Immunodeficiency in Patients with Non-Hodgkin Lymphomas

By Stephen E. Jones, Katherine Griffith, Patricia Dombrowski, and John A. Gaines

Seventy-one previously untreated patients with non-Hodgkin lymphomas were studied with several readily available tests of immune function: number of peripheral blood lymphocytes, serum immunoglobulins, and delayed hypersensitivity to six recall antigens. The results were correlated to histology (Rappaport classification), stage (Ann Arbor classification), the presence of symptoms, and survival. As a group, 38 patients with diffuse lymphomas exhibited marked impairment in reactivity to five of six antigens ($p < 0.03$ to $p < 0.001$). In addition, lymphopenia and reduced levels of serum IgA were found in association with diffuse histiocytic lymphoma. Among patients with diffuse lymphoma, lymphocyte number and skin test reactivity tended to be greater in those with localized disease or without constitutional symptoms, and survival was superior for patients free of symptoms ($p < 0.01$). As a group, 33 patients with nodular lymphoma had normal numbers of lymphocytes, lower levels of serum IgG and IgA, and significant impairment of reactivity to two antigens (streptokinase–streptodornase and mumps; $p < 0.01$); reactivity to three other antigens (Candida albicans, coccidioidin, and tuberculin) was normal. Survival for patients with nodular lymphoma was superior ($p < 0.01$) compared to those with diffuse lymphomas. In summary, severe immunodeficiency was found in patients with diffuse lymphoma (particularly diffuse histiocytic lymphoma), and definite but much less severe immunodeficiency was characteristic of patients with nodular lymphoma.

Immunocompetence correlates with favorable prognosis in several forms of cancer in man, including some of the hematologic malignancies. Although there have been several studies attempting to define immunocompetence in patients with the non-Hodgkin lymphomas (NHL), interpretation of the results of these earlier studies, especially with regard to the relationships between immunocompetence, histology, and prognosis, has been hampered by the lack of uniformity in the histopathologic evaluation of these tumors by different investigators or by the inclusion of both treated and untreated patients. Despite controversy which has recently arisen over what constitutes the proper and most biologically correct classification of these neoplasms, the histopathologic classification proposed in 1956 by Rappaport et al. has gained acceptance and wide usage by clinicians. It is clear that lymphomas with a nodular histologic pattern are associated with unique clinical features, are quite sensitive to
treatment with drugs and radiation, and are associated with a much more favorable natural history than are lymphomas with diffuse histologic patterns. In this study we have investigated the relationship of several readily available parameters of immunologic function to the clinical and histologic features in previously untreated patients with NHL.

**MATERIALS AND METHODS**

**Patients and Control Subjects**

Between December 1972 and March 1976, 71 patients with recently diagnosed NHL were evaluated at the University of Arizona for extent of disease prior to the initiation of treatment. The staging evaluation included standard diagnostic procedures cited elsewhere, as well as the immunologic studies described below. Nineteen patients underwent diagnostic exploratory laparotomy. Pathologic stage of disease for each patient was assigned in accordance with the criteria proposed at the Ann Arbor Conference. All lymphomas were classified histologically by the scheme of Rappaport et al., and most were referred to Dr. James Butler for independent pathology review in accord with the operating guidelines of the lymphoma committee of the Southwest Oncology Group. The clinical and histologic features of these 71 patients are detailed in Table 1.

After completion of staging, patients received a variety of treatments based on the final stage and histologic type of lymphoma. Two patients with extensive intra-abdominal diffuse lymphoma died before treatment could be initiated. Four patients received radiation therapy only (one patient with a nodular lymphoma received two courses of whole-body irradiation). Six patients received combined irradiation and combination chemotherapy, and 41 patients received combination chemotherapy in one of several Southwest Oncology Group lymphoma studies. Eight patients with nodular lymphoma were observed without specific treatment for periods ranging up to 13 years (one patient underwent splenectomy for nodular lymphoma in 1963 and has never received treatment); three have been followed for 39+ months without treatment, and two of the eight patients required treatment with chemotherapy for symptoms and signs of progressive lymphoma at 13 and 28 months after diagnosis. The remaining patients all received individualized programs of single-agent or combination chemotherapy.

In addition, 44 healthy individuals agreed to serve as controls for the immunologic studies. Their mean age was 40 (range, 21–74 yr); 17 were over the age of 40.

