Concanavalin A-induced Agglutination of Human Leukemic and Lymphoma Cells

Richard D. Maca
Division of Hematology-Oncology, Department of Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

SUMMARY

With a newly developed turbidometric method, concanavalin A was shown to agglutinate normal lymphocytes, lymphoma cells, and leukemic cells from chronic lymphocytic leukemia and from acute myelocytic and lymphocytic leukemia. However, there was a marked difference in the kinetics of this agglutination process. Leukemic blast cells and cells from a patient with convoluted lymphoma agglutinated poorly in this system. Conversely, the degree of agglutination for chronic lymphocytic leukemia cells was greater than that for the blast cells and also slightly greater than that for normal lymphocytes. Cultured cells from a Burkitt’s lymphoma (Raji) and from a patient with poorly differentiated lymphoma agglomerated very rapidly with concanavalin A. Prior incubation of all cell types with neuraminidase markedly enhanced the agglutination process similar to that of trypsinization. Thus, these studies illustrate the usefulness of this method in quantitating the kinetics of agglutination of various human neoplastic cell types by concanavalin A.

INTRODUCTION

There is considerable evidence that cell surface properties can be used to classify normal lymphocytes and cells from various malignant lymphoproliferative disorders. These properties include adhesiveness to inert fibers (4), immunological reactivity in specific antisera (1), presence of specific receptor (7, 12), and reactivity to various lectins (14). On the basis of these surface properties, normal lymphocytes have been grouped into thymus-derived, bone marrow-derived, and null lymphocytes. Recently, this classification system has been extended to malignant lymphoproliferative disorders which is providing a new dimension to the traditional morphological approach of classification. Already this approach has added new insight into the pathogenesis of the disorders and is expected to be helpful in guiding specific therapies in the future.

Recently, we have developed an improved method for quantitating lectin-induced agglutination of cultured lymphoblastoid cells (8). We illustrate the usefulness of this method in delineating and quantitating patterns of Con A-induced agglutination of cells from various cancers.

MATERIALS AND METHODS

Preparation of Cell Suspension. Venous blood was collected in a heparinized syringe (25 units/ml) and left inverted for 60 min at room temperature. The leukocyte-rich plasma was expelled, and the cells were separated from the platelets by centrifugation at 400 x g for 5 min. After the erythrocytes were lysed with buffered 0.85% ammonium chloride (pH 7.0), the cells were washed twice with PBS, pH 7.4 (Grand Island Biological Co., Grand Island, N. Y.). For the agglutination studies, the cells were resuspended with serum-free PBS to a concentration of 4 x 10^6 cells/ml. This cell suspension contained over 85% neoplastic cells with viability of over 90%.

To obtain mononuclear lymphocytes from normal subjects, heparinized venous blood was layered on top of a standard Hypaque-Ficoll gradient and centrifuged at 400 x g for 35 min (2). The cells were then removed from the gradient, freed of erythrocytes, washed, and concentrated to a density of 4 x 10^6 cells/ml in PBS. This method of isolation did not alter the agglutination patterns as determined by comparing the agglutination of leukemic cells isolated by the Hypaque-Ficoll and the above, alternate sedimentation methods.

Agglutination Assay. Agglutination was quantitated by using a platelet aggregometer, the details of which have been described elsewhere (8). Fifty μl of PBS containing 50 μg Con A (Sigma Chemical Co., St. Louis, Mo.) were added to 0.5 ml of a cell suspension. Changes in turbidity were recorded immediately thereafter while the cells were being stirred constantly. The recording was continued to 10 min or until the agglutination was maximum. The degree of agglutination was quantitated by measuring the slope of the agglutination curve as previously described (8). A competitive inhibitor, α-methylmannopyranoside (Sigma), at a concentration of 20 mg/ml was used to determine the specificity of Con A-induced agglutination.

