Concanavalin A Receptors on the Surface Membrane of Lymphocytes from Patients with Hodgkin’s Disease and Other Malignant Lymphomas

(lectins/human lymphocytes/cell receptor mobility/fluorescent cap formation/cell agglutination)

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ABSTRACT Concanavalin A (Con A) induces movement of its receptors on the cell surface membrane. This induction results in a concentration of Con A site complexes on one pole of the cell to form a cap. A marked difference was found in the mobility of Con A receptors between lymphocytes from normal persons and lymphocytes from patients with Hodgkin’s disease and other malignant lymphomas. Lymphocytes isolated from tonsils of patients undergoing tonsillectomy and from axillary lymph node biopsies of breast cancer patients exhibited approximately 30% of cells with caps, which is identical with the cap formation ability of normal lymphocytes. In biopsy material from patients with Hodgkin’s disease and other malignant lymphomas, a significant decrease in the ability of the lymphocytes to form caps was observed. This difference in the mobility of Con A sites was even more pronounced in lymphocytes isolated from the peripheral blood. In 123 patients with Hodgkin’s disease and other malignant lymphomas, cap formation ranged between 3 and 12%. The ability of cells, from a normal donor or a lymphoma patient, to form caps was independent of the source from which the lymphocytes were isolated, e.g., lymph node, spleen, or blood. Lymphocytes from patients with lymphoma were also agglutinated by Con A to a higher degree than normal lymphocytes. These findings are discussed in relation to the association of the lymphocytes with these malignancies and as a possible aid in their differential diagnosis.

Concanavalin A (Con A) has been used as a probe to study changes in the mobility of specific receptors on the cell surface membrane (1–5). Studies with this probe have shown differences in the structure and function of the surface membrane of normal and malignant cells (6–9). We have previously reported that lymphocytes from patients with chronic lymphocytic leukemia have a reduced cap-forming ability and a higher Con A-induced agglutinability than normal lymphocytes (5).

The present studies were undertaken in order: (1) to determine whether there are changes in the mobility of Con A receptors on the surface of lymphocytes of patients with Hodgkin’s disease and other lymphomas, (2) to compare the receptor mobility of lymphocytes from malignant lymphoma with that of lymphocytes isolated from other malignancies and diseases, (3) to test whether lymphocytes derived from different tissues of the same individuals exhibit similar Con A receptor mobility.

MATERIALS AND METHODS

Patients. Biopsies and other material for these studies were obtained from ambulatory and hospitalized patients with Hodgkin’s disease and other malignant lymphomas from the following hospitals: the Chaim Sheba Medical Center, the Beilinson and the Hadassah and Rambam Hospitals. The lymph node biopsies were obtained from untreated lymphoma patients undergoing biopsy for diagnostic purposes. Several lymph node biopsies and all spleens were obtained from patients undergoing staging laparotomy. Peripheral blood was obtained from 118 patients with Hodgkin’s disease and five with lymphosarcoma. These included both untreated and treated cases. The patients represented all clinical stages and histologic types of Hodgkin’s disease. Fifty-four of the 118 patients with Hodgkin’s disease were untreated, newly diagnosed patients. The others were patients diagnosed during the years 1969–1972 who had been undergoing radioand/or chemotherapy at various periods of their illness. Bleeding of these patients for our tests was carried out at least 3 months or more after their last treatment.

Preparation of Biopsies and Blood Samples. Biopsies of lymph nodes and spleens were cut into small pieces with scissors into medium RPMI 1640 containing 10% fetal calf serum. The lymphocytes were usually isolated from fresh biopsies; sometimes, however, the biopsies were kept overnight at 4° in medium RPMI 1640 and the cells were isolated next morning. Cells isolated from inflamed tonsils were prepared in a similar manner.

Blood was drawn into sterile bottles containing 10 units/ml of heparin.

Isolation of Lymphocytes. Lymphocytes were isolated from peripheral blood, lymph node or spleen biopsies, and tonsils by Ficoll–Hypaque gradient centrifugation (10), washed twice with phosphate-buffered saline (PBS) (pH 7.2) and diluted in PBS to the appropriate concentration. Viability of the cells was determined by trypan-blue exclusion. In all the experiments viability of the cells was 90–100%.

