The Impaired Responsiveness of Chronic Lymphatic Leukemia Lymphocytes to Allogeneic Lymphocytes

By Martin J. Smith, Elizabeth Browne, and Arne Slungaard

Normal lymphocytes respond to the presence of allogeneic lymphocytes in culture with a high uptake of \(^{3}H\)-thymidine. We present a study of the interaction of chronic lymphatic leukemia (CLL) lymphocytes with normal allogeneic lymphocytes using the one-way mixed lymphocyte culture technique of Bach. The lymphocytes of 11 patients with CLL were tested separately against those of four normal subjects. In all 11 patients an impaired responsiveness was found. The mean response of CLL lymphocytes to those of the control panel was 19,805 cpm, whereas the mean response of panel lymphocytes to CLL lymphocytes was 90,319 cpm (\(p < 0.001\)). There was no significant difference between the mean response of panel lymphocytes to CLL lymphocytes and to those of other panel members (81,630 cpm). Therefore, CLL lymphocytes display an impaired responsiveness to allogeneic lymphocytes, while their capacity to stimulate is intact. These results are in accord with their previously demonstrated impaired reactivity to phytohemagglutinin, a less specific mitogenic stimulus, and serve as further evidence of defective cellular immune response in CLL.

The hallmark of the disease chronic lymphatic leukemia (CLL) is a sustained absolute increase of blood lymphocytes. Despite the abundance of these central cells of the immune mechanism, CLL is a disorder of defective immunoproliferative response. Immunity to bacteria and viruses is often reduced or absent. Serum immunoglobulins are decreased, and the pattern of membrane immunoglobulins is abnormal. The response to phytohemagglutinin and antigens is retarded and/or submaximal, and rejection of skin grafts is delayed.

Normal lymphocytes respond to the presence of allogeneic lymphocytes in culture with a high uptake of tritiated thymidine. This response requires, in most instances, the recognition of and reaction to foreign transplantation antigens under the control of the HL-A locus, hence its use in assessing degree of histocompatibility. We present the first definitive study of the interaction of CLL lymphocytes with normal allogeneic lymphocytes using one-way mixed lymphocyte culture (MLC).
Table 1. Characteristics of Patients With Chronic Lymphatic Leukemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Lymphocyte Count (per cu mm)</th>
<th>Duration of Disease* (yr)</th>
<th>Serum Immunoglobulin Levels†</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. R.</td>
<td>80</td>
<td>M</td>
<td>57,300</td>
<td>2</td>
<td>IgG: 550, IgA: 90, IgM: 17</td>
</tr>
<tr>
<td>V. M.</td>
<td>63</td>
<td>M</td>
<td>21,100</td>
<td>2</td>
<td>IgG: 1400, IgA: 125, IgM: 28</td>
</tr>
<tr>
<td>E. E.†</td>
<td>73</td>
<td>M</td>
<td>31,600</td>
<td>2</td>
<td>IgG: 3700, IgA: 900, IgM: 430</td>
</tr>
<tr>
<td>G. O.</td>
<td>68</td>
<td>M</td>
<td>13,100</td>
<td>1</td>
<td>IgG: 1700, IgA: 140, IgM: 28</td>
</tr>
<tr>
<td>B. O.</td>
<td>73</td>
<td>F</td>
<td>8,750</td>
<td>1</td>
<td>IgG: 640, IgA: 34, IgM: 17</td>
</tr>
<tr>
<td>H. S.</td>
<td>61</td>
<td>F</td>
<td>18,000</td>
<td>3</td>
<td>IgG: 1200, IgA: 140, IgM: 94</td>
</tr>
<tr>
<td>A. L.†</td>
<td>70</td>
<td>M</td>
<td>13,800 (1/2)</td>
<td>1</td>
<td>IgG: 1350, IgA: 130, IgM: 14</td>
</tr>
<tr>
<td>R. McD.</td>
<td>82</td>
<td>M</td>
<td>28,800 (1/2)</td>
<td>1</td>
<td>IgG: 1050, IgA: 500, IgM: 39</td>
</tr>
<tr>
<td>A. W.</td>
<td>61</td>
<td>F</td>
<td>28,700 (1/3)</td>
<td>3</td>
<td>IgG: 1100, IgA: 52, IgM: 24</td>
</tr>
<tr>
<td>H. Pr.</td>
<td>69</td>
<td>M</td>
<td>30,400</td>
<td>5</td>
<td>IgG: 1050, IgA: 180, IgM: 65</td>
</tr>
</tbody>
</table>

