Regulation of Immune Response in Cancer by Plasma: The Postulated Role of Transcortin*

LEONARD AMARAL, Ph.D. AND SEYMOUR WERTHAMER, M.D.

Department of Pathology, The Methodist Hospital and Downstate Medical Center, Brooklyn, NY 11215

ABSTRACT

The effect of plasma from cancer patients on the synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein by human lymphocyte cell line RPMI-1788 was investigated and demonstrated to be inhibitory. Immunologic quantitation of lymphocyte intracellular transcortin of the normal patient and of the cancer patient illustrated that lymphocytes of the cancer patient contain more transcortin than do normal cells. Direct quantitation of plasma transcortin levels of normal patients and cancer patients parallels this increase. Inasmuch as previous studies from our laboratory demonstrate the relationship of plasma transcortin levels to lymphocyte metabolic processes, it is speculated that lymphocytes of cancer patients might be inhibited in their metabolic syntheses by unusually high levels of plasma transcortin.

Introduction

During recent years, numerous studies have supported the hypothesis that the successful development of malignant neoplasm involves the temporary or lasting suppression of the immune system. In vitro studies suggest that the plasma of cancer bearing patients contains protein factors which inhibit the lymphocytes' participation in cell-mediated phenomena. Our previous studies have shown that the concentration of transcortin, the cortisol binding protein of human plasma, appears to be inversely related to the numbers of circulating lymphocytes in chronic lymphocytic leukemia. Furthermore, this protein has been shown by our studies to exist within the cytoplasm of the human lymphocyte. Since the molecule appears to be synthesized exclusively by the liver, the hypothesis presented suggesting the mobilization of this molecule from the plasma compartment to the cytoplasmic compartment of the lymphocyte receives substantial support. The function of transcortin within the cytoplasm of the lymphocyte is postulated to be one of the suppression of lymphocyte protein synthesis. Inasmuch as one study by Matsumoto suggests that transcortin is markedly elevated in plasma of patients bearing a wide assortment of cancers, the possibility that it is this molecule which suppresses the

* Supported by grant ET-47 from the American Cancer Society.
ability of the "T" lymphocyte to function in the recognition and destruction of a spontaneously developed neoplastic cell, appears probable.

The preliminary study reported herein presents evidence that cancer bearing patients exhibit in their plasmas higher levels of transcortin than do comparable normal adults. Intracellular transcortin of the lymphocyte is markedly elevated in the lymphocyte of cancer bearing patient. Evidence is presented of the ability of cancer sera to limit protein and DNA synthesis of a human lymphocyte cell line (RPMI-1788). These results support the hypothesis that transcortin levels have a direct bearing on the cell-mediated functions of the immune system and that such functions in the cancer bearing patient are enhanced as a consequence of increased concentrations of transcortin.

Materials and Methods

Purification of plasma transcortin was by the method of Muldoon and Westphal. Preparation of a transcortin specific antibody has been previously described. Iodination of transcortin specific antibody was conducted by the method described by Hunter. The determination of cortisol binding capacity of human plasmas was conducted by the Sephadex filtration assay of DeMoor. Protein was quantitated by the method of Lowry et al.

Quantitation of Plasma Transcortin by Radioimmunoassay (RIA) Procedures

Twenty μl of 40 to 280 μg of transcortin were incubated with 0.1 ml of I125 labelled transcortin specific antibody for 24 hours at 4°C. At the end of the incubation period, the tubes were centrifuged at 20,000 g for 10 minutes at 4°C, the supernate was discarded and four saline washes were conducted. The last saline wash contained no appreciable radioactivity. Parallel incubations of 20 μl of human serum from 46 normal males, 51 normal females, 34 cancer-bearing males and 41 cancer-bearing females were similarly conducted. Absolute controls consisting of I125 labelled transcortin specific antibody incubated with equivalent volumes of rabbit serum were conducted. I125 activities were obtained with the aid of a gamma counter. Sera were obtained from patients having clinically diagnosed malignancies as shown in table I.