Assay for Binding of Fluorescent Con A. Fluorescein-isothiocyanate-conjugated Con A (F-Con A) was prepared by Miles-Yeda at a mole ratio of 1.86 fluorescein to protein. For the experiments, cells were incubated with different concentrations of fluorescent Con A up to 100 μg/ml for 30 min at 24°, or for 15 min at 37°. The cells were washed with PBS and the fluorescence was determined on a drop of cells with a Reichert Zetopan microscope with transmitted ultraviolet light. Five hundred cells were counted for each point. The results of fluorescent Con A binding in these experiments were obtained with saturation conditions, and only single cells were counted for the percentage of caps.

Assay for Agglutination. Con A was obtained from Miles-Yeda, Rehovoth, Israel; 0.5 ml of Con A at different concen-
Concanavalin A Receptors on Lymphoma Lymphocytes

RESULTS

Cap Formation with Fluorescent Concanavalin A of Lymphocytes from Biopsy Material of Patients with Malignant Lymphoma. Biopsy material was obtained from patients with various types of malignant lymphoma. All the patients were untreated and the biopsies were made in order to establish the histopathological diagnosis. The following patients were included in this series: 29 patients with active Hodgkin's disease, two patients with reticulum cell sarcoma, five with lymphosarcoma and four with non-Hodgkin's lymphoma. All biopsies were from affected lymph nodes and, in the great majority, these were cervical nodes. Lymphocytes were separated by the Ficoll-Hypaque method and examined for cap formation with F-Con A. In addition to the biopsies from the patients with malignant lymphoma, biopsy material was collected from non-lymphoma controls. These were tonsils from 19 patients undergoing tonsillectomy and axillary lymph nodes from six patients with breast cancer. Fig. 1 illustrates the results obtained with 100 µg/ml of F-Con A. Lymphocytes from all lymphoma patients exhibited a reduced ability to form caps with fluorescent Con A. This is true for patients with Hodgkin's disease, as well as for non-Hodgkin's lymphoma. The reduction in cap formation is even more pronounced in the patients with non-Hodgkin's lymphoma than in Hodgkin's disease. Lymphocytes isolated from tonsils of all the patients undergoing tonsillectomy and from lymph nodes of breast cancer patients exhibited a cap formation ability similar to that of normal lymphocytes (ref. 5, and see also Fig. 2). The binding of F-Con A to both normal and lymphoma lymphocytes was inhibited when α-methyl-D-mannopyranoside was added as a hapten inhibitor.

Ability of Lymphocytes from the Peripheral Blood of Patients with Hodgkin's Disease to Form Caps with Fluorescent Con A. Examination of lymphocytes from the peripheral blood of several new untreated patients with Hodgkin's disease revealed that their ability to form caps with fluorescent Con A is significantly reduced. Following this observation we extended this study to a large sample of Hodgkin's disease patients. In addition to the patients with Hodgkin's Disease, we included in this study a group of healthy donors as well as patients with various infections, autoimmune disorders, and other diseases.

Altogether, 118 patients with Hodgkin's disease and five cases with lymphosarcoma were included in the study. These constitute a very nonhomogeneous group of patients. They include patients of both sexes, aged 15-65 years; duration of illness from onset ranges between several weeks and 5 years. All patients were bled either prior to their first treatment or three or more months after they had completed a series of radio- or/and chemotherapy. Although all histological types of Hodgkin's disease are included, the majority are patients with nodular sclerosis and mixed cellularity.

