Changes in the Con-A-Induced Redistribution Pattern of Lymphocytes: A Possible Aid in the Differential Diagnosis Between Malignant Lymphoma and Other Diseases

By Hannah Ben-Bassat, Shmuel Penchas, Aaron Polliack, Stella Mitrani-Rosenbaum, Elizabeth Naparstek, Yaacov Matzner, Amos Kedar, Daniel Shouval, Amiram Eldor, Miron Prokocimer, and Nathan Goldblum

Lymphocytes isolated from the peripheral blood of healthy individuals and of patients with nonmalignant and malignant disorders were studied for redistribution of concanavalin A (Con-A) receptors after ligand contact. Upon ligand contact, Con-A receptors are redistributed in the plane of the membrane and can form a cap at one part of the cell surface. Redistribution of Con-A surface receptors was determined by the cap-forming ability after binding of fluorescent Con-A. The present studies were performed on lymphocytes from 328 patients with malignant lymphomas, 103 patients with chronic lymphocytic leukemia (CLL), 92 healthy individuals, 163 patients with nonmalignant disorders, and 32 patients with carcinomas. The results show a marked reduction in the cap-forming ability of lymphocytes from patients with malignant lymphoma and CLL (mean value 9.9%) as compared with lymphocytes from healthy individuals and from patients with nonmalignant disorders or carcinoma (mean value 22.8%). Lymphocytes isolated from lymphoma patients during remission stages of the disease exhibited an intermediate level of cap-forming ability (16%-20%). The present results that concur with previous observations show that normal lymphocytes and lymphocytes from patients with nonmalignant disorders are characterized by a high degree of mobility of Con-A receptors, whereas lymphocytes from patients with malignant lymphoma and CLL are characterized by a low mobility of Con-A receptors. We suggest the Con-A cap-forming ability test may be useful as an aid in the differential diagnosis between malignant lymphomas and nonmalignant disorders.

The lectin concanavalin A (Con-A) has been widely used as a probe to study changes in the mobility of specific receptors on the cell membrane. Changes in the distribution of Con-A sites, the formation of Con-A-site complexes, and the lateral mobility of these complexes result in the formation of a cap. This cap can be visualized by fluorescence microscopy as concentration of markers localized at one part of the cell surface. With the Con-A probe, the lateral mobility of membrane receptors on the cell surface can be determined. Studies with this probe have shown differences in the structure and function of the surface membrane of normal and malignant cells. In earlier studies, we have shown differences in the mobility of Con-A receptors on the surface membrane of normal lymphocytes and lymphocytes isolated from patients with chronic lymphocytic leukemia (CLL), Hodgkin and non-Hodgkin lymphomas, and Epstein-Barr virus (EBV) carrying lymphoma (African Burkitt lymphoma). The difference in the mobility of Con-A receptors, as measured by the cap-forming ability, was also observed in cells obtained from biopsy material (lymph node or spleen) from patients with the above malignancies. The ability of cells from a normal donor or a patient with lymphoma to form caps appeared to be independent of the source from which the lymphocytes were derived. The present studies were undertaken in order to determine whether the Con-A cap-forming ability reaction can be of value as a laboratory aid in the differential diagnosis between malignant lymphomas and nonmalignant disorders.

MATERIALS AND METHODS

Patients

Blood samples were obtained from hospitalized and ambulatory patients at several hospitals in Israel. The patient population included 92 patients with CLL, 103 patients with the diagnosis of Hodgkin disease, 80 patients diagnosed to have non-Hodgkin type malignant lymphoma, 163 patients with other nonmalignant disorders, 32 patients with diagnosed carcinoma, and 92 normal individuals. Where possible, evaluation was carried out on newly diagnosed patients prior to starting therapy. Evaluation of treated patients was done at least 3 wk following the last administered treatment.

