Monoclonal antibody analysis of human T lymphocyte subpopulations exhibiting autologous mixed lymphocyte reaction

(helper T cells-suppressor T cells/Ia antigens)

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Contributed by Robert A. Good, March 31, 1981

ABSTRACT In autologous mixed lymphocyte reaction (MLR), T cells proliferate in response to the stimulation by autologous non-T cells. In the present study, human T cell subpopulations, defined by murine monoclonal antibodies OKT4, OKT8, or 9.3, were examined for their capacity to proliferate in autologous and allogeneic MLR. It was observed that the treatment of responder T cells with OKT4 or 9.3 antibody (both defining helper/inducer T cells) and complement (C') ablated their proliferative response in autologous MLR and markedly reduced their allogeneic MLR proliferative response. In contrast, treatment of T cells with OKT8 antibody (defining suppressor/cytotoxic T cells) and C' had no or minimal effect on their proliferative response in autologous MLR. Furthermore, OKT4, 9.3, 9.6 (framework specific) but not OKT8 antibody, when present during the entire culture period, in the absence of C' inhibited in a dose-dependent manner the proliferative response in both autologous and allogeneic MLR. Inhibition of proliferation was also observed when the responder T cells were pretreated with OKT4 or 9.3 antibody, washed, and then stimulated with irradiated autologous or allogeneic non-T cells. Pretreatment of T cells with OKT8 or 7.2 anti-Ia antibody in the absence of C' did not influence their proliferative response in autologous MLR. Thus, T cells containing cells with helper/inducer activity defined by OKT4 or 9.3 antibody appear to be the major responder T-cell subpopulation in autologous MLR.

Autologous mixed lymphocyte reaction (MLR) is characterized by T-cell proliferation upon stimulation with autologous non-T cells (1–7). Autologous MLR is considered to be an important regulatory mechanism in the immune response with immunologic specificity and memory (1, 2, 8). Although there is a general agreement that the responder cells are T cells, the nature of the T-cell subpopulation(s) proliferating in autologous MLR has not been clearly established. Distinct subpopulations of human T cells have been defined by using murine monoclonal antibodies (9–17). Helper/inducer T cells react with OKT4 and 9.3 antibodies. OKT4+ cells respond to soluble antigens, help in the differentiation of B cells to plasma cells, and assist in the development of cytotoxic T cells in MLR (11, 12). The cytotoxic/suppressor T cells are identified by OKT8 antibody (15).

In the present study, we have examined the ability of human T-cell subpopulations recognized by OKT4, OKT8, or 9.3 antibodies, to respond in autologous or allogeneic MLR. Our results indicate that T cells reacting with OKT4 or 9.3 antibody proliferate in autologous MLR.

MATERIAL AND METHODS

Cell Suspensions. Mononuclear cells were separated from peripheral venous blood of healthy donors on Ficoll/Hypaque gradient. T cells were separated from non-T cells by rosetting mononuclear cells with neuraminidase-treated 1% sheep erythrocytes at 4°C for 60 min and separating rosetted T cells from non-rosetting non-T cells on Ficoll/Hypaque gradient as described (18). T cells obtained in this way were 96% purified as determined by rosette formation with sheep erythrocytes and by lack of cells with readily demonstrable surface Ig. Non-T cells contained 9Ig+ B cells, plastic-adherent esterase-positive phagocytic monocytes, nonadherent nonphagocytic non-T-non-B cells and less than 2% T cells. Purified T and non-T cells were suspended in RPMI-1640 medium containing 25 mM HEPES, 20% heat-inactivated fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 2mM L-glutamine (complete medium).

Monoclonal Antibodies. OKT4 and OKT8 antibodies were purchased from Ortho Diagnostics (Raritan, NJ). Development, production, and specificity determination of ascites fluids containing antibodies 9.3, 9.6 reacting with the receptor for erythrocyte rosettes, and 7.2 (anti-human-Ia, framework specific) have been described elsewhere (13, 17, 19). All antibodies are complement (C')-fixing IgG class antibodies.