The normal controls were samples from healthy blood donors aged 18-60 years. In this group are also included peripheral blood samples from three newborn babies. In the group designated as “other diseases” are included, as follows:

one patient with infectious mononucleosis; 10 patients with fevers of undetermined etiology, eight patients with carcinoma, and two cases each of rheumatoid arthritis, multiple sclerosis, polycythemia vera, and pancytopenia.
Fig. 3. Agglutinability of lymphocytes from patients with Hodgkin's disease (●) and other malignant lymphomas, reticulum cell sarcoma (▲), lymphosarcoma (♦), and other undetermined types of sarcoma (▼), from normal individuals (O), inflamed tonsils (O), cases of breast cancer (□), polycythemia vera (□), and pancytopenia (▼). Lymphocytes were isolated from biopsy material or from peripheral blood.

Fig. 2 shows the results of this study. Lymphocytes from all patients with Hodgkin's disease exhibit a significant reduction in the ability to form caps with fluorescent Con A. The great majority of the patients with Hodgkin's disease and the five patients with lymphosarcoma are within the range of 1–10% cap-forming ability. This is on the average even lower than the cap-forming ability of lymphocytes isolated from the lymph nodes of patients with Hodgkin's disease and other lymphomas. All healthy donors and patients with other diseases exhibited the pattern of cap formation of normal lymphocytes.

Binding of Fluorescent Con A to Cells from Different Tissues of the Same Patient or Healthy Donor. To test whether lymphocytes derived from different tissues of the same individual exhibit similar Con A receptor mobility, we examined the interaction of fluorescent Con A with cells from different tissues of lymphoma patients and normal donors. Cells were isolated by the Ficoll–Hypaque method from peripheral blood, lymph nodes, and spleens of patients with Hodgkin's disease, and from the tonsils and the peripheral blood of tonsillectomized patients. Lymphocytes from all eight patients with Hodgkin's disease showed a marked reduction in the ability to form caps with fluorescent Con A. In most patients there was an excellent agreement in the percentage of cap formation in lymphocytes derived from the various tissues of the same patient (Table 1). All five tonsillectomized patients showed approximately 30% cap formation, and the results obtained with lymphocytes isolated from the tonsils or from the peripheral blood were identical (Table 2).

Agglutination of Lymphocytes from Lymphoma Patients by Con A. Similarly to the experiments reported for the cap formation ability, the agglutination experiments were also carried out with cells isolated by the Ficoll–Hypaque technique. The final concentration of Con A was 250 mg/ml. The tests were made with lymphocytes from biopsy material—mainly lymph nodes—and from the peripheral blood. The following materials were included in this study: 34 biopsies of malignant lymphomas and 76 samples of peripheral blood. The following samples from non-lymphoma patients were tested: lymphocytes from 20 tonsils of tonsillectomized patients; axillary lymph nodes from eight patients with breast cancer; peripheral blood from 25 healthy donors; and blood from two cases each of polycythemia vera and pancytopenia. The results are shown in Fig. 3. A significant difference in agglutinability is evident for lymphocytes from the patients with malignant lymphoma as compared to the healthy donors and non-lymphoma patients. Agglutinability was specific as determined in each instance by inhibition with 0.1 M α-methylmannoside.

TABLE 1. Cap formation with F-Con A in lymphocytes isolated from various tissues of the same patients with Hodgkin's disease

<table>
<thead>
<tr>
<th>Source of cells, patient no.</th>
<th>Peripheral blood</th>
<th>Lymph node</th>
<th>Spleen</th>
</tr>
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<tbody>
<tr>
<td>25</td>
<td>7.9</td>
<td>6.4</td>
<td>6.6</td>
</tr>
<tr>
<td>26</td>
<td>6.0</td>
<td>6.2</td>
<td>6.1</td>
</tr>
<tr>
<td>52</td>
<td>7.6</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>59</td>
<td>7.6</td>
<td>15.8</td>
<td>14.8</td>
</tr>
<tr>
<td>76</td>
<td>10.8</td>
<td>10.3</td>
<td>10.0</td>
</tr>
<tr>
<td>85</td>
<td>7.6</td>
<td>10.8</td>
<td>7.4</td>
</tr>
<tr>
<td>12</td>
<td>4.0</td>
<td>4.2</td>
<td>N.T.</td>
</tr>
<tr>
<td>58</td>
<td>10.4</td>
<td>14.4</td>
<td>N.T.</td>
</tr>
</tbody>
</table>

Lymphocytes were isolated from patients by the Ficoll–Hypaque method. Cells were incubated with 100 μg of F-Con A/ml for 30 min at 24° or for 15 min at 37° and washed with PBS, and the fluorescence was determined on a drop of living cells. N.T., not tested.

