T Lymphocyte and Monocyte-Macrophage Interaction in Colony-Stimulating Activity Elaboration in Man


Using in vitro culture systems, we have studied the effect of coincubating human monocyte-macrophages with autologous T lymphocytes on colony-stimulating activity (CSA) elaboration in response to methanol extraction residue (MER) of BCG. When monocyte-macrophages were coincubated with T lymphocytes for 4 days at a ratio of 1:3, the resultant colony-stimulating activity, assayed against light density nonadherent human marrow cells, was significantly higher ($p < 0.01$) than that derived from macrophages incubated alone at equivalent cell concentrations. The T lymphocytes did not elaborate any significant CSA in response to MER. On the other hand, when monocyte-macrophages were coincubated with concanavalin A and thymosin (fraction 5) primed T lymphocytes at a ratio of 1:3, the respective resultant CSA was only 51% and 46%, respectively, of that obtained from monocyte-macrophages coincubated with nonprimed T lymphocytes. This suppression was noted for both neutrophil and eosinophil colonies, although it was more marked for the latter. These data suggest that T lymphocytes may augment as well as suppress the CSA elaboration when incubated with monocyte-macrophages. This T-lymphocyte modulation of CSA elaboration may depend on the degree of prior activation of helper and suppressor T lymphocytes.

Pluripotent hemopoietic stem cells (PHSC), after an unknown number of multiplications under the effect of largely unknown stimuli, differentiate into various progenitor cells. These cells are committed to proliferating and differentiating usually into one type of cell lineage. Granulocyte-macrophage progenitor cell (GM-CFC), a bipotent cell committed to giving rise to granulocytes and monocytes, can be cloned in agar culture system in vitro when stimulated with colony-stimulating factor (CSF).

Monocyte-macrophages have been shown to be an important source of CSF. Mitogen-stimulated lymphocytes have also been shown to elaborate colony-stimulating activity (CSA). In addition to neutrophil colonies, this activity has been shown to stimulate eosinophil colony formation. Recently, Cerny has demonstrated that T lymphocytes may also elaborate a factor that stimulates PHSC proliferation. Also, Goodman and Shinpock have amply demonstrated the T-lymphocyte augmentation of hemopoietic recovery in lethally irradiated mice after marrow infusion. However, there are no reports in the literature of T-
lymphocyte modulation of myelopoiesis at the GM-CFC level in man. In this article, we describe T-lymphocyte-mediated modulation of CSA elaboration in man.

**MATERIALS AND METHODS**

*Acquisition of Specimens*

Peripheral blood, a source of monocytes and T lymphocytes, was obtained by venipuncture from normal human volunteers. The marrow cells used for assaying CSA were obtained from patients with no evident hematologic disorder or marrow involvement. Before acquiring any specimens for experimentation, a written, signed consent was obtained from the volunteers, as required by the surveillance committee on human experimentation at The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute.

*Cell Separation Procedures*

**T Lymphocytes.** To obtain mononuclear cells, defibrinated blood was diluted in an equal volume of α-MEM (alpha modification of minimum essential medium) plus 15% fetal calf serum (FCS) and centrifuged at 400 g for 35 min over Ficoll-Hypaque (density 1.077 g/ml) columns in 15-ml plastic tubes. To separate T lymphocytes, we used a modification of the method described by Weiner et al.20 Briefly, 1 ml of 5% sheep erythrocyte suspension was added to 0.2 ml neuraminidase solution (1 μg/ml) and washed 3 times with α-MEM after incubating for 1 hr at 37°C. Subsequently, the mononuclear cells were incubated with neuraminidase-treated erythrocytes (NE), as described,21 and the NE rosettes pelletted by centrifugation over a Ficoll-Hypaque column (density 1.080 g/ml). The NE in the pellet were lysed with Tris buffer, and the remaining cells subjected to carbonyl-iron phagocytosis and plastic adherence procedures to remove contaminating phagocytic and adherent cell populations.22 The cells thus obtained always contained >99% NE rosetting cells.