Neuraminidase and Trypsin Treatment. Washed cells (10^7 cells/ml) in Hanks balanced salt solution containing 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer, pH 6.8, were incubated with Vibrio cholerae neuraminidase (General Biochemical, Inc., Chagrin Falls, Ohio), 50 units/ml. After incubation for 45 min at 37°C, the cells were slightly agitated and the clumps of cells, which were mostly nonviable, were allowed to settle and were discarded. The remaining suspended cells that were over 95% viable as determined by the trypan blue exclusion test were washed twice before they were used in the agglutination assay.

1 The abbreviations used are: Con A, concanavalin A; PBS, Dulbecco’s phosphate-buffered saline; CLL, chronic lymphocyte leukemia; ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia; A'-induced agglutination of cells from various cancers.
Control cells were incubated in the buffer without neuraminidase.

For trypsinization, washed cells, 5 × 10⁶/ml in PBS containing 0.25 mg trypsin (Grand Island Biological Co.), were incubated for 15 min at 37°C. After 2 washings the cells were resuspended in PBS and used immediately in the assay.

**Cells and Culture Conditions.** The continuous lymphoblastoid cell line, Raji, was derived from a Burkitt's lymphoma and grown as a suspension culture in McCoy's Medium 5A supplemented with 10% fetal calf serum (Grand Island Biological Co.) (13). For culturing leukemic cells, blast cells from the blood of leukemia patients were separated aseptically for the agglutination assays. The washed cells were then suspended (10⁶ cells/ml) in McCoy's medium 5A with 20% fetal calf serum and cultured for either 24 or 48 hr at 37°C in a 5% CO₂ atmosphere.

**RESULTS**

**Agglutination of Various Cell Types with Con A.** Con A was found to agglutinate normal lymphocytes and neoplastic cells of both lymphoid and myeloid origin. However, the kinetics of this agglutination process varied significantly with cell types. Normal lymphocytes, which were contaminated with less than 10% monocytes, agglutinated in a linear fashion with an average slope of 0.32 with a range of 0.19 to 0.48 (Chart 1). For the CLL lymphocytes, the average slope was 0.78 (ranging from 0.34 to 0.90) which is significantly greater than for the control lymphocytes (p < 0.01). Two patterns of agglutination were observed for the CLL lymphocytes. For approximately one-half of the samples tested, the curve was biphasic (Chart 1, Curve D). For the remainder, the curves were linear as for the normal lymphocytes (Curve C). On microscopic examination, the cell aggregates were found to be composed of at least 7 to 10 cells with cellular viability of greater than 90%. The agglutination slope was 0.78 (ranging from 0.34 to 0.90) which is significantly greater than for the control lymphocytes (p < 0.01). This effect is also illustrated in Chart 2 and Chart 3 for the cultured cells. This marked enhancement in the agglutination slope has not been observed for the control lymphocytes.

**DISCUSSION**

Con A has been found to agglutinate a variety of malignant cells; however, the kinetics of this process has not been described in detail. The agglutination slope for normal lymphocytes is 0.32 (range, 0.02 to 0.18), respectively. Microscopic examination of the cell suspension confirmed the poor agglutination of these cells. However, if the cell suspension was removed from the aggregometer where they were constantly stirred and left undisturbed at room temperature for an additional 15 to 30 min, agglutination was observed. The cell suspensions were then washed and resuspended in PBS and used immediately in the assay.

**Effect of Neuraminidase Treatment.** Incubation of leukemic blast cells, CLL lymphocytes, and cultured Raji cells with V. cholerae neuraminidase prior to the interaction with Con A resulted in a marked enhancement of agglutination. Staining of the cells in the aggregates with trypan blue revealed them to be over 90% viable. As seen in Table 1, the increase was the greatest for the leukemia cells and least for the cultured cells. This marked enhancement in the agglutination slope is also illustrated in Chart 2 and Chart 3 for the cultured lymphocytes, respectively. This effect is similar to that induced by trypsinization as shown in Chart 3 for CLL cells. Moreover, culturing these blast cells for 24 to 48 hr also enhanced agglutination to about the same degree as trypsinization or neuraminidase treatment.