Identification and quantitation of lymphocyte intracellular transcortin were accomplished by applying varying dilutions of a fluoresceinated transcortin specific antibody to peripheral smears of whole blood. The RPMI-1788 human lymphocytes were cultured in Eagle's spinner-modified essential medium, supplemented with 10 percent heat-inactivated fetal calf serum, 2 mM glutamine, 1 percent non-essential amino acids, 100 μg per ml gentamicin and 0.25 μg per ml Fungizone. Cells were maintained as semi-suspension cultures in one liter capacity T-flasks at 37°C in 5 percent CO2-atmosphere, at an initial cell concentration of 2.5 × 10⁶ per ml and were subcultured at 2 to 3 day intervals. Cell cultures were routinely tested and found to be free from contamination with adventitious agents. Determination of the effect of cancer serum on the synthesis of DNA and protein by the Moore cell line of human lymphocytes was conducted thus: 1.0 ml of minimal essential medium containing 5 × 10⁶ lymphocytes was incubated at 37°C with 0.1 ml of either normal or cancer serum for periods of 2 to 24 hours. At the end of the incubation periods, three microcuries of H³-leucine or H³-thymidine were individually added and the cultures incubated for an additional
TABLE I

<table>
<thead>
<tr>
<th>Squamous Cell Line</th>
<th>Adenocarcinoma</th>
<th>Lymphosarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Endometrium</td>
<td>Medulloblastoma</td>
</tr>
<tr>
<td>Gingiva</td>
<td>Ovary</td>
<td>Synovial sarcoma</td>
</tr>
<tr>
<td>Tongue</td>
<td>Fallopian tube</td>
<td>Acute</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Prostate</td>
<td>lymphoblastic</td>
</tr>
<tr>
<td>Cervix</td>
<td>Appendix</td>
<td>leukemia</td>
</tr>
<tr>
<td>Vulva</td>
<td>Cecum</td>
<td>Acute</td>
</tr>
<tr>
<td>Colon</td>
<td>myelogenous</td>
<td>leukemia</td>
</tr>
<tr>
<td>Rectum</td>
<td>kidney</td>
<td>Chronic</td>
</tr>
<tr>
<td>(hypernephroma)</td>
<td>(lymphatic)</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>(follicular)</td>
<td>leukemia</td>
</tr>
</tbody>
</table>

two hours. At the end of the incubation period, the cultures were centrifuged at 2,000 g for 10 minutes and the supernatants discarded. The cells were resuspended in 0.5 ml of saline after which 10 ml of ice cold 10 percent trichloracetic acid were added. The precipitates formed were collected via millipore filtration procedures. The H\(^3\) activities were obtained with the aid of a scintillation counter\(^\dagger\) (efficiency for tritium of 42 percent), respectively. All incubations were conducted in triplicate.

**Results**

Disc acrylamide electrophoresis of our preparation of pure transcortin indicates the presence of a single electrophoretically homogeneous band (figure 1). The characterization of our transcortin has been presented elsewhere,\(^\dagger\) by all criteria employed, our preparation appears pure. If transcortin is left in the refrigerator at concentrations sufficiently greater than those which exist in plasma (over 3 mg per 100 ml), urea-reversible polymerization results as shown in figure 1 gels labelled “a” and “b.” Double diffusion Ouchterlony procedures employing our transcortin specific antibody against pure transcortin, normal and cancer sera yields two precipitin lines which form continuous lines of identity (figure 2). These two precipitin lines have in previous studies\(^\dagger\) been shown to reflect the formation of a polymer evident in the gels of figure 1. The outer precipitin ring represents the reaction of monomeric transcortin with the antibody.

The application of transcortin specific fluorescein-labelled antibody to smears of peripheral lymphocytes results in fluorescence primarily localized within the cytoplasm of these cells (figure 3). Fluorescence is not due to membrane-bound species of transcortin since parallel incubations.

\(^\dagger\) Picker Ansitron II.
bations of cells with the antibody blocks the fluorescence. The serial dilution of the fluorescein-labelled antibody has been employed as a means of quantitating the amount of transcortin-like protein within the cytoplasm of peripheral lymphocytes of the normal and cancer-bearing patient. Results of such studies suggest that since dilutions of the antibody of 1/320 or greater fail to yield any detectable fluorescence within normal lymphocytes, whereas dilutions as high as 1/640 yield positive fluorescence within the cytoplasm of lymphocytes of cancer patients, the amount of intracellular transcortin must be greater in the lymphocytes of cancer-bearing patients. The lowest dilution whereby 50 percent of the lymphocytes exhibit fluorescence is

![Figure 2](image)

**Figure 2.** Central well contains antitranscortin. Well 1 contains transcortin. Wells 2 and 4 contain normal serum. Wells 3 and 5 contain cancer sera.