**Monocyte-Macrophages.** After the separation of NE-rosetting cells on Ficoll-Hypaque gradient (density 1.080 g/ml), the T-lymphocyte-depleted interface cells were gently aspirated with a Pasteur pipette. These cells usually contained less than 1% E-rosetting cells. To obtain monocyte-macrophages, the interface cells (2 × 10⁶/ml) were subjected to active adherence in 60 × 15 mm polystyrene culture dishes (Corning Glass Works, Corning, N.Y.) by incubation for 3 hr in α-MEM in the presence of 20% FCS at 37°C in 5% CO₂ atmosphere (using lower concentrations of FCS did not increase the proportion of cells adhering to the plastic dishes, as others had reported).23 To obtain the required number of macrophages in the dish, the number of interface cells incubated was adjusted according to the proportion of phagocytic cells obtained by the latex-phagocytosis method.24 For example, with 20% latex-phagocytizing cells in the interface cell suspension, to obtain 2 × 10⁶ in the dish, 10 × 10⁶ interface cells were incubated. Subsequently, the nonadherent cells contained in the supernatant were aspirated and dishes washed twice with α-MEM with 15% FCS. The number of adherent cells remaining in the dish was determined by subtracting the number of nonadherent cells obtained per dish from the total interface cells subjected to adherence procedure per dish. Usually this correlates well with the predicted number of adherent cells based on the percentage of latex-phagocytizing cells in the interface cells. The adherent cells thus obtained in the dish usually contained ≥99% latex-phagocytizing cells. For coincubation experiments, T lymphocytes were added in appropriate numbers to the dishes containing adherent cell monolayers.

**Light Density, Nonadherent Marrow Cells.** Light density, nonadherent human marrow cells for CSA assays were obtained as described elsewhere.25 Briefly, light density cells (1.077 or 1.070 g/ml) obtained by Ficoll-Hypaque gradient centrifugation were subjected to the plastic adherence procedure described above. Subsequently, the nonadherent cells were aspirated, washed once, and resuspended in α-MEM plus 15% FCS at a cell concentration of approximately 10⁶/ml. These cells were later used as target cells for CSA assays.

*Experimental Design*

The effects of coincubation of fresh nonprimed T lymphocytes with monocyte-macrophages on CSA elaboration were studied by incubating the two cell populations separately and together at equivalent cell concentrations (2 × 10⁶/ml) in α-MEM with 15% FCS at 37°C in an atmosphere of 5% CO₂. The
ratios of monocyte-macrophages to T lymphocytes tested were 1:1 and 1:3. To avoid any quantitative and qualitative effects on CSA elaboration due to change in cell numbers per milliliter of culture media, the total cell concentrations were kept constant regardless of the constituting cell populations in the dishes. Thus, at 1:1 ratio, 10⁶ macrophages were incubated with 10⁶ T lymphocytes/ml, and at 1:3 ratio, 0.5 × 10⁶ macrophages were incubated with 1.5 × 10⁶ T lymphocytes/ml. To stimulate CSA elaboration, methanol extraction residue (MER) of BCG (NCI, Bethesda, Md., Lot BV-74-228) was used at 100 µg/ml concentrations. The media were harvested after 96 hr, centrifuged at 1200 g for 10 min to remove cell debris, and dialyzed against α-MEM with 5% FCS for 3 days to remove any low molecular weight inhibitors. These conditioned media (CM) were then assayed for CSA after passing through 0.22-µm Millipore filters. Dose-response curves were not obtained because of the lack of CM in enough quantities.

The effect of concanavalin A (Con-A; Pharmacia, Fine Chemicals, Uppsala, Sweden) and thymosin fraction 5 (Hoffmann-LaRoche Inc., Nutley, N. J.) primed T lymphocytes on monocyte-macrophage-derived CSA was shown by two types of experiments. In the first type, mononuclear cells (2 × 10⁶/ml) were incubated with 20 µg/ml of Con-A or 100 µg/ml of thymosin for 40 hr. Subsequently, the cells were washed thoroughly with α-MEM plus 15% FCS and subjected to the T-lymphocyte separation procedure described above. The T lymphocytes thus obtained were then incubated with autologous monocyte-macrophages to prepare the conditioned media. In the second type of experiment, the purified T lymphocytes (2 × 10⁶/ml) were incubated with Con-A (20 µg/ml) or thymosin (100 µg/ml) for 40 hr and then, after thorough washing, incubated as such with autologous monocyte-macrophages to prepare the conditioned media. The monocyte-macrophage to T lymphocyte ratio used in these experiments was always 1:3. Methanol extraction residue of BCG was again used to stimulate CSA elaboration. Conditioned media were harvested after 96 hr of incubation and then assayed for CSA after dialysis and filtration, as described above.

Colony-Stimulating Activity Assay

Colony stimulating activity was assayed against light density (± 1.077 g/ml for experiment 1 and ± 1.070 g/ml for experiment 2), nonadherent human marrow cells incorporating 0.2 ml of the CM per dish in underlayers, as described elsewhere. Morphological examination of colonies was performed as described by Verma et al. Statistical Methods

The mean colony incidences were compared by two-sample t tests after square-root transformation and using the pooled within-triplicate standard deviation.