Separation of Lymphocytes

Lymphocytes were isolated from peripheral blood (5-10 ml) by Ficoll-Hypaque gradient centrifugation, washed twice in phosphate-buffered saline (PBS) pH 7.2, and diluted to the appropriate cell concentration. A cell concentration of 2 x 10^6/ml is recommended for convenient counting. Viability of the cells used in the experiments was 95% - 100% as determined by the trypan blue exclusion method. Only freshly isolated cells were used in the
experiments, although previous experiments have shown that blood samples stored at +4°C for up to 72 hr can still be used for lymphocyte preparation and examination. Obviously, for routine testing, the use of freshly isolated cells is recommended.

**Binding of Fluorescent Con-A (F-Con-A): Con-A Cap-Forming Ability Test**

F-Con-A was obtained from Miles Yeda, Rehovot, Israel, at a fluorescein-to-protein ratio of 1:68. For the experiment, 0.5 ml of cell suspension (2–4 x 10⁶ cells/ml) were incubated with 0.5 ml F-Con-A (200 μg/ml) for 30 min at 37°C; the cells were washed with 5 ml PBS, and the fluorescence was determined on a drop of cells in a Zeiss microscope with transmitted ultraviolet light. Five-hundred cells were counted for each point, and only single cells and those in very small clumps (2–5 cells) were counted for the percentage of caps.

**Statistical Analysis**

The two-tailed Student t test was used to compare sample means. The percentages of lymphocytes forming caps that were selected as limits were determined by inspecting the frequency distributions of different groups of the cases and selecting values at which the smallest number of cases were misclassified. Standard deviations for these values were approximated by assuming equal variances for all groups.

**RESULTS**

**F-Con-A Cap-Forming Ability of Lymphocytes From Patients With Malignant Lymphoma, CLL, and Nonmalignant Disorders**

Lymphocytes from peripheral blood of patients with malignant lymphomas (Hodgkin and non-Hodgkin type) and chronic lymphocytic leukemia (CLL) were examined for cap formation with F-Con-A. In addition to the healthy individuals, a group of patients with nonmalignant disorders and carcinomas was also examined. Figure 1 illustrates the results obtained with 100 μg/ml F-Con-A. The lymphocytes of the majority of patients with malignant lymphomas and CLL have cap-forming ability within the range of 4%–12%. Con-A cap formation in lymphocytes from the great majority of patients in the control groups was within a range similar to that of normal persons' lymphocytes (20%–30%). Table 1 presents the various groups of malignancies and disorders studied stating the diagnosis, number of cases, and mean and standard deviation of Con-A cap-forming ability of blood lymphocytes for each group of patients. The mean values for malignant lymphomas and CLL lymphocytes are 10.1% and 9.2%, respectively, whereas those for the lymphocytes from the healthy individuals, from the patients with various nonmalignant disorders, and for the carcinoma patients are 24.6%, 21.7%, and 22.4%, respectively.

**Evaluation of Results**

The patients were divided into 3 groups according to the type of disorder. Group I included 431 patients with diagnosed malignant lymphatic disease (Hodgkin disease, non-Hodgkin type lymphoma, and CLL); group II included 163 patients with nonmalignant disorders and 92 healthy individuals; and group III included 32 patients with a diagnosis of carcinoma. The F-Con-A cap-forming ability of peripheral blood lymphocytes presented in Table 1 indicates that lymphocytes from group I exhibit a significant decrease in their cap-forming ability with F-Con-A compared to lymphocytes from group II (p < 0.001). Lymphocytes from group III exhibited a cap-forming ability with F-Con-A similar to that of group II. Table 2 exhibits the subgroups within groups I and II. The F-Con-A cap-forming ability of CLL lymphocytes (mean 9.2%) is significantly lower than for non-Hodgkin malignant lymphoma (mean 11.6%) (p < 0.001). There was no significant difference in the F-Con-A cap-forming ability for the subgroups within group II comprising the normal control (healthy individuals) and various nonmalignant disorders. The nonmalignant disorders group included 28 patients with lymphadenopathy, 24 patients with infectious mononucleosis, and 111 other patients from the internal and surgical departments of the hospital. The lymphadenopathy subgroup was of interest as a control for nonmalignant proliferating lymph node tissue. The patients were children and young adults 2–16 yr old. They had cervical nonspecific reactive lymphadenopathy, usually resulting from viral infection. The diagnosis was confirmed by biopsy. The cap formation with F-Con-A of the lymphocytes was similar to that of healthy donors (Tables 1 and 2). However, there were cases in these subgroups that gave low cap formation. Although it might have been of interest to follow these children after they were discharged, we have not attempted to do so in order to avoid causing stress. Figure 2 illustrates the overall distribution of Con-A cap-forming ability in lymphatic malignancies versus other diseases. The value of F-Con-A cap formation found by inspecting the frequency distribution of these different populations to minimize misclassification was 14%–15% with a standard deviation of 0.25 (Table 3).