![Figure 3](image)

**Figure 3.** Presence of fluorescence, primarily in cytoplasm, is shown.

![Figure 4](image)

**Figure 4.** Incubations contained varying concentrations of purified transcortin in constant volume of 125I labelled antibody. Incubation was at 4°C for 24 hours.

<p>| TABLE II |
| Quantitation of Human Plasma Transcortin by Radioimmunoassay Procedures |
| Average mg of Transcortin per 100 ml |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Plasma</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Male</td>
<td>46</td>
<td>4.9</td>
</tr>
<tr>
<td>Normal Female</td>
<td>51</td>
<td>21.8</td>
</tr>
<tr>
<td>Cancer Bearing Male</td>
<td>34</td>
<td>33.1</td>
</tr>
<tr>
<td>Cancer Bearing Female</td>
<td>41</td>
<td>33.4</td>
</tr>
</tbody>
</table>

Aliquots of 20 μL of serum were incubated at 5°C with 0.1 ml of 125I transcortin antibody for 24 hours. Note: All females assayed for transcortin levels were not pregnant.
POSTULATED ROLE OF TRANSCORTIN

TABLE III
THE EFFECT OF NORMAL AND CANCER SERA ON THE INCORPORATION OF \(^{3}H\)-THYMIDINE AND \(^{3}H\)-LEUCINE BY A HUMAN LYMPHOCYTIC CELL LINE

<table>
<thead>
<tr>
<th>Sera</th>
<th>Average CPM* of (^{3}H)-Thymidine ± S.D.</th>
<th>Percent Difference</th>
<th>No. Average CPM* of (^{3}H)-Leucine ± S.D.</th>
<th>Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum</td>
<td>48,221 ± 1818</td>
<td>-</td>
<td>7,997 ± 396</td>
<td>-</td>
</tr>
<tr>
<td>Cancer serum</td>
<td>32,496 ± 806</td>
<td>-32% (p&lt; 0.001)</td>
<td>6,404 ± 344</td>
<td>-20% (p&lt; 0.001)</td>
</tr>
</tbody>
</table>

*Count per million

1/320 in a normal lymphocyte and 1/640 in a cancer lymphocyte.

The quantitation of absolute levels of plasma transcortin via the procedure of radioimmune assay (figure 4) indicates that normal females exhibit greater amounts of plasma transcortin than do men (table II). In contrast to the respective levels of plasma transcortin in males and females, plasma transcortin levels of all cancer-bearing patients so far studied are much greater than the corresponding normal male or female.

Attempts have been made in our laboratory to determine whether or not the sera of cancer-bearing patients have any effect on the synthesis of DNA or protein by the lymphocyte cell line (RPMI-1788). The results presented in table III indicate that sera obtained from cancer-bearing patients appreciably inhibit the synthesis of DNA and protein in these cells.

Discussion

The results suggest that the lymphocytes and sera of cancer-bearing patients exhibit greater levels of transcortin than do those of corresponding normal humans. These sera are also capable of limiting the synthesis of DNA and protein by cells of a cell line known to retain many of the characteristics of the original lymphocytes from which it had been derived. The relationship of higher levels of plasma transcortin to those high levels found in the cytoplasm of the lymphocyte of cancer-bearing patients suggests that the latter levels are the result of greater numbers of transcortin molecules mobilized from the plasma to the cytoplasmic compartment of the lymphocyte. This possibility receives cogent support in that: the liver is the exclusive site of transcortin biosynthesis and cells of the lymphocyte cell line (RPMI-1788) that are grown in the absence of human serum do not contain transcortin. However, if incubated for a period of 24 or more hours with human serum, molecules of transcortin may be then localized within the cell.

The biological effect of the penetration of transcortin, most likely still containing tightly bound cortisol, has been postulated by us to result in the slowing down of lymphocyte protein synthesis. This hypothesis is further supported by our observations of the inhibitory effect that cancer sera exhibiting high levels of transcortin has on DNA and protein synthesis. If in vivo increases of transcortin levels result in the temporary or lasting inhibition of protein synthesis of lymphocytes of the cancer-bearing patient, then the possibility exists that such increased levels might be the manner whereby immunosuppression in turn permits the successful development and maintenance of a neoplastic malignancy.

Acknowledgments

Thanks are extended to Dr. Kobkul Lin, Dr. R. Khaund, and Mr. B. Denson for technical assistance; to Mrs. H. Compo for the preparation
of the manuscript and to Mr. A. Brown for the preparation of the figures.

References