Isolation of T Cell Subpopulations. Each of the antibodies was dialyzed extensively for 48 hr against RPMI-1640 medium to remove sodium azide preservative. To 0.2 ml of T-cell suspension containing 1 x 10⁶ cells was added 0.4 ml of OKT4, 9.3, or OKT8 antibody, and the cells were incubated for 60 min at 4°C. All three antibodies were used at 1,200 dilution. After incubation, rabbit C' was added to a final C'/cell ratio of 1.5, and further incubation was carried out for 60 min at 37°C in humidified 5% CO₂/95% air. This entire procedure was repeated twice to thoroughly deplete T-cell subsets of the designated subpopulation. Cells of the remaining subpopulation were washed twice with Hanks’ balanced salt solution and resuspended to 1 x 10⁶ cells per ml in complete medium. Analysis of T-cell subsets obtained by this procedure showed that T cells after treatment with OKT4 (reacting with 50–65% peripheral T cells) and C' contained >90% 9.6+, >80% OKT4+, and <4% OKT4− cells. T cells after the treatment with OKT8 and C' contained >94% 9.6− cells, >85% OKT4− cells, and <4% OKT8+ cells.

Autologous and Allogeneic MLR. 1 x 10⁶ responder unfractionated T cells or cells of T-cell subpopulations were cultured in microtiter plates (Nunc, Copenhagen) with an equal number of irradiated (3000 R from a cesium source) autologous or allogeneic non-T lymphocytes at 37°C in humidified 5% CO₂/95% air. The peak proliferative responses in these cultures were studied on day 5 (allogeneic) or day 6 (autologous) by pulsing the cells with 2 μCi (1 Ci = 3.7 x 10¹⁵ becquerels) of [³H]thymidine (New England Nuclear; specific activity, 6.7 Ci/μM).
Experiments). The entire autologous 9.3 mmol) for the final 18 hr of cultures. The cells were then harvested with multiple sampler harvester, and the incorporation of 3H-thymidine was measured by liquid scintillation technique. Results are expressed as net cpm of triplicate cultures (Δcpm) = total cpm in stimulated cultures minus the background cpm in responder cells plus the background cpm of stimulator cells when cultured alone.

Statistical analysis was performed by Student's t test.

RESULTS

Inhibition of Autologous and Allogeneic MLR by T Cell and Anti-Ia Antibodies in the Absence of Complement. T cells were cultured with irradiated autologous or allogeneic non-T lymphocytes in the presence of different concentrations of OKT4, 9.3, OKT8, 9.6, or 7.2 anti-Ia antibody, and their proliferative response was studied. No source of C' was present in the cultures. Presence of OKT4, 9.3, 9.6, and 7.2 anti-Ia antibody in the cultures resulted in a marked reduction in the proliferative response in both autologous MLR (Fig. 1A) and allogeneic MLR (Fig. 1B). However, presence of OKT8 antibody in the culture, did not influence the proliferative response in either autologous or allogeneic MLR. Inhibitory influence of antibodies in culture was dose-dependent and could not be attributed to antibody-mediated cellular cytotoxicity.

Proliferative Response of T-Cell Subpopulations in Autologous and Allogeneic MLR. T cells were separated into mutually exclusive subpopulations by using OKT4, 9.3, or OKT8 antibody and C'. The resultant subpopulations were then studied for their proliferative responses in autologous and allogeneic MLR. Fig. 2 illustrates the results of the proliferative response of T-cell subsets in autologous and allogeneic MLR. Treatment of T cells with OKT4 or 9.3 and C' ablated their proliferative response in autologous MLR and markedly reduced the response in allogeneic MLR. In contrast, depletion of OKT8+ T cells did not significantly influence the proliferative response of the remaining T cells in autologous MLR (P > 0.15). However, allogeneic MLR response of T cells depleted of OKT8+ cells was moderately reduced (P < 0.05).

Table 1. Peak proliferative response in autologous MLR and allogeneic MLR of T cells treated with monoclonal antibodies in the absence of C*.

<table>
<thead>
<tr>
<th>Cells treated</th>
<th>Antibody treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Autologous</td>
<td>Allogeneic</td>
</tr>
<tr>
<td>Responder T cells</td>
<td>None</td>
<td>10,317</td>
<td>18,214</td>
</tr>
<tr>
<td></td>
<td>OKT4</td>
<td>4,631 (54)</td>
<td>9,835 (46)</td>
</tr>
<tr>
<td></td>
<td>OKT8</td>
<td>8,975 (13)</td>
<td>12,569 (31)</td>
</tr>
<tr>
<td>9.3</td>
<td>6,799 (35)</td>
<td>7,649 (58)</td>
<td>3992 (55)</td>
</tr>
<tr>
<td>7.2 (Anti-Ia)</td>
<td>12,108 (17)</td>
<td>18,174 (-3)</td>
<td>9849 (-11)</td>
</tr>
<tr>
<td>Stimulator non-T cells</td>
<td>7.2 (Anti-Ia)</td>
<td>4,229 (59)</td>
<td>6,925 (62)</td>
</tr>
</tbody>
</table>

* Responder T or the stimulator non-T cells were treated with one of the four antibodies at 4°C for one hr, after which the cells were washed twice with Hanks' balanced salt solution, and then mixed-lymphocyte cultures were set.