RESULTS

Table 1 presents the data on the effects of coincubating fresh nonprimed T lymphocytes with monocyte-macrophages. The CM prepared from monocyte-macrophages alone (2 × 10⁶/ml, total 4 × 10⁶/dish) produced 13.7 ± 1.5 (1 SD) colonies and 245 ± 16.8 clusters/10⁵ cells plated. The T-lymphocyte CM produced no colonies or clusters. However, the CM from dishes containing both macrophages and T lymphocytes produced 6.7 ± 1.5 colonies and 556.7 ± 32.1 clusters at the 1:1 ratio (macrophages 1 × 10⁶/ml, total 2 × 10⁶/dish) and 19.7 ± 1.5 colonies and 826.7 ± 32.1 clusters at the 1:3 ratio (macrophages 0.5 × 10⁶/ml, total 10⁶/dish). The colony incidence at 1:3 ratio is significantly higher than that resulting from macrophage-derived CM (p < 0.01 based on triplicate readings), and the difference is probably even more significant when the total macrophages numbers are considered.

Table 2 shows the effect of coincubating Con-A-primed and thymosin-primed T lymphocytes with monocyte-macrophages on subsequent CSA elaboration. The priming of T lymphocytes was achieved by preincubating mononuclear cells prior to T-lymphocyte separation or after they were purified (see Materials and Methods).
Table 1. Comparison of CSA in the Conditioned Media Prepared by Incubating Human Peripheral Blood Monocyte-Macrophages and T Lymphocytes Individually and Together at the Ratios of 1:1 and 1:3, Respectively

<table>
<thead>
<tr>
<th>Cell Source of Conditioned Media</th>
<th>Ratio of Macrophages to T Lymphocytes</th>
<th>Number of Individual Cell Population During Incubation (x 10^7/ml)</th>
<th>Number of Colonies and Clusters*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Macrophages T Lymphocytes</td>
<td>Colonies Clusters</td>
</tr>
<tr>
<td>Monocyte-macrophages</td>
<td>2.0</td>
<td>13.7 ± 1.5 254.7 ± 16.8</td>
<td></td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>2.0</td>
<td>0.0 0.0</td>
<td></td>
</tr>
<tr>
<td>Monocyte-macrophages plus T lymphocytes</td>
<td>1:1</td>
<td>1.0 1.0</td>
<td>6.7 ± 1.5 556.7 ± 32.1</td>
</tr>
<tr>
<td>Monocyte-macrophages plus T lymphocytes</td>
<td>1:3</td>
<td>0.5 1.5</td>
<td>19.7 ± 1.5 826.7 ± 32.1</td>
</tr>
</tbody>
</table>

To stimulate CSA elaboration, MER (100 µg/ml) was always used during the incubation of cells for preparation of CM. At 1:3 ratio a significantly higher (p < 0.01, triplicate readings) CSA is noted compared to that in CM from monocyte-macrophages incubated alone.

* Per 10^5 nonadherent human marrow cells (density ≤ 1.077 g/ml) scored on day 8 of culture; represented as mean of triplicate readings ± 1 SD.
† Aggregates of ≥ 40 cells.
‡ Aggregates of 3-39 cells.

Since the marrow target cells were of lighter density (≤ 1.070 g/ml) than those in the previous experiment (≤ 1.077 g/ml), the colony and cluster incidences were higher in this experiment than in the previous. As measured by the colony and cluster incidences on days 8 and 14, the CSA in the CM from monocyte-macrophages coincubated with nonprimed T lymphocytes was significantly higher.

Table 2. Comparison of CSA in the Conditioned Media Prepared by Incubating Human Peripheral Blood Monocyte-Macrophages Alone and Together With Autologous Nonprimed or Con-A- and Thymosin-Primed T Lymphocytes at 1:3 Ratio

