Phytohemagglutinin-induced Lymphocyte Transformation: 
The Relationship to Prognosis of Hodgkin’s Disease

By Michael P. Corder, Robert C. Young, Robert S. Brown, 
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Forty-three untreated patients with Hodgkin’s disease were evaluated between 1965 and 1967 with PHA-stimulated peripheral leukocyte cultures. No correlation was found between PHA-induced lymphocyte transformation at the time of diagnosis and the clinical course of the patient’s Hodgkin’s disease. There is also no correlation between lymphocyte transformation and the histologic pattern of nodular sclerosis. Although PHA-induced lymphocyte transformation appears to be a general index of immunologic status, it has not proven of value as a prognostic sign in Hodgkin’s disease.

A LARGE LITERATURE has accumulated about the lymphocyte blastogenic transformation induced by phytohemagglutinin (PHA) in patients with Hodgkin’s disease. These studies provide information on the results of testing of the patients at the time of diagnosis, testing after the induction of remission, and testing during the course of the disease. The relation of PHA-induced lymphocyte transformation (PHA-LT) to skin test reactivity and peripheral absolute lymphocyte counts has also been described. Many of the studies previously reported have had few patients who were tested prior to therapy, and there are no published studies that relate PHA-LT at the time of diagnosis to the ultimate course and survival of patients with Hodgkin’s disease.

A prior study from this institution related PHA-LT in 43 untreated patients with Hodgkin’s disease to skin test reactivity, stage, absolute peripheral

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Abbreviations used in text: PHA, phytohemagglutinin; PHA-LT, phytohemagglutinin-induced peripheral lymphocyte transformation.
lymphocyte counts, and to histology. This report is an extension of that earlier study in which additional questions are asked: first, the relation of PHA-LT prior to therapy to survival, remission duration, and time to relapse; second, the relationship of PHA-LT at the time of diagnosis to the nodular sclerosis histologic pattern. This histology was selected for comparison because it has been reported to be associated with a better prognosis, perhaps related to a more profound host defense reaction.15,16

MATERIALS AND METHODS

Patient Populations

From November 1965 to January 1967, 52 previously untreated patients with a tissue diagnosis of Hodgkin’s disease were admitted to the Clinical Center of the National Institutes of Health. The blood of 49 of these patients was studied with leukocyte cultures, and 43 had viable cultures. No patients were lost to follow-up as of May 1, 1971, the date of termination of survival data. One patient who was reported in the earlier study was excluded from the present study because of a revision of his histologic diagnosis from Hodgkin’s disease to lymphosarcoma. The patient group consisted of 27 males and 16 females. The mean age was 30 yr (range 14–68 yr). All but three patients were tested within 3 mo of their initial biopsies.

Clinical staging was performed during the initial evaluation. Patients were staged according to the Rye classification.17 History, physical examination, complete blood counts, liver function tests, chest roentgenograms, metastatic bone surveys, intravenous pyelograms, bone marrow biopsies, inferior venacavagrams, and lymphangiograms were performed on all patients. Whole chest tomograms, bone scans, and liver scans were performed as indicated.

All patients included had biopsy proven Hodgkin’s disease diagnosed by the Pathologic Anatomy Branch using the Lukes-Butler classification18 without knowledge of the immunologic status of the patients. The Lukes and Butler scheme was modified to classify subcategories of the nodular sclerosis group as lymphocyte predominant, mixed cellularity, or lymphocyte depleted.

After staging and immunologic evaluation, patients with stages I, II, and IIIA disease were treated with intensive radiotherapy.19 Patients with stage IIIB disease were randomized to radiotherapy or combination chemotherapy (MOPP).20 and those patients with stage IV disease were treated with combination chemotherapy only.