### Table 1. Clinical Features of 71 Previously Untreated Patients With Non-Hodgkin Lymphomas

<table>
<thead>
<tr>
<th>Feature</th>
<th>Histologic Type*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Diffuse</td>
</tr>
<tr>
<td>Number of patients</td>
<td>38</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>52 (21–74)</td>
</tr>
<tr>
<td>Histologic subtype*</td>
<td></td>
</tr>
<tr>
<td>Histiocytic</td>
<td>25</td>
</tr>
<tr>
<td>Mixed histiocytic and lymphocytic</td>
<td>3</td>
</tr>
<tr>
<td>Lymphocytic (poorly differentiated)</td>
<td>10</td>
</tr>
<tr>
<td>Stage (pathologic)†</td>
<td></td>
</tr>
<tr>
<td>Localized</td>
<td></td>
</tr>
<tr>
<td>I, II, III, IV</td>
<td>11</td>
</tr>
<tr>
<td>Advanced: III, IV</td>
<td>27</td>
</tr>
<tr>
<td>Presence of &quot;B&quot; symptoms†</td>
<td>21</td>
</tr>
</tbody>
</table>

*Classified according to Rappaport et al.
†Classified according to the criteria of the Ann Arbor Conference.
"B" symptoms: unexplained fever, night sweats, or weight loss of more than 10%.
Absolute Peripheral Blood Lymphocytes and Serum Immunoglobulins

Absolute peripheral blood lymphocyte counts were determined on all patients. Serum immunoglobulin levels were determined by radial immunodiffusion for 64 patients prior to treatment.

Skin Testing for Delayed Hypersensitivity

All patients had two or more skin tests with intradermally injected common recall antigens. The test dose for all antigens was 0.1 ml. In the earlier portions of this study, three antigens were usually used: tuberculin purified protein derivative (PPD, 5 tuberculin units; Mantoux, Connaught Laboratories, Willowdale, Toronto, Canada); mumps antigen (Eli Lilly, Indianapolis, Ind.), and coccidioidin (1:100 dilution; Iatric Corp., Tempe, Ariz.). After December 1974 three additional antigens were added to the battery: streptokinase-streptodornase (SK-SD; 10 units streptokinase, 2.5 units streptodornase; Varidase, Lederle Laboratories, Pearl River, N.Y.) Candida albicans (1:20 dilution of stock; Dermatophytin “O”, Hollister-Stier Laboratories, Dallas, Tex.) and trichophytin (1:20 dilution of stock; Dermatophytin, Hollister). Forty-three patients were tested with all six antigens.

Reaction to these antigens was assessed at 48 hr by measuring the indurated area in two diameters (the longest and its perpendicular). A ballpoint pen was employed to help define the induration. Skin test reactions were read by two of us (KG and PD) without knowledge of the histopathology. Any induration whatsoever was recorded and the mean diameter of induration for each test was determined. Any test that measured 4 mm or more in average diameter was also considered a “positive reaction,” i.e., evidence of delayed hypersensitivity to that particular recall antigen. Four millimeters was employed as the minimum induration that we felt could be consistently identified.

Statistical Methods

The clinical and histologic features as well as the results of the immunologic tests were coded and analyzed with the Statistical Package for the Social Sciences on the University of Arizona’s CDC 6400 computer. Initial analyses examined differences between patients with nodular and diffuse lymphomas and normal controls. More detailed analyses of patients with diffuse lymphomas examined differences between those with diffuse histiocytic lymphoma and other histologic types of diffuse lymphomas, those with or without constitutional symptoms (weight loss, night sweats, or unexplained fever), and those with localized (stages I, IE, II, and II_E) versus generalized (stages III, II_E, and IV) disease. Standard analysis of variance was employed and was felt to describe adequately differences between groups without the need for additional mathematical transformations (e.g., logarithmic distributions).

The second stage of the analysis was to look for interrelations between variables. The final stage employed a stepwise discriminant technique to identify those combinations of variables which optimally differentiated between the two groups of patients and healthy controls.

Actuarial survival was calculated for the entire group of patients, and for those with nodular or diffuse lymphomas from the time the patient was first seen at the University of Arizona to the time of death (or if still alive until April 1976) employing the method of Kaplan and Meier. A generalized Wilcoxon test was used to evaluate differences in survival between various populations. For patients with diffuse lymphomas, the effect of stage and the presence of constitutional symptoms in relation to survival were also examined.