**Con-A Cap-Forming Ability Test**

Past and present results suggest that the Con-A cap-forming ability test may be useful in the differentiation between malignant lymphomas and nonmalignant disease. Although the reaction is very reproducible, each patient should be examined twice, and a
control of lymphocytes from a healthy donor should be included in the test. The following limits are recommended: (1) normal reaction, ≥20% lymphocytes with caps; (2) malignant reaction, ≤14% lymphocytes with caps; (3) borderline reaction, 15%–19% lymphocytes with caps. Cases with a borderline reaction should be followed and retested after 2–3 wk. Since it is already well established that the capping phenomena is a metabolic and dynamic feature of the cell surface membrane,

<table>
<thead>
<tr>
<th>Group</th>
<th>Disorder</th>
<th>No. of Patients</th>
<th>Mean Value</th>
<th>Standard Deviation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Lymphatic malignancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Hodgkin lymphoma</td>
<td>248</td>
<td>9.6</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Non-Hodgkin lymphomas</td>
<td>80</td>
<td>11.6</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CLL</td>
<td>103</td>
<td>9.2</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>431</td>
<td>9.9</td>
<td>4.6</td>
<td>0.001</td>
</tr>
<tr>
<td>II.</td>
<td>Nonmalignant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Healthy</td>
<td>92</td>
<td>24.6</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Nonmalignant disorders</td>
<td>111</td>
<td>22.9</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Lymphadenopathy</td>
<td>28</td>
<td>19.0</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Infectious mononucleosis</td>
<td>24</td>
<td>19.7</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>255</td>
<td>22.8</td>
<td>6.8</td>
<td>0.005</td>
</tr>
<tr>
<td>III.</td>
<td>Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Carcinoma</td>
<td>32</td>
<td>22.4</td>
<td>6.5</td>
<td>0.771</td>
</tr>
</tbody>
</table>
(drugs, chemicals, such as vincristine, colchicine, cholesterol, etc.), it is recommended whenever possible to perform the test on untreated patients and always critically evaluate the results.

With a limit of 14%-15%, the sensitivity and specificity of the Con-A cap-forming ability test are 0.85 and 0.86, respectively. For a population with a prevalence of lymphatic malignancy of 50%, the positive predictive value of the test is 0.86 and the negative predictive value is 0.85. For a population with a prevalence of 1%, the respective figures are 0.07 and 0.997.

When lymphatic malignancy is suspected for other reasons and the test is performed late in the diagnostic process, the positive result is very reliable while the negative one is only marginally less reliable. For screening purposes in a population with prevalence of say 0.01 lymphatic malignancy, the very high negative predictive value of 0.999 associated with the suggested limit of 20% makes the test well suited to exclude the diagnosis.