Leukocyte Cultures

Leukocyte cultures were prepared and harvested according to the method of Hersh and Oppenheim3 with the following modifications: duplicate cultures were prepared, two with and two without 0.05 ml phytohemagglutinin-M (Difco Laboratories, Detroit, Mich.); A-gammaglobulinemic newborn calf serum was used (Hyland Labs., Los Angeles, Calif.), if necessary, to achieve a final concentration of 10⁶ leukocytes/ml in a 3-ml volume of culture; and polystyrene particles were omitted from the culture procedure. From each culture, 500 cell differential counts of lymphoblastoid lymphocytes, mitotic cells, small lymphocytes, and macrophages were performed using published morphologic features.3 Per-cent transformed lymphocytes in each culture was calculated from the ratio of lymphoblastoid plus mitotic cells to the total number of cells counted. Average percentages for the two unstimulated and the two stimulated cultures were calculated separately.

Nineteen healthy adult volunteers were evaluated as controls for the leukocyte cultures using the same technique.

Statistical Analysis

Statistical analysis was performed on the life-table data using the Modified Generalized Wilcoxon test.
Fig. 1. Phytohemagglutinin-induced lymphocyte transformation in controls vs. patients with Hodgkin’s disease; 43 patients.

RESULTS

The mean PHA-LT in the normal controls was 72% (range 41–97%). The mean PHA-LT of the entire group of patients with Hodgkin’s disease was 49% (range 0–93%), and the mean transformation of unstimulated cultures of patients was less than 1% (range 0–8%). The difference between the mean PHA-LT of normal controls and the group of patients with Hodgkin’s disease is significant ($p = 0.001$) (Fig. 1).

When PHA-LT is examined by stage (Fig. 1), stage I patients had a mean of 62% PHA-LT, with only one patient outside of the normal range. As can be seen from the figure, there was no significant difference in mean PHA-LT between stages I, II, and III, but stage IV patients had a mean PHA-LT of 27%. The difference between stage IV patients and controls is significant ($p < 0.0005$), as is the difference between stage IV patients and the combined stages I, II, III ($p < 0.0005$). When the patients within each stage are separated into those who remained in continuous remission after initial therapy and those who relapsed or died of Hodgkin’s disease, there is no difference in mean PHA-LT comparing each subgroup (Fig. 2).

Because of the similarity of the PHA-LT values, the small number of patients involved, and the similarity of prognosis of Hodgkin’s disease, stages IIIB and IV were combined and compared to combined stages I, II, and IIIA.

Fig. 2. Phytohemagglutinin-induced lymphocyte transformation in Hodgkin’s disease (patients in continuous remission vs. patients relapsed or dead).
in the remainder of the analysis. Combined stage I, II, and IIIA patients had a mean lymphocyte transformation that was not significantly different from normals.

As can be seen from Fig. 3, the mean PHA-LT in combined stages I, II, and IIIA was 58% with no significant difference between the group of patients in continuous remission after initial therapy and those who died or relapsed with Hodgkin's disease. The mean for combined stages IIIB and IV is 28%, with again no significant difference between the group of patients in continuous remission and those relapsing or dying with Hodgkin's disease. The difference of the mean PHA-LT value between the combined stages I, II, and IIIA and combined stages IIIB and IV is significant ($p = 0.0005$). Worthy of note is the marked variation of PHA-LT values within each category of Fig. 3.

It was also of interest to see if PHA-LT values predicted the time of onset of relapse. Accordingly, patients in both combined groups who relapsed or died of Hodgkin's disease were divided into those who relapsed or died early (in the first 13 mo after diagnosis) and those who relapsed or died late (after the first 13 mo after diagnosis). There is no significant difference between the PHA-LT of those patients who relapse or die early and those who relapse or die late in either of the combined stage groups (Fig. 4). Cumulative life-table analysis was performed on both combined groups (Fig. 5). There is no significant difference in survival from time of diagnosis between those patients with
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Fig. 5. Cumulative life-table plot of Hodgkin's patients (combined stages I, II, IIA and combined stages IIIB, IV). Numbers in parentheses are numbers of patients.

a PHA-LT value less than 40% (2 SD below the mean normal) and the survival of patients with transformation over 40% in either group of combined stages. Specifically, there is no increase in survival of the combined group of stages I, II, and IIA patients with a PHA-LT value over 40%, and, in fact, it appears that the survival of this group is decreased, although this is not significant ($p = 0.075$) (Fig. 5A). For patients with PHA-LT values less than 40%, there is a slightly shortened survival in the early follow-up period in the combined stage IIIB-IV group (Fig. 5B), but this difference is not significant ($p = 0.17$).

