Effect of Activated Lymphocytes on the Regulation of Hematopoiesis: Enhancement and Suppression of In Vitro BFU-E Growth by T Cells Stimulated by Autologous Non-T Cells

By Mine Harada, Shinji Nakao, Kunio Kondo, Kazuaki Odaka, Mikio Ueda, Shintaro Shiobara, Kosei Matsue, Takao Mori, and Tamotsu Matsuda

Autologous mixed lymphocyte culture (AMLR) is an immunologic response with memory and specificity and plays a role in immune regulation. Effects of T cells activated by AMLR were studied in the regulation of in vitro erythropoiesis. AMLR-activated T cells were cocultured with autologous non-T, nonphagocytic peripheral blood mononuclear cells for assaying erythroid progenitor cells (BFU-E). T cells activated for 3 days in AMLR showed significant enhancement of in vitro colony growth by BFU-E. In contrast, activated T cells from day 7 AMLR caused significant suppression of BFU-E growth. Both enhancing and suppressing activities of AMLR-activated T cells were mediated by an la-positive and radiosensitive population within the OKT4⁺ subset. These observations suggest that AMLR-activated T cells may play a role in the immune-mediated regulation of in vitro erythropoiesis. It is also suggested that heterogeneous T-cell subsets may exert regulatory functions in the regulation of in vitro hematopoiesis.

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MATERIALS AND METHODS

Preparation of T cells and non-T cells. Heparinized peripheral blood from normal volunteers was diluted with an equal volume of RPMI 1640 medium (GIBCO, Grand Island, NY), layered over a Ficoll-Hypaque density gradient, and centrifuged at 400 x G for 30 minutes. Peripheral blood mononuclear cells (PBMC) were obtained from the interface and washed twice with RPMI 1640. Then T cells and non-T cells were separated from PBMC by using the rosette sedimentation technique.

Isolation of T-cell subsets. An OKT4- or OKT8-positive cell fraction was isolated by negative selection. T cells (1 x 10⁷/mL) were treated with an OKT4 monoclonal antibody (Ortho Pharmaceutical Corp., Raritan, NJ) staining on a FACS analyzer (Becton Dickinson, Mountain View, Calif) while non-T cells contained ≤5% OKT3-positive cells.

Preparation of non-T, nonphagocytic PBMC. For the separation of nonphagocytic cells, 5% silica particle solution (KAC-2, Japan Immunoresearch Laboratory, Takasaki, Japan) was added to heparinized peripheral blood at a 1:9 ratio and incubated at 37°C for 60 minutes. The mixture was then layered over a Ficoll-Hypaque density gradient and centrifuged at 400 x G for 30 minutes. Furthermore, T cells were removed from this nonphagocytic cell fraction by using the rosette sedimentation technique. The resulting non-T, nonphagocytic PBMC were then treated with a OKT4 monoclonal antibody for nonspecific esterase staining and contained ≤9% OKT3-positive and ≤15% la-positive cells, respectively. These cells were cryopreserved until the use for assaying BFU-E.

Autologous mixed lymphocyte reaction (AMLR). A bulk AMLR was performed by culturing 5 x 10⁶ irradiated (20Gy) autologous non-T cells in 10 mL of RPMI (GIBCO, Grand Island, NY) supplemented with 15% pooled human serum at 37°C in a humid 5% CO₂ atmosphere. For unstimulated cultures, 5 x 10⁶ irradiated autologous T cells were added instead of non-T cells. In some experiments, OKT4⁺ or OKT8⁺ cells were used as responder cells. Responder cells were harvested on days 3, 7, and 12 of AMLR and T cells were separated again by using the rosette sedimentation technique. These responder T cells were washed three times with RPMI 1640 before coculturing with non-T, nonphagocytic autologous PBMC for BFU-E assays.

Treatment with OKIal monoclonal antibody and radiation. In some experiments, responder T cells harvested from AMLR (1 x 10⁷/mL) were treated with an OKIal monoclonal antibody (50 eight percent to 95% killing of each T-cell subset occurred following treatment with the corresponding antibody and complement.)