RESULTS

Peripheral blood lymphocytes. The results of the absolute peripheral blood lymphocyte determination are given in Table 2. Although lymphopenia or lymphocytosis was occasionally observed in patients with other histologic types of lymphoma, the mean absolute count was significantly lower in patients with diffuse histiocytic (DH) lymphoma than other types of lymphoma. Moreover, 8 of 25 patients (32%) with DH lymphoma had absolute lymphocyte counts of
less than 1000/cu mm, whereas this level was observed in only 2 patients with nodular lymphoma and in 3 patients with other types of diffuse lymphoma. Among the patients with diffuse lymphoma, patients with constitutional symptoms or advanced disease tended to have decreased numbers of peripheral blood lymphocytes, but there were too few patients for the differences to be significant.

**Serum immunoglobulins.** The results of the immunoglobulin determinations are summarized in Table 3. Although considerable variation in values of individual patients was observed, the mean value for IgG for patients with nodular lymphoma was significantly less than for patients with diffuse lymphomas or controls. In addition, average serum IgA levels were found to be significantly lower in two groups of patients: those with DH lymphoma and those with nodular lymphoma. Serum IgM levels did not vary significantly with respect to the histologic type of lymphoma.

**Delayed hypersensitivity to recall antigens.** The skin test data are summarized in Table 4. The results were analyzed both quantitatively and in terms of the frequency with which various groups of individuals reacted to particular antigens. The conclusions from both methods of analysis were identical.

*Trichophyton* proved to be of no value in assessing delayed hypersensitivity. In fact, only 25% of healthy individuals reacted positively to this antigen. Coccidioidin proved to be a useful recall antigen for our patients because they lived in an endemic area.

Previously untreated patients with diffuse lymphomas manifested markedly impaired ability to react to five common recall antigens compared to healthy individuals (*p* values for differences ranged from *p* = 0.02 for PPD to *p* < 0.001 for SK-SD, mumps, and *C. albicans*). Patients with nodular lymphoma as a group reacted in a near normal fashion to three antigens (*C. albicans*, coccidioidin, and PPD), but could be distinguished from healthy subjects on
Table 4. Delayed Hypersensitivity to Recall Skin Test Antigens* in Previously Untreated Patients With Lymphoma

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Controls</th>
<th>Diffuse Lymphoma</th>
<th>Nodular Lymphoma</th>
</tr>
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<tbody>
<tr>
<td>Streptokinase-streptodornase</td>
<td>20.7 (86%)†</td>
<td>1.4 (12%)†</td>
<td>6.6 (47%)†</td>
</tr>
<tr>
<td></td>
<td>N†</td>
<td>44</td>
<td>24</td>
</tr>
<tr>
<td>Mumps</td>
<td>13.6 (96%)†</td>
<td>4.2 (27%)†</td>
<td>8.6 (48%)†</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>44</td>
<td>37</td>
</tr>
<tr>
<td>C. albicans</td>
<td>13.5 (81%)§</td>
<td>5.1 (34%)§</td>
<td>13.5 (76%)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>44</td>
<td>29</td>
</tr>
<tr>
<td>Coccidioidin</td>
<td>6.2 (43%)§</td>
<td>1.6 (10%)§</td>
<td>5.4 (39%)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td>PPD (tuberculin)</td>
<td>5.6 (30%)§</td>
<td>1.8 (13%)§</td>
<td>4.5 (25%)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td>Trichophyton</td>
<td>2.7 (25%)§</td>
<td>2.0 (17%)§</td>
<td>2.4 (25%)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>44</td>
<td>24</td>
</tr>
</tbody>
</table>

*Expressed as the mean diameter of induration in millimeters at 48 hr. Number in parentheses indicates the percentage of individuals reacting ≥ 4 mm of induration at 48 hr.

†Differences between groups significant (p < 0.01).
§Number of individuals tested with each antigen.
§Differences between groups significant (p < 0.03).

the basis of their depressed reactivity to SK-SD (p < 0.001) and mumps antigen (p < 0.01). The reactivity to the latter two antigens in patients with nodular lymphoma was intermediate between that observed with healthy individuals and the severe impairment characteristic of patients with diffuse lymphomas. The differences observed between patients with nodular and diffuse lymphomas were significant for SK-SD (p < 0.01) and nearly significant for mumps antigen (p = 0.10). In summary, five different antigens independently demonstrated evidence of severely impaired cell-mediated immunity in patients with diffuse lymphomas and a significant but much less severe type of impairment in patients with nodular lymphoma.