**DISCUSSION**

Lymphocytes isolated from patients with malignant lymphoma have a reduced mobility of Con-A receptor sites as determined by their cap-forming ability. This is true for lymphocytes isolated from biopsy material of the patients, as well as for lymphocytes isolated from peripheral blood. The reduced mobility of Con-A receptors of lymphocytes isolated from blood of patients with CLL and from affected lymph nodes of patients with Hodgkin disease, Burkitt lymphoma, and other malignant lymphomas concur with the findings in mouse leukemia and lymphoma. The reduced mobility of Con-A receptors has hitherto been interpreted as a characteristic associated with malignancy. This explanation might be relevant for malignant tissue, such as the affected lymph node or spleen. We have previously shown that lymphoma cells from tumor tissues in Burkitt lymphoma, as well as the permanent lines derived from those cells, exhibit a reduced mobility of Con-A receptor sites. Surprisingly, lymphocytes isolated from peripheral blood of patients with lymphoma also exhibit reduced Con-A receptor mobility, although according to presently available evidence, these lymphocytes are not categorized as malignant. We have previously shown that the
cap formation with F-Con-A of several samples of lymphocytes from the same patient is generally very consistent.\textsuperscript{10,20} Six patients with Burkitt lymphoma and one with carcinoma were examined several times during a period of 3 wk–5 mo. During this period, the patient had been already under treatment. No obvious differences in the absolute numbers of cells from the same patient or from identical blood samples were observed.\textsuperscript{10} However, we have observed, and our results have been confirmed by others,\textsuperscript{21} that during remission of leukemia or lymphoma there might be an increase in the cap-forming ability of lymphocytes from these patients, although the values do not reach the mean of normal values (range 16%–20%, H. Ben-Bassat, unpublished results).

The reduced mobility of Con-A receptor sites is not due to differences in the proportion of B or T lymphocytes in patients with lymphoma compared to normal donors, since both B and T lymphocytes give similar numbers of cells with caps.\textsuperscript{8}

Another possible explanation may be the immunologic state of the lymphocyte. In patients with Hodgkin disease, as well as in patients with other malignant lymphoma or leukemia, impairment of cellular immunity has been repeatedly demonstrated.\textsuperscript{14} However, the nature of this immunologic defect has not yet been 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.\textsuperscript{17} More recent studies have demonstrated an in vitro manifestation of this defect: a decreased lymphocyte response to nonspecific mitogens.\textsuperscript{16,19}

We have recently shown differences in the membrane of lymphocytes from patients with malignant lymphomas and normal healthy persons by using yet another fluorescence probe (1,6-diphenyl-1,3,5 hexatriane) embedded in the membrane lipid core of intact cells.\textsuperscript{20} With the aid of this probe, a quantitative method based on fluorescence polarization analysis of the probe was developed for analyzing the membrane fluidity of intact cells.\textsuperscript{21} Studies with this probe have indicated that in leukemic, as well as in malignant lymphoma lymphocytes, there is a marked increase in the fluidity of the surface membrane lipid core.\textsuperscript{22,23} Using this probe it was shown that the increase in membrane fluidity correlates with the activity of the disease. Patients with malignant lymphoma and leukemia in stages of clinical remission exhibited an increase in the rigidity of the membrane lipid core.\textsuperscript{20}

Thus, in the lymphocyte system, an inverse correlation between the lipid fluidity and the mobility of Con-A receptor sites was observed. The mobility of Con-A receptors decreases with increased fluidity of the lipid core. This dynamic feature concurs with the hypothesis that Con-A receptors become more exposed to the aqueous surroundings with increased fluidity of the lipid layer.\textsuperscript{24} There are various possibilities of using the Con-A probe in clinical practice. We hope that it might be used as an aid in the differential diagnosis between malignant lymphoma and other diseases.

ACKNOWLEDGMENT
The authors appreciate the excellent technical assistance of Lea Muznick-Goldstein.

REFERENCES
15. Inbar M, Ben-Bassat H, Fibach E, Sachs L: Mobility of carbohydrate-containing structures on the surface membrane and the normal differentiation of myeloid leukemic cells to macrophages and granulocytes. Proc Natl Acad Sci USA 70:2577, 1973


24. Gurtler LG, Emmerich B: Cap formation on lymphocytes from patients with leukemic diseases induced by four different lactins. Blut 36:239, 1978

Changes in the Con-A-induced redistribution pattern of lymphocytes: a possible aid in the differential diagnosis between malignant lymphoma and other diseases