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Table 1. Effect of T Cells Activated by Autologous MLR (AMLR) on BFU-E Growth

<table>
<thead>
<tr>
<th>Type of T Cells Added*</th>
<th>No. of BFU-E /2 x 10^5 Cells</th>
<th>% of Control BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No cells added)</td>
<td>77 ± 20†</td>
<td>100</td>
</tr>
<tr>
<td>T/Tx from day 3 AMLR</td>
<td>83 ± 17</td>
<td>114 ± 9.8</td>
</tr>
<tr>
<td>T/non-Tx from day 3 AMLR</td>
<td>110 ± 21</td>
<td>164 ± 21.0</td>
</tr>
<tr>
<td>Control (No cells added)</td>
<td>94 ± 16†</td>
<td>100</td>
</tr>
<tr>
<td>T/Tx from day 7 AMLR</td>
<td>96 ± 16</td>
<td>102 ± 1.5</td>
</tr>
<tr>
<td>T/non-Tx from day 7 AMLR</td>
<td>53 ± 19</td>
<td>50 ± 12.0</td>
</tr>
<tr>
<td>Control (No cells added)</td>
<td>113 ± 32†</td>
<td>100</td>
</tr>
<tr>
<td>T/Tx from day 12 AMLR</td>
<td>124 ± 31</td>
<td>111 ± 5.2</td>
</tr>
<tr>
<td>T/non-Tx from day 12 AMLR</td>
<td>64 ± 25</td>
<td>50 ± 11.3</td>
</tr>
</tbody>
</table>

T cells stimulated by autologous T cells or non-T cells were harvested from day 3, 7, or 12 AMLR. 1 x 10^6 AMLR-activated T cells were cocultured with 2 x 10^6 autologous non-T, non-phagocytic PBMC for assaying BFU-E. Each value represents M ± SEM in 5 experiments.

†Statistically significant at P < 0.025 level.
‡Percent of control BFU-E-derived colonies was calculated as follows: (No. of BFU-E colonies in coculture with AMLR-activated T cells/No. of BFU-E colonies in culture with no added T cells) x 100.

RESULTS

Table 1 shows effects of AMLR-activated T cells on in vitro BFU-E growth. When T cells stimulated by irradiated autologous T cells (T/Tx) were added to autologous non-T, non-phagocytic PBMC for BFU-E assays, they did not affect BFU-E growth. T cells activated by irradiated autologous non-T cells (T/non-Tx) increased BFU-E growth at a significant level (P < 0.025) when they were harvested on day 3 of AMLR. In contrast, T cells activated by autologous non-T cells for 7 or 12 days in AMLR showed a significant suppression of BFU-E growth (P < 0.025). A dose-dependent enhancement or suppression of BFU-E growth was observed. In a representative experiment, percentages of control BFU-E-derived colonies were 117, 160, and 167 with the addition of 0.5 x 10^5, 1.0 x 10^5, and 2.0 x 10^5 day 3 AMLR-activated T cells, respectively, while they were 62, 54, and 27 with the addition of 0.5 x 10^5, 1.0 x 10^5, and 2.0 x 10^5 day 7 AMLR-activated T cells, respectively.

Table 2 provides the data to determine whether AMLR-activated T cells will affect allogeneic BFU-E growth or not. Activated T cells harvested from day 3 AMLR showed a significant increase (P < 0.05) of not only autologous but also allogeneic BFU-E growth when they were added to BFU-E assays. However, activated T cells harvested from day 7 AMLR suppressed only autologous BFU-E growth (P < 0.05). Allogeneic BFU-E growth was unaffected by adding day 7 AMLR-activated T cells to BFU-E assays.

Next, effects of AMLR-activated OKT4+ or OKT8+ cells on BFU-E growth were studied. As shown in Fig 1, OKT4+ cells activated by autologous non-T cells exerted regulatory functions while AMLR-activated OKT8+ cells did not. Activated OKT4+ cells harvested from day 3 AMLR increased BFU-E growth in comparison with the unactivated control (P < 0.025). This increase in BFU-E growth was comparable to that exerted by day 3 AMLR-activated T cells. In contrast, activated OKT4+ cells harvested from day 7 AMLR showed a significant suppression of BFU-E growth (P < 0.025), which was almost identical to that exerted by day 7 AMLR-activated T cells.

