Monocyte-mediated Inhibition of Lymphocyte Blastogenesis in Hodgkin Disease

By Geraldine P. Schechter and Frances Soehnlen

Mononuclear leukocytes isolated from the blood of previously treated patients with advanced active Hodgkin disease contained high concentrations of monocytes and showed poor lymphocyte blastogenesis to mitogens. In five of eight patients with disseminated disease, blastogenesis became normal or improved markedly when the leukocyte suspensions were depleted of monocytes before culture. Addition of autologous macrophages to the monocyte-depleted lymphocytes resulted in a reappearance of the inhibition of blastogenesis. Monocyte inhibition was associated with the presence of active disease, lymphocytopenia, and low lymphocyte/monocyte ratios in the peripheral blood. The role of previous treatment is uncertain, since inhibition tended to disappear when the patients were retreated. Inhibitory monocyte-lymphocyte interactions may be one of the causes of impaired cell-mediated immunity in Hodgkin disease.

MANY LABORATORIES have found in the last decade that monocytes and macrophages potentiate the lymphocyte blastogenic response to mitogens and antigens in both human and animal lymphocyte cultures. Recently, however, there have been a number of reports of suppression of lymphocyte transformation by macrophages or adherent cells from animals thought to have “activated” macrophages. Rodents bearing tumors, undergoing graft-versus-host (GVH) reactions, or treated with BCG or Corynebacterium parvum have been found to have “suppressor” macrophages.

The present report describes evidence for analogous monocyte-mediated suppression of lymphocyte transformation in some patients with advanced Hodgkin disease. Severe impairment of lymphocyte blastogenesis to phytohemagglutinin, pokeweed mitogen, and antigen could be reversed by depletion of monocytes from the mononuclear cells of these patients.
MATERIALS AND METHODS

Patients and controls. The leukocytes of 12 adults with Hodgkin disease and 12 healthy adults were studied. The extent of disease in the patients was determined according to the Ann Arbor staging classification.9 Nine of the patients had advanced Hodgkin disease (stages IIIB, IVA, IVB), and three had localized disease (IIA, IIB, and a late marginal recurrence after radiotherapy in a stage IIIB patient). (The patients are listed individually in Table 2 and are denoted by the same numerals in the later tables.) The patients with advanced disease had been previously treated with radiotherapy and/or chemotherapy. The interval between the completion of any previous therapy and the time of initial study ranged from 1 mo to 3 yr, the median interval being 5 mo and the average 10 mo. All but one patient (No. 9) had active disease at the time of study. Two patients (Nos. 1 and 3) were studied during periods of both remission and relapse. The patients gave informed consent for the studies; the normal subjects were hospital employees who were paid volunteers.

Each of the patients was skin tested with 0.1 cc of at least two of four antigens, including mumps antigen (Lilly), streptokinase-streptodornase (Lederle), Candida albicans (Hollister-Stier), and intermediate strength PPD tuberculin (Connaught). All of the patients with advanced disease and two of the three patients with localized disease were anergic to these antigens.

Preparation of leukocyte cultures. A heparinized blood specimen from each subject was divided into two parts. One part was incubated with carbonyl iron powder (General Aniline and Film, Dyestuff and Chemical Division, Linden, N.J.), 150 mg to 10 cc blood at 37°C for 60 min. Thereafter the two portions of blood were treated identically. The mononuclear cell fractions from each were isolated by Hypaque-Ficoll density gradient centrifugation using the method of Boyum.10 The monocyte-rich lymphocyte suspensions are denoted in the tables as Hypaque-Ficoll (HF) cells and the monocyte-depleted lymphocyte suspension derived from the carbonyl iron treated fraction are called the iron Hypaque-Ficoll (FeHF) cells. Monocyte-depleted lymphocyte suspensions were also prepared by passage of leukocyte-rich plasma over glass bead columns as previously described.3 Differential counts were done by phase-contrast microscopy using a hemocytometer. These counts were checked in selected instances by examination of Wright-Giemsa stained smears and preparations stained for nonspecific esterase activity using α-naphthyl acetate as the substrate.11