<table>
<thead>
<tr>
<th>Cell Source of Conditioned Media</th>
<th>Ratio of Macrophages to T Lymphocytes</th>
<th>Number of Individual Cell Population During Incubation (x 10^7/ml)</th>
<th>Number of Colonies and Clusters*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Macrophages T Lymphocytes</td>
<td>Colonies Clusters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 8 Day 14</td>
<td></td>
</tr>
<tr>
<td>Monocyte-macrophages</td>
<td>2.0</td>
<td>40.0 ± 1.5 250.0 ± 9.0</td>
<td>43.7 ± 2.3 270.0 ± 10.0</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>2.0</td>
<td>0.0 10.0 ± 1.0</td>
<td>2.0 ± 0.7 43.0 ± 3.5</td>
</tr>
<tr>
<td>Monocyte-macrophages plus T lymphocytes</td>
<td>1:3</td>
<td>0.5 1.5</td>
<td>66.3 ± 3.5 430.0 ± 12.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 8 Day 14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colonies Clusters</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.0 ± 1.5 250.0 ± 9.0</td>
<td>43.7 ± 2.3 270.0 ± 10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0 10.0 ± 1.0</td>
<td>2.0 ± 0.7 43.0 ± 3.5</td>
</tr>
<tr>
<td>Monocyte-macrophages plus Con-A-primed T lymphocytes</td>
<td>1:3</td>
<td>0.5 1.5</td>
<td>35.7 ± 2.5 250.0 ± 22.0</td>
</tr>
<tr>
<td>Monocyte-macrophages plus Con-A-primed T lymphocytes</td>
<td>1:3</td>
<td>0.5 1.5</td>
<td>33.7 ± 1.5 310.0 ± 10.0</td>
</tr>
<tr>
<td>Monocyte-macrophages plus Con-A-primed T lymphocytes</td>
<td>1:3</td>
<td>0.5 1.5</td>
<td>32.0 ± 1.5 280.0 ± 10.0</td>
</tr>
<tr>
<td>Monocyte-macrophages plus Con-A-primed T lymphocytes</td>
<td>1:3</td>
<td>0.5 1.5</td>
<td>30.0 ± 4.0 280.7 ± 7.6</td>
</tr>
<tr>
<td>Monocyte-macrophages plus thymosin-primed T lymphocytes</td>
<td>1:3</td>
<td>0.5 1.5</td>
<td>38.7 ± 1.5 220.0 ± 10.0</td>
</tr>
<tr>
<td>Monocyte-macrophages plus thymosin-primed T lymphocytes</td>
<td>1:3</td>
<td>0.5 1.5</td>
<td>35.0 ± 2.3 150.0 ± 9.0</td>
</tr>
</tbody>
</table>

To stimulate CSA elaboration, MER (100 µg/ml) was always used during the incubation of cells for preparation of CM. The CSA in CM from monocyte-macrophages plus nonprimed T lymphocytes is again significantly higher (p < 0.01, triplicate readings) than that in monocyte-macrophage-CM. However, priming of T lymphocytes with Con-A or thymosin fraction 5 prior to their coincubation with autologous monocyte-macrophages causes significant (p < 0.01, triplicate readings) suppression of CSA elaboration as represented by decrease in the number of colonies and clusters in this table.

* Priming was done by incubating mononuclear cells (2 x 10^7/ml) with Con-A (20 µg/ml) or thymosin (100 µg/ml) for 40 hr.
† Priming was done by incubating purified T lymphocytes (2 x 10^7/ml) with Con-A or thymosin as above.
Table 3. Comparison of the Ability to Stimulate Various Types of Colonies by the CM Prepared by Incubating Human Peripheral Blood Monocyte-Macrophages With Autologous Nonprimed T Lymphocytes and Con-A- or Thymosin-Primed T Lymphocytes

<table>
<thead>
<tr>
<th>Cell Source of Conditioned Media</th>
<th>Day of Score</th>
<th>Neutrophil (%)</th>
<th>Neutrophil Macrophage (%)</th>
<th>Macrophage (%)</th>
<th>Eosinophil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte-macrophages plus</td>
<td>8</td>
<td>57.1 (86.1)</td>
<td>6.2 (9.3)</td>
<td>0.0</td>
<td>3.0 (4.6)</td>
</tr>
<tr>
<td>nonprimed T lymphocytes</td>
<td>14</td>
<td>4.9 (6.8)</td>
<td>36.0 (50.0)</td>
<td>4.9 (6.8)</td>
<td>26.2 (36.4)</td>
</tr>
<tr>
<td>Monocyte-macrophages plus</td>
<td>8</td>
<td>27.8 (82.6)</td>
<td>4.4 (13.0)</td>
<td>0.0</td>
<td>1.5 (4.4)</td>
</tr>
<tr>
<td>Con-A-primed T lymphocytes</td>
<td>14</td>
<td>3.2 (7.7)</td>
<td>31.8 (77.0)</td>
<td>1.6 (3.8)</td>
<td>4.7 (11.5)</td>
</tr>
<tr>
<td>Monocyte-macrophages plus</td>
<td>8</td>
<td>26.5 (86.3)</td>
<td>2.8 (9.2)</td>
<td>0.0</td>
<td>1.4 (4.5)</td>
</tr>
<tr>
<td>thymosin-primed T lymphocytes</td>
<td>14</td>
<td>3.0 (7.7)</td>
<td>28.3 (73.1)</td>
<td>2.9 (7.7)</td>
<td>4.5 (11.5)</td>
</tr>
</tbody>
</table>

Since a majority of neutrophil colonies grow fully by day 7 or 8 and a majority of eosinophil colonies develop fully by day 14 as described before, the morphological examination of colonies was performed on both day 8 and 14 of the culture. A significant decrease in both neutrophil (day-8 cultures) and eosinophil (day-14 cultures) colonies is noted; the latter, however, are suppressed more.