*Duration of disease is defined as time elapsed from date of diagnosis to date of study.
†Normal values of serum immunoglobulins in our laboratory (Proceedings XIV International Congress of Hematology [in press]), mean (mg/100 ml) ± 1 SD, are: Male—1450 ± 503, IgG; 296 ± 131, IgA; 72 ± 40, IgM; Female—1390 ± 506, IgG; 256 ± 116, IgA; 104 ± 43, IgM.
‡These patients were receiving chlorambucil at the time of study.

MATERIALS AND METHODS

The one-way mixed lymphocyte culture technique of Bach10 was used with certain modifications. The cell button was suspended in 199-bicarbonate tissue culture medium (Grand Island Biological Co., Grand Island, N.Y.) containing normal pooled plasma 15%–25% by volume. The cells and medium were mixed well, were layered onto 10 ml of Ficoll-Hypaque gradient mixture (specific gravity 1.077) in a 50 ml conical glass centrifuge tube, and were spun at 400 g (at the interface) for 40 min at 20–22°C. The white cell layer at the interface was then removed and divided into two aliquots. Contamination by nonmononuclear cells was uniformly less than 5% (phase microscopy).

The final concentration of responding cells was $0.5 \times 10^6$ and of stimulating cells was $1.5 \times 10^6$ mononuclear cells/ml.

The mixed lymphocyte cultures were incubated for 140 hr, the time of peak reactivity, at 37°C in 5% CO₂ atmosphere. Viability, determined by eosin dye exclusion just prior to labeling, was uniformly greater than 90%.

Counting was performed in a Nuclear-Chicago Mark II liquid scintillation spectrometer, with a counting efficiency of 61% for tritiated thymidine determined by channels-ratio method. Response is defined as the number of counts per minute of tritiated thymidine incorporated into acid-precipitable material by $1 \times 10^6$ mononuclear cells and is expressed as mean values of triplicate cultures.

Patients with chronic lymphatic leukemia were selected on the basis of persistent blood lymphocytosis (greater than 8000/cu mm) and infiltration of bone marrow by lymphocytes. Peripheral blood monocyte differential counts varied between 1% and 3%. Characteristics of the patient population are given in Table 1.

Mitomycin-treated lymphocytes of 11 patients with CLL were tested separately against nonmitomycin-treated lymphocytes of four normal control subjects for their capacity to stimulate in MLC. Obversely, nonmitomycin-treated lymphocytes from the CLL patients were mixed with mitomycin-treated lymphocytes from control subjects to measure their capacity to respond. Age-matching was not attempted, since previous studies by Bach et al.11 and preliminary experiments in our own laboratory had failed to demonstrate a significant effect of age on lymphocyte behavior in mixed culture.
IMPAIRED RESPONSIVENESS OF CLL LYMPHOCYTES


table 2. Responsiveness of Allogeneic Cell Mixtures

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>CELL MIXTURE</th>
<th>ACTIVITY (cpm)</th>
<th>PATIENT</th>
<th>CELL MIXTURE</th>
<th>ACTIVITY (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ISOGENEIC</td>
<td>&lt;2,000</td>
<td>G.R.</td>
<td>76,578</td>
<td>116,877</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37,006</td>
<td></td>
<td>53,435</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7,319</td>
<td>V.M.</td>
<td>151,420</td>
<td>50,838</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3,169</td>
<td>R. McD.</td>
<td>65,886</td>
<td>30,254</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43,363</td>
<td>B.O.</td>
<td>108,622</td>
<td>162,340</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12,067</td>
<td>H. Pa.</td>
<td>54,133</td>
<td>102,438</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74,125</td>
<td>E. E.</td>
<td>19,805 ± 17,106</td>
<td>90,319 ± 41,990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17,690</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

= NORMAL LYMPHOCYTES RESPONDING TO CLL LYMPHOCYTES

= CLL LYMPHOCYTES RESPONDING TO NORMAL LYMPHOCYTES

RESULTS

In each of the 11 patients an impaired responsiveness relative to the control panel was found (Table 2). The mean response of CLL lymphocytes from the 11 patients to those of the control panel was 19,805 ± 17,106 (cpm ± 1 SD), whereas the mean response of panel lymphocytes to CLL lymphocytes was 90,319 ± 41,990. The difference between the means is significant at p <0.001. There was no significant difference between the mean response of panel lymphocytes to CLL lymphocytes and to those of other panel members: 81,630 ± 36,196 (p >0.5). Baseline counts, obtained by reacting lymphocytes with mitomycin-treated isogeneic lymphocytes, were uniformly under 2000 cpm. In this small series of patients, no effect of chemotherapy, duration of disease, or lymphocyte count on the response to allogeneic lymphocytes was noted.