The stimulants incubated with the cells included phytohemagglutinin (PHA) 1 μg/ml (Burroughs Wellcome, N.C.), pokeweed mitogen (PWM) 40 μg/ml (Grand Island Biological, Grand Island, N.Y.), and streptolysin O (SLO) 0.01", (Difco Laboratories). The monocyte-rich and -depleted cultures were adjusted to contain the same number of lymphocytes. The PHA-stimulated cultures contained 200,000 lymphocytes/well. Cultures with other mitogens contained 400,000 lymphocytes/well. In certain experiments, cells were irradiated with a cobalt γ source as previously described.3 The cells were cultured in flat-bottomed microtiter plates (Falcon, Oxnard, Calif.) in 10% autologous plasma and 90% RPMI-1640 with HEPES buffer (Grand Island Biological) in a final volume of 200 μl. The plates were sealed with a plastic adhesive cover and incubated at 37°C. The culture period depended on the mitogens used: PHA cultures were incubated for 3 or 4 days, PWM and SLO cultures for 4 or 5 days. For the final 4 hr of incubation 1 μCi of 3H-thymidine (5 Ci/mole) (Amersham-Searle, Arlington Heights, Illinois) was added to each culture. The cells were then deposited on a glass fiber filter disc with a multichannelled cell harvester (Model M-12, Biomedical Research Institute, Rockville, Md.), and scintillation counting was done.

Experiments were performed in triplicate, and individual cultures were generally well within 10% of the mean. Cultures selected for microscopic examination were grown in wells containing a glass cover slip that was removed at the end of the incubation period and stained with Wright-Giemsa.

The ability of the lymphocytes in the monocyte-rich and monocyte-depleted suspensions to bind sheep red cells (E rosetting) was examined by the method of Bentwich et al.12 using heat-inactivated AB serum (Grand Island Biological) and an overnight incubation at 4°C. The absolute peripheral blood lymphocyte and monocyte levels were calculated from the white blood cell count (Coulter Counter, Model S) and the percentage of lymphocytes and monocytes determined from a Wright-Giemsa-stained smear. The data were analyzed according to the t-test for independent samples.13
**Table 1. Monocytes (%) in Mononuclear Cell Suspensions Isolated from Peripheral Blood**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>HF Monocytes (%)</th>
<th>FeHF Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>12</td>
<td>12.6 ± 3 (6-16)</td>
<td>1.3 ± 0.5 (1-2)</td>
</tr>
<tr>
<td>Hodgkin disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>8</td>
<td>38.5 ± 4.3 (23-61)*</td>
<td>5.5 ± 5.0 (1-14)</td>
</tr>
<tr>
<td>Localized</td>
<td>3</td>
<td>16.6 ± 5.8 (10-20)</td>
<td>2.0 ± 1.0 (1-3)</td>
</tr>
<tr>
<td>Inactive</td>
<td>3</td>
<td>19.8 ± 4.5 (14-25)</td>
<td>1.8 ± 1.0 (1-3)</td>
</tr>
</tbody>
</table>

Mean ±SD, range in parentheses. HF, mononuclear cells prepared by Hypaque-Ficoll centrifugation of whole blood. FeHF, mononuclear cells depleted of monocytes by incubation of whole blood with carbonyl iron powder prior to Hypaque-Ficoll centrifugation.

*p < 0.001 compared to normal controls.

**RESULTS**

**Characteristics of the Isolated Leukocyte Suspensions**

The percentage of monocytes in the mononuclear cell suspensions from the Hodgkin disease patients and normal controls is shown in Table 1. These percentages were determined by phase-contrast microscopy differential counts of the cell suspensions cultured in the experiments reported below in Tables 2 and 3. Patients with active, advanced disease had the highest mean concentration of monocytes (39%) in the Hypaque-Ficoll leukocyte suspensions, which was significantly different from the mean (13%) of the normal controls (p < 0.01).

