Endotoxin Stimulation in Patients With Lymphoma: Correlation With the Myelosuppressive Effects of Alkylating Agents

By Ronald C. DeConti, Stephen R. Kaplan, and Paul Calabresi

Estimates of the bone marrow granulocyte reserve were made in 97 patients with lymphoma utilizing the endotoxin Pyrexal. Normal granulocyte responses were observed in most patients who had not received prior myelosuppressive therapy. Patients with lymphosarcoma responded as well as those with Hodgkin's Disease. All patients with leukopenia due to previous therapy or marrow infiltration failed to respond. In the absence of leukopenia only 47% of previously treated patients achieved normal responses. The severity of granulocytopenia produced by intravenous alkylating agent therapy was correlated with pretreatment Pyrexal responses. Patients with normal granulocyte reserves, as demonstrated by this test, tolerated subsequent chemotherapy with significantly less severe granulocytopenia (p < 0.02) than patients with abnormal test responses. Nadir granulocyte values below 1000 cells/cu mm developed in 7 of 33 patients with normal Pyrexal responses and in 11 of 15 abnormal responders, despite comparable pretreatment granulocyte values.

The successful chemotherapy of patients with lymphoma is frequently limited by the degree of bone marrow suppression that effective agents regularly produce. While our ability to prevent the complications of anemia and thrombocytopenia with red blood cell and platelet transfusion has improved, the dangers of increased morbidity and potential mortality due to the infectious complications of granulocytopenia have remained less amenable to replacement therapy. Despite advances in antibiotic therapy, infectious complications remain the chief hazard of aggressive chemotherapy; frequently the extent of the granulocytopenia produced by a given dose of drug may not be reliably predicted from the peripheral blood count or the cellularity of a bone marrow aspirate.

Attempts to evaluate more accurately the bone marrow granulocyte reserve and to predict the myelosuppressive effects of antineoplastic agents have been made with purified bacterial endotoxin (pyrexal) and more recently etiocholanolone, a naturally occurring pyrogenic steroid. Administration of these compounds results in mobilization of granulocytes from the marrow into the...
ENDOTOXIN STIMULATION IN LYMPHOMA

This acute leukocytosis has been shown to be dependent on an adequate bone marrow reserve of granulocytes and normal release mechanisms.3-7

This report describes our experience with measurements of the bone marrow reserve using endotoxin (the pyrexal test) in patients with generalized lymphoma and correlates these results with the degree of subsequent myelosuppression induced by intravenous alkylating agent therapy.

MATERIALS AND METHODS

The Pyrexal Test

Pyrexal, a highly purified lipopolysaccharide endotoxin derived from Salmonella abortis equi,8 was administered intravenously to patients with lymphoma in dosages of 0.1 µg/day. All injections were given between 8 and 9 a.m., in order to avoid fluctuations in the total leukocyte count resulting from possible diurnal variations in the rate of granulocyte release from the bone marrow.9 Immediately prior to the injection, blood was taken for baseline total and differential leukocyte counts. These determinations were repeated 3, 4, and 5 hr after the injection of endotoxin. Prior experience1 had demonstrated maximal granulocytosis during this period. Each patient received 600 mg of aspirin immediately before the test in order to minimize the febrile and chilling response to the endotoxin.

Total leukocyte counts were performed in duplicate using a Model B Coulter counter after saponin lysis of red blood cells. Differential counts of 100 cells were performed on cover slip smears of capillary blood specimens stained by the Wright-Giemsa method, and the absolute number of circulating mature granulocytes was calculated.

Previous experience in this laboratory1 with this dosage of endotoxin has established an increment of 2000 granulocytes as the lower limit of normal in patients without hematologic abnormalities. Rises greater than this were considered normal and lesser responses abnormal and an indication of compromised granulocyte reserve.

Patients

Ninety-seven patients with generalized lymphoma ranging in age from 14-69 yr were evaluated by the Section of Oncology and Cancer Chemotherapy and constitute the test group. No patients with local disease were studied. Both in-patient and out-patient tests were done; patients remained at bed or chair-rest during the study period and were observed for evidence of fever, chills, or other possible untoward reaction. Both previously treated and untreated patients were evaluated. A bone marrow aspirate was customarily obtained at the time to assess marrow cellularity and possible infiltration. Bone marrow biopsy was not routinely done.

Response to pyrexal was analyzed in terms of tumor histology, the presence of marrow involvement, history of prior therapy with drugs and/or irradiation, and the peripheral white blood cell count. Leukopenia was defined as less than 5000 cells/cu mm.

