Lymphocyte Receptors for Concanavalin A in Hodgkin Disease

By Alan C. Aisenberg, Sigmund Weitzman, and Barbara Wilkes

The number of lymphocytes with mobile receptors for concanavalin A (Con A) on their surface membrane (forming visible caps after the addition of fluorescein-conjugated Con A) was determined in the peripheral blood of 53 patients with Hodgkin disease. Of 29 individuals studied prior to treatment, the level of capped cells was found to be below the normal range in 9 of 13 in stages I and IIA, 6 of 8 in stage IIIA, and all 8 in stages IIIB and IV. Even among patients in remission 2 yr after successful treatment the level was below the lower normal limit in 9 of 16. The number was also reduced in 7 of 8 individuals with recurrent lymphoma. The level of lymphocytes that cap with Con A may prove to be a more sensitive measure of active Hodgkin disease than the total peripheral lymphocyte count or the level of T cells. This lymphocyte parameter merits further study as a correlate in vitro of cellular immunity.

Active Hodgkin disease is accompanied by a deficiency in cell-mediated immunity. In an effort to establish the pathogenesis of this defect, a variety of lymphocyte functions has been investigated over the past decade. Recently profound depression of the percentage of peripheral lymphocytes forming fluorescent caps in the presence of fluorescein-conjugated concanavalin A (Con A) has been reported. The present investigation was undertaken to delineate the effect of pathologic stage and disease status on this property of the Hodgkin disease lymphocyte.

Materials and Methods

Fifty-three patients with Hodgkin disease seen at the Massachusetts General Hospital from 1975 to 1977 were investigated. The Ann Arbor staging classification was employed, and all biopsies were reviewed by the Pathology Dept. and subclassified according to the Rye system. Three groups of patients were studied. Twenty-nine individuals were untreated; 26 had been pathologically staged with exploratory laparotomy and splenectomy. Surgically staged patients were investigated either prior to exploration or immediately before discharge from the hospital. (The operation did not alter the parameters under investigation, but lymphocyte separations may be unsatisfactory in the first few postoperative days.) A second group of 16 patients was examined 2 or more yr after treatment with radiation, combination chemotherapy (MOPP), or both, when in complete remission. Finally, 8 individuals in clinical relapse were studied, 6 undergoing single-agent or combination chemotherapy. Twenty-five determinations performed on normal laboratory personnel served to establish control values.

The standard Ficoll-Hypaque gradient employed to isolate lymphocytes from defibrinated venous blood and the rosetting technique have both been described in detail earlier. The total peripheral lymphocyte count was calculated from the white blood cell count and the differential count of a Wright-stained blood smear. Cells forming spontaneous rosettes were as-
sessed by adding freshly prepared lymphocytes to sheep erythrocytes in the presence of 9", AB serum, incubating at 37°C for 10 min, centrifuging, reincubating at 4°C overnight, resuspending, and enumerating the rosettes in a hemocytometer chamber. The number of T lymphocytes (rossetting cells) per cu mm was calculated with the aid of the total lymphocyte count.

The method of Ben-Bassat and Goldblum was followed closely to measure the number of cells capping in the presence of Con A. First, 2 x 10^6 lymphocytes were washed twice with pH 7.4 phosphate-buffered saline (PBS) and the cells resuspended in 0.5 ml of the same medium. (Fluorescein isothiocyanate conjugated Con A was purchased from Miles Laboratories, Elkhart, Ind., and stored at -70°C in 0.1-ml aliquots.) Then 100 µg freshly prepared and centrifuged (15 min at 850 g) Con A in 0.5 ml PBS was added to the cells, which were then incubated at 37°C for 15 min in a shaking water bath. The cells were washed three times with PBS, suspended in PBS glycerine, and mounted on a glass slide. The number of lymphocytes with fluorescent caps was determined with a Zeiss ultraviolet microscope equipped with an Osram HBO 200 mercury arc lamp and an interference primary filter. A minimum of 200 cells was examined in each preparation. From the percentage of capped cells and the total lymphocyte count, the number of lymphocytes that capped with Con A per cu mm of peripheral blood was calculated. Preparations contaminated with more than 2", nonlymphoid cells were excluded from study, since phagocytosis of the fluorescent reagent may be difficult to distinguish from fluorescent caps.