**Table 2. ³H-Thymidine Incorporation of PHA-stimulated Monocyte-rich and Monocyte-depleted Lymphocyte Cultures**

<table>
<thead>
<tr>
<th>Patients*</th>
<th>Monocyte-rich</th>
<th>Monocyte-depleted</th>
<th>Inhibition Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. IVB relapse</td>
<td>100</td>
<td>4450§</td>
<td>44.5</td>
</tr>
<tr>
<td>2. IVA relapse</td>
<td>170</td>
<td>3110§</td>
<td>18.3</td>
</tr>
<tr>
<td>3. IVB relapse</td>
<td>200</td>
<td>770§</td>
<td>3.9</td>
</tr>
<tr>
<td>4. IIIB relapse</td>
<td>470</td>
<td>2790§</td>
<td>5.9</td>
</tr>
<tr>
<td>5. IIIB relapse</td>
<td>1050</td>
<td>5290§</td>
<td>5.0</td>
</tr>
<tr>
<td>6. IVB relapse</td>
<td>850</td>
<td>760</td>
<td>0.9</td>
</tr>
<tr>
<td>7. IVB relapse</td>
<td>1960</td>
<td>2530</td>
<td>1.3</td>
</tr>
<tr>
<td>8. IIIB relapse</td>
<td>3100</td>
<td>2720</td>
<td>0.9</td>
</tr>
<tr>
<td>9. IVB remission</td>
<td>2070</td>
<td>3075</td>
<td>1.5</td>
</tr>
<tr>
<td>Localized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. IIIB marginal recurrence</td>
<td>2970</td>
<td>2320</td>
<td>0.8</td>
</tr>
<tr>
<td>11. II A untreated</td>
<td>5230</td>
<td>4940</td>
<td>0.9</td>
</tr>
<tr>
<td>12. II B untreated</td>
<td>4240</td>
<td>2780</td>
<td>0.7</td>
</tr>
<tr>
<td>Normal Controls (n = 12, mean ± SD)</td>
<td>4600 ± 2690</td>
<td>5310 ± 2050</td>
<td>1.4 ± 0.7</td>
</tr>
</tbody>
</table>

*Patients 2, 5, and 7-10 had nodular sclerosis Hodgkin disease; the remaining had mixed cellularity Hodgkin disease.

§Disintegrations per second. ³H-thymidine incorporation of cultures without PHA was 60 DPS in all cases and 20 DPS in 85% of the cultures.

†Ratio of the ³H-thymidine incorporation of the monocyte-depleted culture to that of the monocyte-rich culture.

§³H-thymidine incorporation of the monocyte-depleted culture was significantly greater than that of monocyte-rich culture.
Table 3. Consecutive Studies in Three Patients with Active Hodgkin Disease and Monocyte Inhibition of PHA-stimulated Lymphocyte Blastogenesis: Correlation With Monocytes (%) in Monocyte-rich Cultures

<table>
<thead>
<tr>
<th>Patient</th>
<th>Exp.*</th>
<th>Monocytes (%)</th>
<th>³H-Thymidine Incorporation (DPS)</th>
<th>Inhibition ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Monocyte-rich</td>
<td>Monocyte-depleted</td>
</tr>
<tr>
<td>1t</td>
<td>1</td>
<td>26</td>
<td>170</td>
<td>2370</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>61</td>
<td>100</td>
<td>4450</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>56</td>
<td>150</td>
<td>3450</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>25</td>
<td>2050</td>
<td>2030</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>44</td>
<td>140</td>
<td>3450</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>56</td>
<td>50</td>
<td>5170</td>
</tr>
<tr>
<td>2t</td>
<td>1</td>
<td>45</td>
<td>170</td>
<td>3110</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43</td>
<td>210</td>
<td>1850</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>32</td>
<td>1300</td>
<td>2630</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>36</td>
<td>1360</td>
<td>1550</td>
</tr>
<tr>
<td>4t</td>
<td>1</td>
<td>23</td>
<td>470</td>
<td>2790</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>32</td>
<td>1530</td>
<td>7080</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>54</td>
<td>1260</td>
<td>2040</td>
</tr>
</tbody>
</table>

*These experiments were performed during a 4-9-mo period for each patient. The percentage of monocytes in the monocyte-rich Hypaque-Ficoll-separated mononuclear cell fraction is given. The inhibition ratio is the PHA-stimulated ³H-thymidine incorporation of the monocyte-rich culture divided by that of the monocyte-depleted culture.