DISCUSSION

The results of these studies clearly indicate that lymphocytes isolated from patients with Hodgkin's disease and other malignant lymphomas have a reduced mobility of Con A receptor sites. This is true for lymphocytes isolated from biopsy material of the patients, as well as for lymphocytes isolated from the peripheral blood. In this respect these findings are similar to those reported by us previously in cases of chronic lymphocytic leukemia (5).

The reduced mobility of Con A receptor sites of lymphocytes isolated from the blood of patients with chronic lymphocytic leukemia and from the affected lymph nodes of patients with Hodgkin's disease and other malignant lymphomas are also in accord with the findings in mouse leukemia and lymphoma (2–4).

This reduced mobility of Con A receptor sites has hitherto been interpreted by others and by ourselves as a characteristic association with malignancy (2–5). This explanation might be relevant for the malignant tissue, such as the affected lymph node or spleen. It cannot, however, account for the behavior of the lymphocytes isolated from the peripheral blood of patients with Hodgkin's disease and other malignant lymphomas. As far as present evidence is available, the lymphocytes in the peripheral blood of such patients cannot be categorized as malignant (11). The reduced mobility of Con A receptors is not due to differences in the number of B or T lymphocytes in patients with lymphoma as compared to nor-
Table 2. Cap formation with P-Con A in normal lymphocytes of tonsillectomized patients

<table>
<thead>
<tr>
<th>Source of cells, donor no.</th>
<th>Peripheral blood</th>
<th>Tonsils</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.0</td>
<td>30.6</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
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<tr>
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<tr>
<td>5</td>
<td>28.9</td>
<td>32.0</td>
</tr>
</tbody>
</table>

ternal donors, since both B and T lymphocytes give a similar number of cells with caps (5).

Another possible explanation may be sought in the immunologic state of the lymphocyte. In many patients with Hodgkin's disease impairment of cellular immunity has been repeatedly demonstrated. The nature of this immunologic defect is not yet precisely established. It is characterized by the loss of several manifestations of cell-mediated immunity, without any apparent defect in the synthesis of humoral antibody (12-14). Patients with Hodgkin's disease have been repeatedly proven to have diminished delayed-hypersensitivity functions to both natural antigens and to chemical contact allergens (15, 16). More recent studies have demonstrated yet other in vitro manifestations of the defect in cellular immunity in Hodgkin's disease; a decreased lymphocyte responsiveness in vitro to nonspecific mitogens such as phytohemagglutinin has been repeatedly demonstrated (17-19). Moreover, Gaines et al. have recently shown that lymphocytes from patients with Hodgkin's disease are deficient in their recognition of certain specific antigenic stimuli (20). Thus, much attention in Hodgkin's disease has been focused on the lymphocyte; many, though not all experimental and clinical observations support the view that the lymphocyte from patients with Hodgkin's disease functions abnormally (21).

The question which is then posed is: is there an association, in patients with Hodgkin's disease, between this abnormal behavior of the lymphocyte and the reduced cap-forming ability with Con A? This is not easy to establish. We thought that study of lymphocytes from certain autoimmune diseases would give us a clue to this problem, but all these patients turned out to behave as normal with this Con A probe. It seems that other avenues must be explored to resolve this question.

There are various possibilities of using the Con A probe in clinical practice. First, it can be used as an aid in the differential diagnosis between malignant lymphomas and other diseases. We have attempted to do so in a number of double blind tests with very promising results. However, there is room for more tests to be carried out by investigators other than ourselves. Another avenue which is worth exploring is the follow-up with the Con A probe of patients during remission. It may turn out that there are changes in the cap-forming ability during reactivation of disease symptoms. All these suppositions can and will now be experimentally tested.

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