We conclude that CLL lymphocytes display an impaired responsiveness to allogeneic lymphocytes, whereas their capacity to stimulate is intact.

DISCUSSION

This study does not reveal why the responsiveness of CLL lymphocytes to allogeneic lymphocytes is impaired. Since mixed lymphocyte culture is considered to be an in vitro model of cell-mediated immunity, attention must be focused on the thymic-dependent lymphocyte (T cell). Is there a qualitative or quantitative defect of the T cell in CLL; i.e., is T cell function faulty, or is there a decreased ratio of T cells to bone-marrow-derived lymphocytes (B
cells)? The weight of currently available evidence favors the latter hypothesis. We are unaware of any studies that demonstrate defective function of specifically identifiable T cells in CLL. In the guinea pig L5C lymphatic leukemia, Shevach et al. conclude that the lymphocytes are probably bone marrow-derived because they possess surface immunoglobulins and complement receptors and are not stimulated by mitogens capable of stimulating thymus-derived lymphocytes. In the human, Grey et al. found surface immunoglobulins on 28% of lymphocytes from normal subjects and on 30%-100% of lymphocytes from CLL patients. Whereas the normal lymphocytes had all major immunoglobulin classes and both kappa and lambda light-chain types, the CLL lymphocytes contained only one heavy-chain class, usually IgM, and one light-chain type. If the premise is correct that bone marrow is the major source of lymphocytes with detectable surface immunoglobulins and thymus is the source of those cells that lack surface immunoglobulins, then these studies suggest that CLL represents a malignancy of the B cell with a decreased ratio of T cells to B cells.

Recent studies have raised the possibility that recognition of antigens by lymphocytes expressing cell-mediated immunity is also effected by surface immunoglobulins. Therefore, the cell-mediated immune mechanism may be dependent, at least in part, on lymphocytes that bear surface immunoglobulins and are possibly bone marrow-derived rather than thymic-derived. The lack of heterogeneity of membrane immunoglobulins on CLL lymphocytes demonstrated by Grey et al. may be, at least partially, responsible for their impaired MLC responsiveness.

What of the finding that serum immunoglobulin levels in CLL are reduced? If serum immunoglobulin production is an independent function of B cells, yet these cells are increased in CLL, then their function must be impaired. However, the induction of specific antibody by B cells against many antigenic determinants requires the cooperation of T cells. B cells may not produce antibody to haptenic determinants on an immunogen unless other determinants on the immunogenic molecule are recognized by T cells. Segal et al. have presented evidence in mice that there are indeed two separate populations of T cells, one participating in humoral immune reactions (hydrocortisone-inhibitable) and the other mediating cellular immune reactions (hydrocortisone-resistant). Therefore, a deficiency of T cells may be sufficient to explain the decrease in immunoglobulin production, as well as the impaired responsiveness to transplantation and other antigens in CLL.

The intact capacity of CLL lymphocytes to stimulate in mixed lymphocyte culture probably indicates that there is no loss of major histocompatibility antigens. However, alternative possibilities, such as the presence of leukemic antigens or minor non-HLA antigens capable of stimulating allogeneic lymphocytes, have not been ruled out.

In view of the studies demonstrating a delayed responsiveness of CLL lymphocytes to phytohemagglutinin, it would be of interest to determine if CLL lymphocytes are more responsive to allogeneic lymphocytes in 10-day cultures than in 6-day cultures. Technical difficulties, such as loss of viability and bacterial contamination, have hampered such studies in our laboratory.
Further work is required to determine if impairment of lymphocyte function antedates the development of lymphocytosis and clinical manifestations in CLL. The slight familial tendency suggests the possibility of genetic predisposition, as well as the merit of studying lymphocyte function in apparently healthy family members.

ACKNOWLEDGMENT

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REFERENCES

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