†Patient 1 was in untreated clinical relapse during experiments 1–3. He was then treated and had a short-lived response at the time of experiment 4. Experiments 5 and 6 were done when the patient relapsed again. Patients 2 and 4 had active disease at the time of each study. Experiments 3 and 4 in patient 2 were done 3 mo after local radiotherapy and 1 mo after combination chemotherapy. Patient 4 had received combination chemotherapy 1–3 mo before each study.

Patients in remission or with localized disease had only a slightly higher monocyte concentration (mean 18%) than the controls. Treatment of blood with carbonyl iron before Hypaque-Ficoll centrifugation consistently depleted the monocytes in the cell suspensions of both the patients and the normal controls. Passage of leukocyte-rich plasma over glass bead columns also depleted monocytes to less than 2%.

The phase-contrast microscopy differential counts of the leukocyte suspensions correlated well with differentials based on Wright-Giemsa–stained smears and preparations stained for nonspecific esterase activity. Leukocyte suspensions from patients with advanced disease also contained occasional myelocytes, atypical lymphocytes, and cells resembling promonocytes. These cells, however, were not depleted by carbonyl iron treatment. The cells that were depleted in all cases were nonspecific esterase-positive cells or mature monocytes. The numbers of T lymphocytes determined by E rosetting in the monocyte-rich and -depleted leukocyte suspensions from the patients were not significantly different in eight experiments (mean ±SD: 36% ± 19% and 39% ± 19%, respectively.) However, the mean percentage of T lymphocytes in the patients was significantly lower than the percentage of T cells in a group of normal controls (58% ± 10%, n = 15, p < 0.01).

The survival of lymphocytes in the two preparations was assessed by counting the number of lymphocytes that excluded trypan blue after being cultured for 3 days without mitogens at 37°C and comparing that number to the original
number of lymphocytes planted. In an experiment done in triplicate with the cells of a patient (No. 1) with advanced Hodgkin disease, there was no difference between the percentages of lymphocytes surviving after 3 days in the monocyte-rich cultures (26%–30%) and in the monocyte-depleted cultures (28%–31%). However, the number of viable lymphocytes from the patient’s cultures was half that of the cells of a normal subject (53%–61%).

**Lymphocyte Blastogenesis in Monocyte-rich and Monocyte-depleted Leukocyte Cultures**

The effect of removal of monocytes on blastogenesis was studied. The patients with advanced disease generally had depressed lymphocyte transformation in response to PHA in the monocyte-rich cultures (Table 2). However, monocyte depletion with carbonyl iron was associated with a marked increase in \(^{3}\text{H}\)-thymidine incorporation by the cultured mononuclear cells from five of eight patients with active advanced disease. In four of the five patients the incorporation rose from being severely impaired to levels within the normal range. In contrast, there was no difference between the thymidine incorporation of the monocyte-rich and the monocyte-depleted cultures from patients with localized or inactive disease or from normal controls. The highest inhibition ratio (ratio of \(^{3}\text{H}\)-thymidine incorporation of monocyte-depleted to that of monocyte-rich cultures) for a normal control was 2.6, and the median for the normals was 1.0. Monocyte depletion had no effect on the \(^{3}\text{H}\)-thymidine incorporation of the unstimulated cultures from either the patients or the controls.

The PHA response of the patients’ cultures was also assessed morphologically. The monocyte-rich cultures from patients 1 through 4 contained many macrophages, few lymphocytes, and only occasional blasts (Fig. 1). In contrast, the cultures prepared after monocyte depletion showed masses of lymphoblasts and few macrophages, similar to PHA-stimulated monocyte-rich cultures from normal individuals (Fig. 2).