Forty-four patients were deemed suitable for therapy with intravenous alkylating agents. White blood cell counts in these patients were obtained at least twice weekly for 3-4 wk after treatment to establish the nadir of leukopenia and granulocytopenia produced by the chemotherapy and to correlate it with the results of the pretreatment pyrexal test. Mechlorethamine hydrochloride (HN2) was administered at dosages of 0.3 mg/kg or 0.4 mg/kg, and cyclophosphamide at 20 mg/kg or 30 mg/kg. The choice of agent and the dosage given were usually made by the attending staff, using the information of the pyrexal test together with an over-all assessment of the patient's clinical condition. Patients were excluded from this program if they appeared to be candidates for extended field radiotherapy, or required immediate local palliation with X-ray therapy, were already apparently resistant to alkylating agents, or if in their physicians' judgment they were more likely to benefit from alternative chemotherapy.
RESULTS

Table 1 demonstrates the results of the initial pyrexal test performed in each patient and relates this to the histologic classification of the lymphoma and the presence of bone marrow infiltration, as well as to previous therapy with both irradiation and cytotoxic drugs. No differences in response were noted among patients with Hodgkin’s disease, lymphosarcoma, or reticulum cell sarcoma. Untreated patients, those having had prior supradiaphragmatic X-ray therapy or drugs more than 6 mo before, responded well in each histologic group. Normal responses were obtained in 40 or 48 tests (83%), while in patients with marrow infiltration, only five of nine tests were normal (55%).

The effect of subdiaphragmatic irradiation on granulocyte reserves was demonstrable only in patients still leukopenic (WBC <5000 cells/cu mm) after such therapy. None of six such patients showed a normal response. Comparable effects were also seen in patients who had received drug therapy within the last month. None of 12 tests in leukopenic patients was normal; in the absence of leukopenia only six of 14 (43%) achieved an adequate response.

The absolute granulocyte increment after endotoxin was related to the baseline granulocyte count in all patients with advanced Hodgkin’s disease independent of prior therapy. Figure 1 demonstrates that only one of 13 patients with fewer than 5000 granulocytes/cu mm manifested an adequate response and that an occasional patient with 20,000 granulocytes/cu mm also failed to respond.

The quantity and quality of the granulocyte response to endotoxin was compared in patients with Hodgkin’s disease and lymphosarcoma. Only previously untreated patients, those with prior irradiation confined to supradiaphragmatic areas or those who had had short-term chemotherapy in the remote past were included. Figure 2 demonstrates lower baseline granulocyte values in patients with lymphosarcoma compared to those with Hodgkin’s disease, but granulocyte increments at least as great were achieved.

The previous treatment of 44 patients who subsequently received 48 courses of intravenous alkylating agent therapy was reviewed. Twenty-one patients had previously received cervical, axillary, mediastinal, or mantle radiotherapy.

Table 1. Initial Pyrexal Test Results* in Patients With Lymphoma

<table>
<thead>
<tr>
<th>Prior therapy</th>
<th>Disease Hodgkin’s</th>
<th>Reticulum Cell Sarcoma</th>
<th>Lymphosarcoma</th>
<th>Leukolymphosarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7/9</td>
<td>6/8</td>
<td>8/9</td>
<td>4/7</td>
</tr>
<tr>
<td>Supradiaphragmatic irradiation</td>
<td>10/12</td>
<td>—</td>
<td>2/3</td>
<td>—</td>
</tr>
<tr>
<td>Drugs &gt;6 mo previous</td>
<td>4/4</td>
<td>1/1</td>
<td>2/2</td>
<td>1/2</td>
</tr>
<tr>
<td>Subdiaphragmatic irradiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without leukopenia</td>
<td>1/1</td>
<td>0/1</td>
<td>2/3</td>
<td>—</td>
</tr>
<tr>
<td>With leukopenia</td>
<td>0/1</td>
<td>—</td>
<td>0/5</td>
<td>—</td>
</tr>
<tr>
<td>Recent drug therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without leukopenia</td>
<td>5/9</td>
<td>0/1</td>
<td>1/4</td>
<td>0/1</td>
</tr>
<tr>
<td>With leukopenia</td>
<td>0/8</td>
<td>0/1</td>
<td>0/3</td>
<td>0/2</td>
</tr>
</tbody>
</table>

*Results are expressed as number of normal responses/total tested.
Fig. 1. Relationship of peak granulocyte increment after endotoxin to granulocyte count in all patients with Hodgkin's disease irrespective of treatment status. Normal granulocyte rises (>2000 cells/cu mm) rarely occurred with granulocyte levels below 5000 cells/cu mm.

with or without additional drug or X-ray treatment. Twenty-eight patients had received either no therapy or radiotherapy confined to cervical, axillary, or mediastinal areas. Twenty-two of these 28 patients (78%) showed normal pyrexal responses. Of eight patients who had had radiotherapy to pelvic, inguinal, or paraaortic disease, pyrexal responses were normal in five (62%). Thirteen patients had had prior therapy with myelosuppressive agents; seven of these demonstrated normal pyrexal responses (54%). Five patients had received both drug and subdiaphragmatic irradiation; three (60%) revealed normal responses.