AMLR-activated T cells expressing regulatory functions for BFU-E growth were studied for the presence of Ia antigens and radiosensitivity. When AMLR-activated T

Table 2. Effect of AMLR-Activated T Cells on Autologous or Allogeneic BFU-E

<table>
<thead>
<tr>
<th>Type of T Cells Added*</th>
<th>PBMC Cultured for BFU-E Assay</th>
<th>No. of BFU-E/2 x 10^5 Cells</th>
<th>% of Control BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No cells added)</td>
<td>autologous</td>
<td>72 ± 24†</td>
<td>100</td>
</tr>
<tr>
<td>T/non-Tx from day 3 AMLR</td>
<td>autologous</td>
<td>132 ± 31</td>
<td>156 ± 7.5</td>
</tr>
<tr>
<td>Control (No cells added)</td>
<td>allogeneic</td>
<td>74 ± 25†</td>
<td>100</td>
</tr>
<tr>
<td>T/non-Tx from day 3 AMLR</td>
<td>allogeneic</td>
<td>129 ± 31</td>
<td>156 ± 6.3</td>
</tr>
<tr>
<td>Control (No cells added)</td>
<td>autologous</td>
<td>77 ± 30</td>
<td>100</td>
</tr>
<tr>
<td>T/non-Tx from day 7 AMLR</td>
<td>autologous</td>
<td>37 ± 8</td>
<td>49 ± 6.4</td>
</tr>
<tr>
<td>Control (No cells added)</td>
<td>allogeneic</td>
<td>116 ± 45</td>
<td>100</td>
</tr>
<tr>
<td>T/non-Tx from day 7 AMLR</td>
<td>allogeneic</td>
<td>115 ± 48</td>
<td>94 ± 8.5</td>
</tr>
</tbody>
</table>

1 x 10^6 AMLR-activated T cells were cocultured with 2 x 10^6 autologous or allogeneic non-T, non-phagocytic PBMC for assaying BFU-E. Each value represents M ± SEM in 4 experiments.

*See footnote in Table 1.
†Statistically significant at P < 0.05 level.
‡Not significant.
§See footnote in Table 1.
Fig 1. Effect of OKT4⁺ cells and OKT8⁺ cells activated by AMLR on BFU-E growth. OKT4⁺ or OKT8⁺ T cells were cultured with autologous non-T cells in AMLR. 1 × 10⁶ AMLR-activated OKT4⁺ or OKT8⁺ cell were harvested on day 3 or day 7 and cocultured with 2 × 10⁶ autologous non-T, nonphagocytic PBMC for assaying BFU-E. For percent of control BFU-E-derived colonies, see footnote in Table 1. Data indicate M ± SEM in 5 experiments. □ Control; □, AMLR activated OKT4⁺ cells added; □, AMLR activated OKT8⁺ cells added.

Fig 2. Effect of OKTal plus complement treatment on AMLR-activated T cells in the regulation of BFU-E growth. 1 × 10⁶ AMLR-activated T cells were treated with complement alone or OKTal monoclonal antibody and complement before cocultures, both BFU-E enhancing and suppressing activities were completely abolished (Fig 2). Enhancement and suppression of BFU-E growth were unaffected by addition of AMLR-activated T cells treated with complement alone. Similarly, both of the two opposing functions were completely abolished when these activated T cells were 20 Gy irradiated before cocultures (Fig 3).

When T cells were stimulated by allogeneic non-T cells instead of autologous ones in a conventional one-way MLR, T cells activated by alloantigens for 3 or 7 days in MLR had no effects on in vitro growth of BFU-E as shown in Fig 4. On

Fig 3. Effect of radiation on AMLR activated T cells in the regulation of BFU-E growth. 1 × 10⁶ AMLR activated T cells were 2,000 rad irradiated before culturing with 2 × 10⁶ autologous non-T, nonphagocytic PBMC for assaying BFU-E. For percent of control BFU-E-derived colonies, see footnote in Table 1. Data indicate M ± SEM in 4 experiments. □ Control; □, nonirradiated AMLR activated T cells added; □ irradiated AMLR activated T cells added.

Fig 4. Effect of T cells activated by alloantigens in MLR on BFU-E growth. 1 × 10⁶ alloantigen-activated T cells or AMLR-activated T cells were cocultured with 2 × 10⁶ autologous non-T, nonphagocytic PBMC for assaying BFU-E. For percent of control BFU-E-derived colonies, see footnote in Table 1. Data indicate M ± SEM in 8 experiments for the day 3 harvest and 6 experiments for the day 7 harvest. □ Control; □, AMLR activated T cells added; □, MLR activated T cells added.
the other hand, T cells activated by AMLR expressed dual functions for BFU-E growth depending on the culture period of AMLR.

DISCUSSION

The present study demonstrates that T cells activated by autologous non-T cells in AMLR exert both enhancing and suppressing activities in the regulation of in vitro erythropoiesis; those from day 3 AMLR enhanced and those from days 7–12 AMLR suppressed BFU-E growth. Both of these regulatory functions were mediated by an Ia-positive and radiosensitive population within the AMLR-activated OKT4+ cells. Furthermore, activated T cells harvested at an early stage of AMLR enhanced only autologous BFU-E growth, whereas those at a late stage of AMLR suppressed only autologous BFU-E. These observations suggest that in vitro differentiation and proliferation of erythroid progenitor cells may be regulated at least partly by autoreactive T cells.