Longitudinal studies of the lymphocyte reactivity of the patients showing monocyte inhibition were performed (Tables 3 and 4). During periods of active disease the studies showed high monocyte concentrations in the mononuclear cells prepared without carbonyl iron pretreatment and frequently showed evidence of monocyte inhibition (inhibition ratios >3) of PHA-stimulated cultures. After chemotherapy and during periods of remission, monocyte concentrations in the mononuclear preparations tended to decrease and evidence of inhibition to disappear. However, in not all patients was a direct relationship between the monocyte concentration and inhibition shown (see, for example, patient 4, Table 3), suggesting that a high concentration of monocytes was not the sole requirement for blastogenesis inhibition. Inhibition of PWM- and SLO-stimulated cultures was also seen and reversed with monocyte depletion. A representative experiment from patient 3 is shown in Table 4.

It is well known that patients with widespread Hodgkin disease often have lymphocytopenia and monocytosis. Therefore, the peripheral blood lymphocyte and monocyte levels of the patients with monocyte inhibition were compared to those without (Table 5). Both the lymphocyte levels and the lymphocyte/monocyte ratios were significantly lower (\(p < 0.025\) and \(p < 0.01\), respectively) in the patients who showed monocyte inhibition than in those who
Fig. 1. PHA-stimulated monocyte-rich leukocyte culture from patient 1. Note increased numbers of macrophages and the paucity of lymphocytes or lymphoblasts. Wright-Giemsa. × 630.

Fig. 2. PHA-stimulated monocyte-depleted leukocyte culture from patient 1. Masses of lymphoblasts and rare macrophages are seen. Wright-Giemsa. × 630.
Table 4. Activity of Hodgkin Disease and Monocyte Inhibition of Mitogen- and Antigen-stimulated Lymphocyte Blastogenesis

<table>
<thead>
<tr>
<th>Patient No. 3*</th>
<th>Monocytes (%)</th>
<th>^3H-Thymidine Incorporation (DPS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Remission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF'</td>
<td>20</td>
<td>1390 1040 570 30</td>
</tr>
<tr>
<td>FeHF</td>
<td>2</td>
<td>920 780 210 7</td>
</tr>
<tr>
<td>(2) Relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>56</td>
<td>200 40 40 4</td>
</tr>
<tr>
<td>FeHF</td>
<td>2</td>
<td>770 1350 640 7</td>
</tr>
<tr>
<td>(3) After Therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>18</td>
<td>760 8</td>
</tr>
<tr>
<td>FeHF</td>
<td>1</td>
<td>900 3</td>
</tr>
</tbody>
</table>

*The first study (1) was one of two that showed similar results. The patient had last received chemotherapy 30 mo previously. Relapse occurred 6 mo later, when study (2) was done. Study (3) was done 2 mo after a 5-mo course of combination chemotherapy, at which time there was no evidence of progressive disease.

†See footnote to Table 1. HF, monocyte-rich, FeHF, monocyte-depleted mononuclear cells.

did not. Although a moderate absolute monocytosis was generally noted at the time of monocyte inhibition, the mean level of monocytes was not significantly higher than when monocyte inhibition was not found. Therefore the most relevant index of monocyte inhibition appears to be the ratio of lymphocytes to monocytes in the circulation.

A number of experiments were undertaken to ensure that the monocyte inhibition was not due to trivial reasons, such as sequestration of stimulant by macrophages, and to confirm that the suppression was mediated by monocytes. Increasing the PHA concentration from 1 to 100 μg/ml did not improve the blastogenesis of the monocyte-rich cultures in the patients with reversible suppression. Decreasing the number of cells per culture while holding the lymphocyte/monocyte ratio constant did not change the ^3H-thymidine incorporation in three experiments. Depletion of macrophages by another technique, passage over glass bead columns, also resulted in monocyte-depleted (<2%) lymphocyte suspensions with an increased blastogenic response to PHA.

In an effort to rule out “overcrowding” of the cultures as a cause of monocyte inhibition, the role of monocyte concentration was tested. Mixing the monocyte-rich and -depleted cell suspensions so that the monocyte concentration was halved while the lymphocyte concentration remained constant still resulted in

Table 5. Peripheral Blood Lymphocytes, Monocytes, and Lymphocyte/Monocyte (L/M) Ratio

<table>
<thead>
<tr>
<th>Monocyte Inhibition*</th>
<th>Lymphocytes/μl</th>
<th>Monocytes/μl</th>
<th>L/M†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>590 ± 230</td>
<td>1220 ± 380</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>Absent</td>
<td>1290 ± 600</td>
<td>870 ± 720</td>
<td>1.9 ± 0.9</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.025</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*^3H-thymidine incorporation of PHA-stimulated monocyte-depleted cultures three or more times greater than that of monocyte-rich cultures. Mean ± SD of peripheral blood absolute lymphocyte and monocyte counts and lymphocyte/monocyte ratio is given for five patients at the time of six experiments showing monocyte inhibition and ten patients at the time of ten experiments without monocyte inhibition.