Additionally, there appeared to be some differences in reactivity to skin tests among patients with diffuse lymphoma in relation to histologic subtype, presence of symptoms, and stage of disease. For example, patients with diffuse mixed and diffuse lymphocytic lymphomas appeared to react less well to PPD and coccidioidin antigens than did patients with diffuse histiocytic lymphoma (p = 0.08 and 0.05, respectively). In addition, patients with diffuse lymphomas who were free of symptoms or who had more localized disease tended to manifest more reactivity to recall skin test antigens. However, too few cases were present in each group for the differences to be significant.

Relationship of skin test reactivity to lymphocyte counts and immunoglobulins.

No real differences could be found between the peripheral blood absolute lymphocyte count and serum immunoglobulins or four of the recall antigens. There was a positive correlation between the lymphocyte count and reactivity to C. albicans (r = 0.32, p < 0.02) or SK-SD (r = 0.29, p < 0.03).

Multivariate analysis. Except for the relationship between the peripheral blood lymphocytes and reactivity to C. albicans and SK-SD, the other clinical and immunologic variables were found to be essentially independent of one another.16 Taking the dependencies into consideration, stepwise discriminate
Fig. 1. Comparison of actuarial survival for 33 patients with nodular lymphoma and 38 patients with diffuse lymphoma ($p < 0.01$).

Survival. The actuarial survival for the 71 patients is shown in Fig. 1; the difference between patients with nodular and those with diffuse lymphoma is significant ($p < 0.01$). For patients with diffuse lymphoma, survival was superior for those with localized, compared to those with advanced disease, but too few cases were available for the differences to be significant. However, among patients with diffuse lymphomas there was a significant difference ($p < 0.01$) in survival in relation to the presence or absence of constitutional symptoms (Fig. 2).

DISCUSSION

Impairment of skin test reactivity (a sensitive T-lymphocyte function) has been observed in some patients with NHL for many years, but the published results have been highly variable when the Rappaport classification was not used and when both treated and untreated patients were included. In this study we included only previously untreated patients and employed the Rappaport classification. The clinical features and distribution of pathologic subtypes were quite similar to those in other recently published series. Survival of
these patients from the time they were first seen at the University of Arizona also agreed closely with other series, except that patients with diffuse lymphomas but no constitutional symptoms survived significantly better than patients with symptoms. In previous reports from Stanford no difference in survival in relation to systemic symptoms was apparent. In this series not only were the clinical features, pathology, and prognosis representative of patients with NHL, but the significant abnormalities of both humoral (B-lymphocyte) and cell-mediated (T-lymphocyte) immunity were also probably characteristic of certain groups of patients. For example, patients with DH lymphoma manifested lymphopenia, depressed levels of serum IgA, and severely impaired reactivity to five recall skin test antigens. As a group, patients with the other types of diffuse lymphoma also manifested severe impairment to recall antigens but not the abnormalities of lymphocyte number or immunoglobulins. In addition, although the number of patients was small, lymphopenia and the impairment of skin test reactivity appeared to be more severe in patients with diffuse lymphoma of advanced extent or in those who manifested systemic symptoms.

As a group, patients with nodular lymphoma had less severe but definite impairment in their ability to react to two recall antigens compared to healthy controls, but retained normal or near normal reactivity to three other antigens. In this regard SK-SD (Varidase) and mumps proved to be the two most useful skin tests because the results with these antigens distinguished patients with nodular lymphoma from those with diffuse lymphoma and patients with lymphoma from healthy individuals. Patients with nodular lymphoma also had depressed average values for two major immunoglobulin classes: IgG and IgA. This observation was of interest because virtually all nodular lymphomas have
proved to be monoclonal B-cell neoplasms. Depression of normal immunoglobulin synthesis has been a common finding in other monoclonal B-cell neoplasms, including multiple myeloma, macroglobulinemia, and chronic lymphocytic leukemia, and it has been suggested that the immunoglobulin deficiency in these disorders may well be due to tumor burden, secretion of a "chalone," or a population of "suppressor" cells. We can only speculate that a similar phenomenon may be occurring in some patients with nodular lymphoma.