Several investigators have shown that in vitro growth of erythroid progenitor cells can be enhanced by coculturing them with normal or unactivated T cells and T-cell subsets.1-3 Similarly, recent studies indicate that unactivated T-cell subsets, both OKT4+ and OKT8+ cells, could stimulate in vitro growth of BFU-E without collaboration between them.4,5 In the present study, only OKT4-positive T cells activated by 3-day AMLR increased in vitro growth of peripheral blood BFU-E. These AMLR-induced BFU-E-enhancing T cells were found to be Ia-positive and radiosensitive. Therefore, this AMLR-activated T-cell subset is probably different from T-cell subsets affecting BFU-E growth reported by others.2,3,22,23 Mechanisms by which AMLR-activated T cells exerted the BFU-E growth-enhancing activity are unknown. One possible explanation is that they are capable of producing burst-promoting activities, as described by Winisiewski et al.22

The present study provides evidence suggesting that AMLR-activated T cells may exert dual functions in the regulation of BFU-E growth. Torok-Storb et al.12,26 have also demonstrated that normal T cells affecting BFU-E growth can be divided into at least two functionally different subsets defined by monoclonal antibodies and that a population of Ia-positive T cells limits BFU-E growth. Similarly, Mangan et al.13 reported the presence of BFU-E-regulating T-cell subpopulations defined by Fc receptors and monoclonal antibodies. However, these T-cell subsets with regulatory functions were not activated in vitro. Our data show that in vitro colony formation by BFU-E was affected by the two functionally different T-cell subsets. Both of them were Ia-positive and radiosensitive within the OKT4+ phenotype but they were harvested at different stages of AMLR for activation. From our data, however, it is not determined whether these T-cell subsets with opposing functions were identical or not before activation of AMLR, one possibility is that there may exist two distinct subpopulations before activation, and another is that the same T-cell population may be induced to express the two opposing functions successively upon activation in AMLR.

In contrast to nonspecific or genetically unrestricted stimulation of BFU-E growth by unactivated or activated T cells in our study and others,6,12-24 T cells activated by 7-day AMLR suppressed only autologous, but not allogeneic BFU-E. This suggests a possible presence of genetic restriction in expressing a regulatory function by AMLR-induced BFU-E-suppressor T cells. Genetic restriction of T cells suppressing erythroid colony growth has been reported by Torok-Storb and Hansen26 and Lipton et al.27 These erythroid suppressor T cells were Ia-positive, although they were not activated in vitro. Data from the former study26 suggest that a subset of Ia-positive T cells may suppress BFU-E growth by limiting a monocyte-dependent T-cell stimulation activity for BFU-E growth through HLA-DR antigen recognition.28 This is unlikely in our study since OKIa1 plus complement treatment of AMLR-activated T cells abolished suppression of BFU-E growth but did not result in increased stimulation of BFU-E growth. Since activated T cells are able to generate a variety of lymphokines,11,29,30 and interferons can inhibit proliferation of hematopoietic progenitor cells,11,31 it is possible that suppression of BFU-E growth may be mediated by interferon(s) produced by activated OKT4+ cells from day 7 AMLR. Recently we have shown that concanavalin-A–stimulated T cells can suppress in vitro growth of erythroid and myeloid progenitor cells;2 they were OKT8-positive but Ia-negative. Thus, our data together with others indicate that T cells involved in the regulation of in vitro erythropoiesis are heterogeneous in their immunologic phenotype.

AMLR has been shown to be abnormal or defective in a variety of diseases characterized by disturbed immune regulations.18,32 Recently Kotani et al.13 have demonstrated that OKT4+ cells activated for 3 days in AMLR exert helper activity for pokeweed mitogen-induced immunoglobulin synthesis while those from day 7 AMLR express suppressor activity. These observations suggest that AMLR may play an important role in maintaining normal immune regulation. In this regard, our data indicate a possibility that AMLR may be actively involved in the immune-mediated regulation of in vitro erythropoiesis; dual functions successively exerted by AMLR-activated T cells could provide a “regulatory circuit” in which AMLR-induced BFU-E-suppressor T cells may exert a “feedback suppression” for BFU-E growth stimulated by AMLR-induced BFU-E-enhancing T cells.

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