†L/M (mean ± SD) for seven normal controls was 4.4 ± 0.9, which was significantly different from both groups of patients (p < 0.001).
Table 6. Effect of Addition of Adherent Cells on PHA Response of Monocyte-depleted Leukocytes

<table>
<thead>
<tr>
<th>Cells</th>
<th>Monocyte Inoculum per Culture</th>
<th>3H-Thymidine Incorporation (DPS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF*</td>
<td>300,000</td>
<td>60</td>
</tr>
<tr>
<td>FeHF*</td>
<td>2,000</td>
<td>2010</td>
</tr>
<tr>
<td>FeHF + adherent cells†</td>
<td>42,000</td>
<td>830</td>
</tr>
<tr>
<td>Patient 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>265,000</td>
<td>1260</td>
</tr>
<tr>
<td>FeHF</td>
<td>2,000</td>
<td>2040</td>
</tr>
<tr>
<td>FeHF + adherent cells</td>
<td>30,000</td>
<td>710</td>
</tr>
<tr>
<td>FeHF + adherent cells</td>
<td>100,000</td>
<td>770</td>
</tr>
<tr>
<td>Normal Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>20,000</td>
<td>3290</td>
</tr>
<tr>
<td>FeHF</td>
<td>4,000</td>
<td>4460</td>
</tr>
<tr>
<td>FeHF + adherent cells</td>
<td>34,000</td>
<td>4440</td>
</tr>
<tr>
<td>FeHF + adherent cells from patient 4</td>
<td>34,000</td>
<td>3190</td>
</tr>
</tbody>
</table>

Lymphocyte number was constant at 200,000/culture. 3H-Thymidine incorporation of the allogeneic mixture without PHA was 13 DPS.

*See footnote of Table 1. HF, monocyte-rich, FeHF, monocyte-depleted cells.
†Adherent cells (>99% monocytes) were prepared by allowing HF cells to adhere to a plastic surface for 1 hr, after which the nonadherent cells were washed free. The adherent cells were harvested by scraping cells off the surface with a “rubber policeman.” Adherent cells are autologous, unless otherwise stated.

marked inhibition of the PHA response. The inhibition persisted after irradiating the monocyte-rich cells with 3000 R.

The effect of the addition of purified macrophages to monocyte-depleted lymphocytes was tested. Preparations of adherent cell consisting of >99% macrophages from patients 1 and 4 reduced the PHA-stimulated 3H-thymidine incorporation of their autologous monocyte-depleted lymphocytes by greater than 50% (Table 6). On the other hand, adherent cells from a normal individual had no effect on the proliferation of his autologous lymphocytes at similar lymphocyte/macrophage ratios. Furthermore, the adherent cells of a patient whose macrophages inhibited his own lymphocyte transformation had little effect on the response of normal lymphocytes (Table 6).

DISCUSSION

Impairment of lymphocyte blastogenesis of patients with Hodgkin disease has been known for more than a decade,16-18 but the mechanisms involved are not well understood. Twomey et al.19 noted that mononuclear cells from patients with Hodgkin disease suppressed the response of normal lymphocytes in the mixed leukocyte culture. This effect was at first attributed to a T suppressor lymphocyte; however, experiments recently reported suggest that the suppression is mediated by monocytes.20 In the present study we have shown that leukocyte suspensions prepared by density gradient centrifugation of the blood of patients with symptomatic advanced Hodgkin disease contain high concentrations of monocytes that inhibit the lymphocyte blastogenic response to PHA, PWM, and streptococcal antigen. In five of six patients with severe im-
pairment of blastogenesis in monocyte-rich cultures, there were striking increases in $^3$H-thymidine incorporation and morphologic blastogenesis when the leukocyte suspensions were depleted of monocytes prior to culture. The monocyte-rich and -depleted leukocyte suspensions did not differ in lymphocyte viability or in number of E-rosetting lymphocytes. Marked inhibition was noted even in mixtures of monocyte-rich and -depleted cells where the number of monocytes was decreased by half, suggesting that nonspecific inhibition due to overcrowding was unlikely. Irradiation of the monocyte-rich population did not affect the inhibition, suggesting that it was not dependent on a proliferative event. Finally, addition of isolated macrophages to autologous monocyte-depleted lymphocytes again caused inhibition of blastogenesis.

