $T4^+$ cells; however, the former may require a second signal necessary for proliferation. The $T4^+$ population may provide such a signal.

These data further suggest that the AMLR itself represents an intrinsic inducer pathway, or a part thereof. The $T4^+$ cells proliferate in the AMLR and may induce $T8^+$ cells to proliferate, whereas the $T8^+$ cells do not suppress the proliferative response of the $T4^+$ cells. Thus, the overall proliferative response in the AMLR may be regarded as a measure of such a pathway. This inducer pathway may lead to the induction of a number of immune functions, including suppressor-effector functions. Dissection of the AMLR in various diseases (8–10) may lead to a better understanding of the cellular bases for immune abnormalities in those disorders.

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