The mean percent incidences and incidences per 10⁵ cells plated (calculated as percent incidence of colony subtype × mean incidence of a total of all types of colonies ÷ 100). The figures in parentheses represent percentage values.

DISCUSSION

Bolin and Robinson and Robinson et al. recently demonstrated that human peripheral blood lymphocytes may play a role in monocyte-macrophage-derived CSF elaboration. However, these authors have not reported on the types of lymphocytes involved in this interaction. Recently, we reported that for a maximum CSA elaboration by human marrow cells, a light density adherent and nonadherent cell interaction is required. As a further extension of this study, we performed the experiments described in this paper.

We find that fresh, nonprimed T lymphocytes do not elaborate any significant CSA when stimulated with MER, although they do so when incubated with PHA.
(data not shown). When these T lymphocytes were incubated with monocyte-macrophages at a ratio of 1:1, the cluster incidence was higher than that obtained with macrophage-CM, but the colony incidence was lower. The decreased colony-stimulating activity at 1:1 ratio could be due to a combination of lower number of monocyte-macrophages (10⁶/ml compared to 2 x 10⁶/ml in control) coincubated with the less than optimum concentration of T lymphocytes required for maximizing the CSA elaboration. On the other hand, when the proportion of T lymphocytes was increased to 3:1, a significant increase in both colony and cluster incidence was noted. Since the total cell concentration in the dishes containing both types of cells was the same as in the dishes with individual cell populations alone, and the dishes containing both types of cells had a significantly lower concentration of monocytes (10⁶/ml at 1:1 ratio and 0.5 x 10⁶/ml at 1:3 ratio) than to the dishes with macrophages alone (2 x 10⁶/ml), this increase in the colony and cluster incidences provides a clear evidence for synergistic interaction between T lymphocytes and macrophages. It is possible that T lymphocytes, which do not elaborate any CSA alone in response to MER, do so when incubated with monocyte-macrophages and thereby add to this synergism. Alternatively, T lymphocytes may stimulate monocyte-macrophages and enhance CSA elaboration by humoral or cell-cell interactions.

In recent years, T lymphocytes have been classified into those that stimulate (helper cells) and those that suppress (suppressor cells) B-lymphocyte antibody synthesis. At high dose levels, Con-A and thymosin (fraction 5) have been demonstrated to stimulate suppressor T-cell activity. Therefore, we decided to investigate the effect of coincubating Con-A-primed and thymosin-primed T lymphocytes with monocyte-macrophages on subsequent CSA elaboration.

We find that Con-A-primed and thymosin-primed T lymphocytes unlike nonprimed T lymphocytes when coincubated with monocyte-macrophages cause remarkable suppression of CSA elaboration. Achieving the priming by incubation prior to, rather than after, the purification of T lymphocytes does not make any significant difference in terms of this suppression. The T-lymphocyte effect may be related to the enhanced suppressor T-cell activity caused by Con-A and thymosin (fraction 5), or it may be due to Con-A or thymosin's rendering the T lymphocytes resistant to the possible monocyte-macrophage-mediated stimulating of CSA elaboration as suggested earlier. Alternatively, it is possible that Con-A- or thymosin-stimulated suppressor cells could inhibit CSA elaboration by some other subsets of T lymphocytes. The elaboration of low molecular weight inhibitors causing this decrease in CSA can be ruled out as we always dialyzed the CM prior to assaying them for CSA; however, the presence of high molecular weight inhibitors in these CM cannot be ruled out. Morphological examination of the colonies produced by various CM reveals that the T-lymphocyte-induced increase in colony formation is due to a parallel increase in all types of colonies, and the suppression seen with Con-A-primed and thymosin-primed T lymphocytes is due to a decrease in both neutrophil and eosinophil colonies, primarily the latter.

We have demonstrated that T lymphocytes coincubated with monocyte-macrophages in the presence of MER may augment or suppress the resultant CSA. This is the first report of suppressor lymphocyte mechanism in CSA elaboration. These interactions may be related to the presence of a dominant subtype of T lymphocytes, i.e., helper cells or suppressor cells, during the coincubation period.
Further studies are needed to delineate the exact mechanisms involved in these interactions.

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REFERENCES


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