The inhibitory effect of isolated Hodgkin disease macrophages even at low monocyte/lymphocyte ratios suggests that the phenomenon may be due to a qualitative difference between these cells and normal macrophages, perhaps because of activation. Although recent evidence favors the idea that the Reed-Sternberg cell is of macrophage origin, the morphology of the circulating monocytes does not suggest that they stem from the malignant clone. Activation of patients' monocytes by the systemic disease seems a more likely basis for the difference from normal cells. Increased macrophage activation measured by a number of parameters has been reported in patients with Hodgkin disease.

Alternatively, the inhibitory effect of the Hodgkin disease monocytes may be primarily quantitative. The patients' lymphocytes may have an increased sensitivity to the high number of macrophages introduced into the cultures owing to the altered peripheral blood lymphocyte/monocyte ratio associated with advanced disease and extensive previous treatment. Previous treatment alone is unlikely to cause this phenomenon, since inhibition tended to disappear with treatment of recurrent disease.

The fact that the PHA response of normal lymphocytes was not affected by similar numbers of autologous macrophages or Hodgkin disease macrophages also favors the idea that abnormal lymphocyte function plays a role in monocyte inhibition. We have preliminary data that high concentrations of normal macrophages (three times higher than that required to suppress the PHA blastogenic response of Hodgkin disease lymphocytes) can inhibit antigen-stimulated blastogenic responses of autologous normal lymphocytes. Laughter et al. also have shown that large numbers of stimulating mononuclear cells inhibit responding allogeneic cells in the normal mixed leukocyte culture, which they ascribe to a monocyte effect.

A recent finding of Goodwin et al. may account, at least in part, for the monocyte inhibition noted here. They found that the hyporesponsiveness of PHA-stimulated lymphocytes from Hodgkin disease patients could be reversed by prostaglandin synthetase inhibitors and that increased levels of prostaglandin E$_2$ (PGE$_2$) were present in the cultures. When glass-adherent cells, presumably monocytes, were removed, prostaglandin production decreased. We also found large levels of PGE in the unstimulated monocyte-rich cultures of patient No. 1 that increased further after stimulation with lipopolysaccharide.

The relevance of the monocyte suppression in vitro noted here to the well-documented deficiency in delayed hypersensitivity of patients with Hodgkin disease is intriguing. All of the patients with suppressive monocytes were
anergic, but a number of our patients who did not exhibit monocyte suppression in vitro were also anergic. Furthermore, we found that monocyte suppression is not limited to Hodgkin disease. We have shown suppression of the response in vitro to tuberculin in monocyte-rich cultures from patients with active tuberculosis who retained delayed hypersensitivity to tuberculin.29

Recently, Markenson et al. also reported a similar phenomenon in vitro in patients with systemic lupus erythematosus; however, they did not correlate their findings with the patients’ capacity to mount delayed hypersensitivity reactions.30 Similarly, Zembala et al. also recently showed adherent cell inhibition of lymphocyte transformation from patients with advanced cancer.31

It is clear that cellular interaction between monocytes and lymphocytes must be evaluated further before impaired lymphocyte blastogenesis is attributed solely to an intrinsic defect of the lymphocyte or macrophage. Moreover, the aberrant cellular interaction noted here could be responsible for, or a result of, impaired immunity in Hodgkin disease.

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REFERENCES


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Monocyte-mediated inhibition of lymphocyte blastogenesis in Hodgkin disease

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