† Numbers in parentheses represent percentage reduction; negative values indicate percentage enhancement.
carried out with T cells, which were pretreated with OKT4, 9.3, OKT8, or 7.2 anti-Ia antibody in the absence of C', washed, and then cultured with autologous or allogeneic non-T lymphocytes. Binding of OKT4 or 9.3 antibody to T cells resulted in significant reduction in their proliferative response in both autologous and antigenic MLR (Table 1). Binding of OKT8 antibody to T cells, which resulted in a modest reduction in the proliferative response in allogeneic MLR, did not influence the proliferation in autologous MLR. Treatment with anti-Ia antibody without C' of stimulus non-T lymphocytes but not of responder T cells resulted in a markedly diminished proliferative response in both autologous and allogeneic MLR. These results suggest that binding of OKT4 or 9.3 antibody to T cells results in significant inhibition (P < 0.05) of their proliferative response in both autologous and allogeneic MLR.

**DISCUSSION**

Using monoclonal antibodies, we have defined a subpopulation of T cells that exhibit proliferative response upon stimulation with autologous non-T cells. T cells reacting with OKT4 or 9.3 antibody (both react with T cells containing helper/inducer activity) appear as the major responder cells in autologous MLR. The depletion of T cells that react with OKT4 or 9.3 antibody resulted in almost total loss of the proliferative response in autologous MLR. The depletion of T cells reacting with OKT8 antibody either had minimal or no influence on the proliferative response in autologous MLR. Similar results were obtained when cells were cultured in the presence of antibody (no C') for the entire culture period or when T cells were pretreated with OKT4, 9.3, or OKT8 antibody (no C'), washed, and then stimulated with autologous or allogeneic non-T cells. These results also suggest that antigens on T-cell membranes that are recognized by OKT4, 9.3, or 9.6 antibody may be involved directly in step(s) in the process of T-cell activation leading to cellular proliferation in autologous MLR. Treatment of fresh T cells with anti-Ia antibody had no effect on their proliferative capacity in autologous or allogeneic MLR. However, anti-Ia antibody inhibited both autologous and allogeneic MLR when present in the culture containing both T and non-T cells or after treatment of the non-T cells in the system with the anti-Ia antibody. Therefore, it is apparent that Ia antigens, expressed by non-T cells, are involved in the stimulation of T cells in both autologous and allogeneic MLR.

Autologous MLR-activated T cells have been shown to exhibit helper and suppressor cell functions (20–25). It has been demonstrated that OKT4+ cells act as helpers in the differentiation of B cells to immunoglobulin-synthesizing and -secreting plasma cells and also help in the development of cytotoxic OKT4+ cells in allogeneic MLR (11, 12). OKT8+ cells, on the other hand, have been shown to contain precursors of concanavalin A-inducible suppressor cells (15). Sakane and Green (6) have shown that T cells that proliferate in autologous MLR also contain precursors of concanavalin A-induced suppressor cells. Therefore, one would have expected that OKT8+ cells would proliferate in autologous MLR. However, in the present study, T cells enriched for the OKT8+ phenotype failed to respond or responded only poorly in autologous MLR. It also was demonstrated clearly here that T cells enriched for OKT4+ or 9.3+ cells proliferate vigorously in autologous MLR. The reason for this lack of correlation between T-cell subsets proliferating in autologous MLR and T-cell subsets containing precursors of concanavalin A-induced suppressor activity as reported (6, 15) remains unclear. Our own preliminary experiments seem to indicate that cells of both the OKT4+ phenotype and OKT8+ phenotype contain precursors that can develop into cells capable of expressing suppressor activity upon stimulation with concanavalin A. Further, selection of autologous MLR-activated T cells into distinct subpopulations by using monoclonal antibodies such as OKT4, 9.3, or OKT8 and studying their regulatory function could resolve this apparent controversy.

We thank A. Prajapati for excellent technical assistance. This work was supported by U.S. Public Health Service Grants AG-00541, AI-11843, CA-08748, CA-17404, CA-19267, NS-11456, CA-18029, CA-29548, and HL-17265; the Fund for the Advanced Study of Cancer; and the Judith Harris Selig Memorial Fund.