Our multivariate analysis for the 39 patients on whom we had complete sets of data (lymphocyte count, immunoglobulins, and skin test results) clearly demonstrates that the results of these simple tests can be used together to discriminate three distinct populations: healthy individuals, patients with nodular lymphoma, and patients with diffuse lymphomas. Despite variation in the results of some of these tests for an individual patient and despite the fact that a 79% predictive rate is too low to be clinically useful, this multivariate analysis reconfirms that immunocompetence of patients with nodular lymphoma differs from that of diffuse lymphoma patients and that both groups of patients differ from normal controls.

Our observation on the relationship of lymphocyte number and skin test reactivity in patients with NHL parallels similar findings described in patients with Hodgkin’s disease with respect to histologic type, stage of disease, and the presence or absence of systemic symptoms. Recent observations employing other assays of T-lymphocyte function have demonstrated immunodeficiency in even the most favorable types of Hodgkin’s disease (lymphocyte-rich histologic types or localized disease). Such impairment is correspondingly more severe in lymphocyte-depleted histologic types or in advanced disease. However, despite the potential importance of these factors in determining prognosis, the markedly improved results of treatment of Hodgkin’s disease seem in most cases to outweigh any adverse effect of immunodeficiency on prognosis.

In the NHL, although we have been able to correlate lymphocyte count, impairment of skin test reactivity, and serum immunoglobulin levels with histologic type, we have not yet been able to relate immunodeficiency per se to prognosis (independent of histology, stage, symptoms, or treatment). For instance, we have attempted to relate prognosis in patients with nodular lymphoma (almost all of whom have widespread disease at the outset and most of whom are free of "B" symptoms) to skin test reactivity, number of peripheral blood lymphocytes, etc.; yet too few patients with deficiencies have been available in this group to assess the potential impact of impaired immunity. This observation is a critical issue but one which can only be answered by studying a larger number of untreated patients and by performing serial testing of immunity in relation to response to treatment, survival, etc. However, there is some circumstantial evidence and considerable speculation to support the view that immunocompetence and prognosis are closely linked in patients with NHL.

For example, Sokal and his colleagues have demonstrated a difference in prognosis in relationship to skin test reactivity and, furthermore, have argued that successful nonspecific immunopotentiation with bacillus Calmette-Guerin (BCG) vaccination is associated with improved prognosis in all types of lymphomas. This concept will require further testing, and clinical trials using...
nonspecific immunotherapy or chemoimmunotherapy for lymphoma are underway.

By cell surface marker criteria, many lymphomas (even many of the "histiocytic" tumors) seem to be malignant B-cell neoplasms. Nonetheless, malignant cells which appear to be similar by morphologic or even immunologic criteria proliferate in discrete nodules of tumor within normal lymph nodes (nodular lymphomas) in about one-half of cases and in the remaining cases proliferate in an invasive manner which completely effaces normal lymph node architecture (diffuse lymphomas). We therefore suggest that normal T-cell immunocompetence may be a central factor in determining whether a given lymphoma has a nodular or diffuse morphologic pattern and thus may influence the clinical course. It may be that some of the lymphocytes surrounding the nodules of neoplastic cells are normal T lymphocytes with specific immunity or suppressive activity directed against these malignant cells.

Although the above remarks are purely speculative, there are several clinical and pathologic observations which tend to support this view: the nodular lymphomas are associated with relatively intact cell-mediated immunity, favorable prognosis, occasional spontaneous regressions, and, in some cases, the patients can be observed for long periods with stable disease not requiring treatment (eight patients in this series). Coalescence of tumor nodules, the existence of "composite lymphomas," and the known progression of some nodular lymphomas to lymphomas with diffuse patterns all suggest that progressive paralysis of cell-mediated immunity occurs with the loss of immunologic containment of the tumor in nodules. In contrast, diffuse lymphomas are associated with much more severe impairment of cell-mediated immunity, generally have an unfavorable prognosis, and appear to be the terminal form of certain nodular lymphomas. In renal transplant recipients who have their cellular immunity intentionally suppressed, lymphomas of diffuse (not nodular) histologies occur at a much greater than expected incidence.

Thus, the clinical and morphological manifestations of the NHL might well depend on the degree of T-cell immunocompetence and might be favorably altered with immunotherapy that stimulates normal T-cell function. Further studies in vitro and in vivo of immune function of patients with NHL and further clinical trials of various types of immunotherapy will be necessary to define the exact role of immunocompetence in these disorders.

REFERENCES
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SE Jones, K Griffith, P Dombrowski and JA Gaines