RESULTS

Total Lymphocyte Counts (Fig. 1)

Peripheral lymphocyte counts of untreated patients in stages I and IIA fell either within the normal range or were slightly reduced. The mean level for patients in stage IIIA was quite similar to that of patients in stages I and IIA, but several patients in the more advanced stage displayed profound lymphocytopenia. Lymphocytopenia was uniform among individuals in stages IIIB and IV. Patients in remission for 2 or more yr showed a spread of lymphocyte counts within the normal range, with a normal mean. The values in the treated group could not be related to the type of therapy, i.e., extended field or total nodal irradiation and/or MOPP chemotherapy. Six patients with recurrent disease undergoing single-drug or combination chemotherapy showed marked lymphocytopenia, while two in whom the disease recurred but who were not yet treated (one in stage IIA and one in IIIA) displayed normal levels.
**T Lymphocyte Counts** (Fig. 2)

The levels of T lymphocytes (cells forming spontaneous rosettes with sheep erythrocytes) were quite similar to the total peripheral lymphocyte counts in the various patient groups. An occasional individual in each group exhibited a depressed percentage of T lymphocytes, but in the majority of patients in each category the percentage was in the range observed in normal controls (52%–78%).

**Lymphocytes Capping With Concanavalin A** (Fig. 3)

The mean and individual levels of peripheral lymphocytes that cap with Con A were reduced more, compared to the range of normal controls, than the total or T lymphocyte levels. Thus 9 of 13 patients in stages I and IIA, 6 of 8 in stage IIIA, and all 8 in stages IIIB and IV were found to have levels of lymphocytes capping with Con A below the lower limit of the normal range. Even among patients in remission 2 yr after successful treatment 9 of 16 fell below the lower normal limit. The 8 individuals with recurrent disease were exceptional in that...
Table 1. Lymphocyte Studies in Untreated Patients With Stage I, II, and IIIA Hodgkin Disease

<table>
<thead>
<tr>
<th></th>
<th>Normal Range</th>
<th>Range in Patients</th>
<th>p</th>
<th>t</th>
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<tbody>
<tr>
<td>Total lymphocytes</td>
<td>1953 ± 484</td>
<td>1427 ± 742</td>
<td>&lt;0.01</td>
<td>2.89</td>
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<tr>
<td>E rosette lymphocytes</td>
<td>1300 ± 323</td>
<td>865 ± 527</td>
<td>&lt;0.01</td>
<td>3.42</td>
</tr>
<tr>
<td>Con A lymphocytes</td>
<td>357 ± 98</td>
<td>183 ± 123</td>
<td>&lt;0.001</td>
<td>5.35</td>
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their levels of cells capping with Con A were not reduced, proportional to normal values, more than the reduction of total and T lymphocytes.

Statistical Comparison

Table 1 shows the mean peripheral lymphocyte values observed in the group of patients in stages I, II, and IIIA. All three measured parameters were significantly lower than normal, but the higher t value (representing a higher degree of probability of difference between means) and the greater statistical significance (p < 0.001 versus p < 0.01) in the Con A group suggests that this may prove a more sensitive measurement. All mean values were even more strikingly depressed in patients with advanced (stages IIIB and IV) disease (see Figs. 1–3).

DISCUSSION

Concanavalin A has been employed extensively to probe the mobility of specific receptors on the surface membrane of lymphocytes of experimental animals and man. While in birds the Con A receptors on T lymphocytes are more mobile than those on B cells, in man both B and T lymphocytes are reported to form caps with fluorescent Con A. Thus the population of cells with mobile Con A receptors, i.e., those that cap, cannot be exclusively related to either B or T lymphocytes. Despite the voluminous Con A literature, a separate functional significance of the subpopulation of lymphocytes that cap with Con A has not been established.

We agree with earlier investigators that the number of peripheral lymphocytes with mobile Con A receptors is reduced in Hodgkin diseases, although we found more overlap between patients and normal controls than has been reported. However, a diminution in the number of cells that cap with Con A was found in most patients with active disease, and the deficit was severe and consistent in those with advanced stage. The depletion of cells with mobile Con A receptors correlates better with disease activity than does either total peripheral lymphocyte count or the level of T lymphocytes (rosetting cells). Indeed, we found little advantage of T-lymphocyte determination over the simpler total lymphocyte count in this investigation.

It is attractive to relate the depletion of lymphocytes with mobile Con A receptors to the impaired cellular immunity observed in Hodgkin disease, however, without concurrent assessment of delayed hypersensitivity in vivo (and such skin testing remains difficult to quantitate) this relationship remains conjectural. Similarly, it is plausible that the depletion of lymphocytes with mobile Con A receptors, and the diminished responsiveness of these cells in vitro to phytohemagglutinin observed in Hodgkin disease, reflect the
same lymphocyte abnormality. In this connection it should be noted that determination of cells capping with fluorescent Con A is far less demanding of time and technique than is measurement of responsiveness in vitro to phytohemagglutinin by lymphocyte culture. Thus if the level of cells with mobile Con A receptors were to prove a measure of delayed hypersensitivity in vitro, it would be a most convenient determination. However, for the present we can conclude only that the level of lymphocytes that cap with Con A may prove to be a more sensitive measure of active Hodgkin disease and pathologic stage than the total peripheral lymphocyte level or the level of T cells. As a correlate in vitro of cellular immunity this lymphocyte parameter merits further study.

REFERENCES

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