Bone marrow aspirates disclosed infiltration in two patients with lymphosarcoma; the pyrexal response was normal in one. Normal responses were seen in three of seven patients from whom hypocellular aspirates were obtained.

The effects of pretreatment total white blood cell and granulocyte counts on response to pyrexal were studied in these 44 patients. Baseline total white blood cell counts ranged from 2800 to 27,400 cells/cu mm with granulocyte values from 600 to 25,200 cells/cu mm. Thirty-three normal and 15 abnormal responses were obtained. The character of these responses is shown in Fig. 3. The mean total white blood cell count in pyrexal responders was slightly

Fig. 2. Comparison of granulocyte increments after endotoxin in patients with Hodgkin's disease and lymphosarcoma. In both groups prior therapy was limited to irradiation above diaphragm, and no myelosuppressive chemotherapy had been employed within last 6 mo. Patients with lymphosarcoma tend to have lower baseline granulocyte counts but normal increments after pyrexal.
higher than in abnormal responders, 9288 cells/cu mm compared to 8148 cells/cu mm. The mean pretreatment granulocyte counts of both groups were strikingly similar—7059 cells/cu mm compared to 6903 cells/cu mm.

The predictive value of the pyrexal test in assessing the myelosuppressive effects of alkylating agents is summarized in Table 2. More severe myelosuppression was noted in patients with abnormal test responses. Eleven of the 15 patients with abnormal test responses developed granulocytopenia below 1000 cells/cu mm, and in seven of these values below 500 cells/cu mm were found. Only seven of 33 patients with normal responses reached nadirs below 1000 granulocytes/cu mm, and none fell below 500/cu mm, despite the fact that more patients in this group received the usual rather than reduced dosages of drug. The correlation between pyrexal response, drug dosage, and the degree of subsequent granulocytopenia is shown in Table 3. Reduction in dosage of alkylating agent was reflected in lesser degrees of granulocytopenia in both pyrexal responders and nonresponders. The most severe granulocytopenia occurred in patients with abnormal pyrexal responses who received customary doses of drug. Less depression developed in this group after attenuated doses of drug. The mean granulocyte count of all the normal pyrexal responders was 2070 cells/cu mm ± 168 (SE) at maximal suppression, while the nadir in patients with abnormal responses was 1170 cells/cu mm ± 322 (SE). This difference is statistically significant with a p value of <0.02. The relationship between the pretreatment granulocyte count, the pyrexal response, and the severity of granulocytopenia is analyzed further in Table 4.

**Table 2. Relationship Between Pretreatment Pyrexal Test Responses and Severity of Granulocytopenia Produced by Alkylating Agent Therapy**

<table>
<thead>
<tr>
<th>Pyrexal Test Response</th>
<th>Number</th>
<th>Nadir of Granulocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>500-1000</td>
</tr>
<tr>
<td>Normal</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

Fig. 3. Relationship of granulocyte level to response to endotoxin in 44 patients who subsequently received intravenous alkylating agent therapy. Abnormal responses occurred at all granulocyte values.
Table 3. Correlation of Pyrexal Test Response With the Degree of Subsequent Granulocytopenia Produced by Alkylating Agents

<table>
<thead>
<tr>
<th>Pretreatment Pyrexal Test Response</th>
<th>Dosage Alkylating Agent Used</th>
<th>Number of Patients</th>
<th>Granulocyte Count (cells/cu mm) Pretreatment</th>
<th>Nadir After Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Reduced*</td>
<td>10</td>
<td>6828 ± 1466†</td>
<td>2220 ± 545</td>
</tr>
<tr>
<td></td>
<td>Usual†</td>
<td>23</td>
<td>6947 ± 1003</td>
<td>2013 ± 127</td>
</tr>
<tr>
<td>Abnormal</td>
<td>Reduced</td>
<td>10</td>
<td>8053 ± 2154</td>
<td>1345 ± 482</td>
</tr>
<tr>
<td></td>
<td>Usual</td>
<td>5</td>
<td>4956 ± 700</td>
<td>819 ± 299</td>
</tr>
</tbody>
</table>

*Single intravenous dose of nitrogen mustard 0.3 mg/kg or cyclophosphamide 20 mg/kg.
†Means ± SE.
‡Single intravenous dose of nitrogen mustard 0.4 mg/kg or cyclophosphamide 30 mg/kg.

Cytopenia below 1000 cells/cu mm developed with the same frequency in patients with pretreatment values greater than 10,000 cells/cu mm as in those with values below 2500 cells/cu mm. Though the numbers in each group are small, pretreatment granulocyte values alone did not predict the degree of myelosuppressive effects of drug therapy.