**Table 1**

Enhancement of Con A-induced agglutination by neuraminidase treatment

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Slope of agglutination curve for untreated cells</th>
<th>Increase in slope by neuraminidase treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal lymphocytes</td>
<td>0.32 ± 0.02 (10)*</td>
<td>3.9 (9)*</td>
</tr>
<tr>
<td>CLL</td>
<td>0.78 ± 0.10 (14)</td>
<td>5.8 (4)</td>
</tr>
<tr>
<td>AML</td>
<td>0.10 ± 0.02 (7)</td>
<td>5.8 (4)</td>
</tr>
<tr>
<td>ALL</td>
<td>1.11 ± 0.02 (5)</td>
<td>9.2 (3)</td>
</tr>
<tr>
<td>Raji</td>
<td>1.66 ± 0.29 (6)</td>
<td>1.9 (7)</td>
</tr>
</tbody>
</table>

* Ratio of the average slope for neuraminidase-treated cells to the average slope for controls (untreated cells).
* Mean ± S.E
* Numbers in parentheses, number of samples tested.

**Chart 1.** Agglutination slopes of various lymphoid cell types. A, Raji cells; B, lymphocytic lymphoma cells; C, normal lymphocytes; D, CLL; E, ALL.
been carefully studied (3, 5). The method of quantitation most frequently used is a rough scoring technique whereby the agglutination is graded on a scale of 0 to + + + + (3).

Using this technique, it is difficult to quantitate small differences in agglutination and to quantitate the rate or degree of agglutination. Recently, a spectrophotometric method has been described whereby the rate and extent of cell agglutination by Con A can be ascertained (6). Using this method, differences in the kinetics of agglutination between 2 sarcoma cell lines were shown. Likewise, our method, which is similar in many respects, has also proved useful in detecting and quantitating differences in the kinetics of Con A-induced agglutination of human neoplastic cells as illustrated in this study.

It appears that the clustering of receptor sites is an important step for agglutination to occur. This phenomenon, which is dependent upon the mobility of these sites on the cell surface, is believed to be responsible for the agglutination of malignantly transformed cells (10, 15) and of trypsinized, normal cells by Con A (11). Agglutination is also enhanced by neuraminidase treatment as shown in this study and by others (9). Perhaps terminal sialic acid impedes the mobility of surface components, and thus by its removal the mobility and clustering of Con A receptor sites may be enhanced. However, other possible mechanisms such as elimination of surface negative charge and unmasking of membrane "cryptic" binding sites must be considered. Perhaps these studies define another role played by surface sialic acid, which would be to modulate or regulate the mobility of surface components.

This study provides evidence that variations do indeed exist in the kinetics of agglutination of various human lymphoproliferative disorders. Normal mononuclear cells, about 90% of which are lymphocytic, agglutinate in a linear fashion, only varying in the degree of agglutination. For CLL, the agglutination curves were either linear or biphasic. Conversely, blast cells from either ALL or AML were agglutinated poorly by Con A. These cells did not appear to lack the receptor for Con A since agglutination occurred if the cells were left undisturbed with the lectin or if treated with trypsin or neuraminidase prior to agglutination. Instead, these studies suggest that the Con A receptors on blast cells are present but are relatively immobile and are unable to cluster readily when exposed to Con A. Consequently, agglutination of these blast cells is impaired as compared to the other cell types studied.

The variability of agglutination patterns for the various cell types studied may well reflect variation in the membrane fluidity and thus the rate of clustering of Con A sites after the addition of this lectin. In taking advantage of this cell surface property, it may be possible to group the different lymphoproliferative disorders based on the type of agglutination curves produced by various lectins, such as Con A, wheat germ agglutinin, and others. If such subgroups could be defined, then this information in conjunction with information from other classification systems may provide helpful prognostic information and perhaps a guide to specific therapies for lymphoproliferative disorders in man.

**REFERENCES**

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