When patients with the nodular sclerosis histologic pattern are compared to those with nonnodular histologic patterns of Hodgkin's disease, there is no difference in mean PHA-LT for all patients grouped, or for combined stages I, II, and IIA. There is an increased mean PHA-LT value in the three patients with stages IIIB and IV, but this observation is not statistically significant ($p = 0.18$) (Table 1).

Table 1. Relationship of PHA-LT to Histology

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean PHA-LT Values</th>
<th>Nodular Sclerosis</th>
<th>Other Histologic Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>All stages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II, IIA</td>
<td>54% (20)*</td>
<td></td>
<td>43% (23)</td>
</tr>
<tr>
<td>IIIIB and IV</td>
<td>56% (17)</td>
<td>44% (3)</td>
<td>60% (14)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>22% (9)</td>
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</tbody>
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*Numbers in parenthesis are number of patients in that category.
DISCUSSION

The method of Hersh and Oppenheim for evaluating PHA-LT was used at the time of initiation of this study (1965) because it was the best method available and had the advantage of determining per-cent transformation of only the living cells. This is particularly important in Hodgkin’s disease, due to the high rate of cell death in leukocyte cultures. The PHA-LT values for normal controls and the total group of Hodgkin’s disease patients in this study very closely approximate those published by Han and Sokal and Trubowitz et al. The control values also agree with those published by Thomas et al. and Hersh and Oppenheim.

The PHA-LT has been studied extensively by other authors, and the following information is well documented: there is a general inverse correlation with extent of disease, and the PHA-LT is most strikingly depressed in patients with stage IV disease; there is a positive correlation with skin test reactivity, however, there is considerable overlap and variation; there is a lack of correlation between absolute peripheral lymphocyte count and PHA-LT; the PHA-LT returns to normal levels in remission if patients are without therapy for 6 mo or more.

This is the largest reported group of untreated patients with Hodgkin’s disease in whom PHA-LT has been measured. This study answers the question posed by Han and Sokal regarding the clinical significance of the depressed PHA-LT levels in Hodgkin’s disease. The advantage of the absence of prior therapy is obvious due to the documented ability of radiotherapy and chemotherapy to suppress PHA-LT.

This study confirms the previously noted depressed PHA-LT in patients with Hodgkin’s disease and emphasizes that this change is most pronounced in stage IV disease. It also demonstrates that the PHA-LT at the time of diagnosis is of no value in predicting the survival time, frequency of relapse, or remission duration over and above the clinical staging of the disease. Figure 5 demonstrates the invalidity of the PHA-LT as a predictor of clinical course. The apparent shortened survival early in the follow-up period seen in the group of IIIB and IV patients with strikingly depressed PHA-LT (Fig. 5B) is not significant, and the survival curves are actually reversed for combined stages I, II, and IIIA (Fig. 5A).

It is possible that the lack of usefulness of the PHA-LT in predicting clinical course is due to the availability of effective therapy in Hodgkin’s disease that masks the prognostic significance of immunologic parameters at the time of diagnosis. Additional evidence that this may be the case in Hodgkin’s disease is provided by another study from this institution that demonstrated the lack of correlation between skin test reactivity and clinical course.

The nodular sclerosis histology has been reported to be associated with an improved prognosis. If this is the case, the improved prognosis is not directly related to the immunologic responsiveness of the peripheral lymphocyte as measured by the PHA-LT.

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REFERENCES


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