Serial pyrexal tests were performed in a small number of patients after intravenous therapy with HN2 and while receiving maintenance therapy with chlorambucil. In general, normal responses became abnormal during the 2–3 wk after intravenous therapy (even in the absence of leukopenia) and reverted to normal thereafter. Continued normal responses to pyrexal during chlorambucil therapy tended to be associated with less myelosuppression and longer periods of therapy without severe granulocytopenia. Abnormal pyrexal responses usually indicated progressive granulocytopenia. In all, 186 tests were performed. The febrile and chilling response was usually well controlled by aspirin. One patient experienced a transient fall in blood pressure without sequelae. No evidence of tolerance to repeated endotoxin administration was obtained.\(^{10}\)

**DISCUSSION**

In recent years, pyrexal, etiocholanone, and piromen have been used as means of assessing the bone marrow granulocyte reserve in a variety of disease states and in relationship to the effects of both radiotherapy and chemotherapy.\(^{11-15}\)

Despite the differences in the origin of these agents, the dosages used, and

Table 4. Effect of Pretreatment Granulocyte Count on Pyrexal Response and Tolerance of Myelosuppressive Effect of Alkylating Agent Therapy

<table>
<thead>
<tr>
<th>Baseline Granulocyte Count (cells/cu mm)</th>
<th>No. Normal Pyrexal Tests/Total No. Tested</th>
<th>Granulocyte Nadir After Alkylating Agent Therapy (cells/cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;500 500-1000 1001-2000 &gt;2000</td>
</tr>
<tr>
<td>&gt;10,000</td>
<td>7/10 70%</td>
<td>2 3 2 3</td>
</tr>
<tr>
<td>5,001-10,000</td>
<td>12/16 75%</td>
<td>1 2 7 6</td>
</tr>
<tr>
<td>2,500-5,000</td>
<td>12/18 67.7%</td>
<td>3 5 5 4</td>
</tr>
<tr>
<td>&lt;2,500</td>
<td>2/4 50%</td>
<td>1 1 0 2</td>
</tr>
</tbody>
</table>
the standard of normalcy applied, each of these agents has been shown to be capable of mobilizing granulocytes into the circulation. Pyrexal was used in this study as an extension of earlier work in patients with nonlymphomatous neoplasms. This test demonstrated that despite active generalized disease, patients whose marrow granulocyte reserves had not previously been compromised by extensive radiotherapy or chemotherapy usually responded in a manner quite similar to that of patients with other nonhematologic malignancies. Interestingly, while patients with Hodgkin's disease tended to have higher baseline granulocyte counts, the increment after stimulation by endotoxin was neither quantitatively or qualitatively different from that of patients with lymphosarcoma. A history of recent marrow suppressant therapy and particularly residual leukopenia were more reliably associated with negative responses. It is not surprising that prior radiotherapy to supradiaphragmatic areas was not associated with abnormal responses in view of the elapsed time and the small volume of active marrow previously irradiated. Hellman and Fink have estimated that at least 40% of the bone marrow must be irradiated with an excess of 1500 rads in order to depress the response to pyrexal. In patients who received irradiation to areas below the diaphragm, it is much more likely that such volumes were so treated, and this, together with possible recovery accounts for the mixed results seen in this group of patients.

The demonstrated ability of the pyrexal test to gauge the tolerance of a patient with lymphoma to the myelosuppressive effects of usual doses of intravenous alkylating agent cannot be equaled by evaluation of the cellularity of aspirates of bone marrow or the pretreatment blood count. In this study, eight of 18 patients (44%) with circulating granulocyte levels between 2500–5000 cells/cu mm developed fewer than 1000 granulocytes/cu mm after drug therapy. The normal blood granulocyte level is considered to range from <2000 to >7000 cells/cu mm. This same degree of granulocytopenia occurred in only seven of 33 patients (21%) with normal pyrexal test responses. Not only may pretreatment tests be of benefit in selecting patients for more aggressive therapy, but abnormal test results may warn of the need for reduced drug schedules to lessen the likelihood of severe granulocytopenia. The increased risks of infection with marked granulocytopenia have been best documented in patients with acute leukemia, though they are well appreciated in patients with lymphoma and nonhematological malignancies as well. This study has dealt with the evaluation of the pyrexal test in assessing myelosuppression after administration of a single intravenous dose of alkylating agent. Serial tests may be of value in indicating impending compromise of marrow granulocyte reserve after prolonged courses of drug therapy and particularly in the management of patients receiving investigational agents whose potential myelosuppressive effects are incompletely understood.

ACKNOWLEDGMENT

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REFERENCES


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