ABSTRACT

Sera of normal individuals or patients with lung cancer were assayed for Forssman-like antibody by a quantitative and specific method using ethylenediaminetetraacetate-containing buffer to inactivate complement in the test serum. It was shown that although Forssman-like antibody levels were distributed widely, (a) the levels of young (20 to 45 years of age) normal subjects of Blood Groups O and B were lower than those of Blood Groups A and AB, (b) the levels of old (60 to 80 years of age) normal subjects were lower than those of young normal subjects of Blood Groups O and B, and (c) the levels of old lung cancer patients were lower when compared to age-matched normal individuals of their blood group.

INTRODUCTION

Several reports have described the presence of blood group antigens in tumor tissue. Levine et al. (9, 11) in 1952 were the first to demonstrate the presence of P antigen in gastric tumor tissue of a patient with the rare genotype pp. Subsequently, Hakkenin (5) reported the presence of an A-like blood group antigen on gastric cancer cells of patients with Blood Groups O and B. More recently, Springer et al. (13) found T-antigen, which they assumed to be the precursor of the MN system, on tissue of a patient with the rare genotype pp. Subsequently, Levine (10) therefore speculated that if F.Ag is present on tumor tissue of Forssman-negative individuals (sfs), Forssmann antibody will be found in the serum and will be absorbed on the cancer tissue as antigen-antibody complexes or removed from the circulation as antigen-antibody complexes by phagocytes.

In this paper, we have compared levels of F.Ab in sera of normal persons of different ages and blood groups to the levels in sera of age-matched, blood group-matched cancer patients. It will be shown that F.Ab titers are lower in the sera of patients with lung cancer than in age-matched controls.

MATERIALS AND METHODS

Buffers

GGVB++ and GVB−− were prepared as described previously (8). EDTA-GVB was prepared by diluting 0.2 M EDTA solution (7) with GVB−−.

Sera

Normal human sera were obtained from healthy normal laboratory staff and from donors of the New York Blood Center. The age group of these donors was between 20 and 45 years. Sera from adults 60 to 80 years of age were obtained through the courtesy of the "Immunology of the Aging Study Group" (Cornell University and Sloan-Kettering Institute for Cancer Research). Sera from patients with operable lung cancer were obtained from the thoracic service of Memorial Hospital, New York, N. Y. The patients had not been treated previously. Blood samples were drawn just prior to surgery.

Titration of F.Ab

The titration of F.Ab in sera was carried out using 2 methods. In the first method, heat-inactivated serum (56°; 30 min) was used, and in the second method, EDTA was used to inactivate complement in the test sera.

Method Using Heat-Inactivated Serum. This procedure is based upon the method used by Davidsohn (3). To tubes containing 0.5 ml of heat-inactivated test serum serially diluted in GGVB++ starting from a dilution of 1:20, 0.5 ml of diluted guinea pig complement (20 CH50 units/ml) and 0.5 ml of sheep erythrocytes (1.0 × 10⁸/ml) in the same buffer were added. After the further addition of GGVB++ to bring the total volume to 2.5 ml, the tubes were incubated at 37° for 60 min followed by centrifugation at 2000 rpm for 10 min. Supernatants were read for release of oxyhemoglobin at 412 nm in a spectrophotometer. Two controls, one consisting of sheep erythrocytes and guinea pig complement and the other consisting of sheep RBC and buffer alone, were treated similarly.

Method Using EDTA. The test serum was diluted serially from 1:50 to 1:3200 in EDTA-GVB. To the tubes containing these serum dilutions, 0.5 ml of sheep RBC (1.0 × 10⁹/ml) in the same buffer was added, and the tubes were incubated at 37° for 15 min. Following centrifugation at 2000 rpm for 10 min, the cells were carefully washed once with GVB−− and suspended in 2.0 ml of GGVB++ starting from a dilution of 1:20, 0.5 ml of diluted guinea pig complement (20 CH₅₀ units/ml) and 0.5 ml of sheep erythrocytes (1.0 × 10⁸/ml) in the same buffer were added. The mixtures were incubated at 37° for 60 min with occasional shaking and then were centrifuged at 2000 rpm for 10 min. The degree of hemolysis in the supernatant was determined.

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Determination of Circulating Immune Complexes and Total Hemolytic Complement

The Raji cell radioimmunoassay was carried out for the titration of circulating immune complexes in sera according to the method of Theofilopoulos et al. (15). The levels of immune complexes in sera were expressed as μg aggregated human γ-globulin/ml (12). Total hemolytic complement (TCH50) in sera was titrated as described previously and expressed as CH50 units/ml (12).

RESULTS

Effect of Heat Inactivation on Normal Serum. Varying dilutions of guinea pig complement (0.5 to 0.8 CH50 units/ml) in 0.5 ml of GGVB** were added to the tubes containing 0.5 ml of heat-inactivated normal human serum diluted 1:50 with the same buffer. After incubation at 37° for 5 min., sheep erythrocytes coated with rabbit antibody against sheep erythrocytes (1.0 x 10⁹/ml) in GGVB** and 1.0 ml of GGVB** were added. The mixtures were incubated at 37° for 60 min followed by centrifugation, and the degree of hemolysis in the supernatants was evaluated by spectrophotometric analysis. As shown in Chart 1, the degree of hemolysis with guinea pig complement in the presence of heated human serum was much less than in the absence of heated serum. This experiment was reproducible using 4 different normal human sera. These observations suggest that heat-inactivated sera can activate the exogenous guinea pig complement used in this experiment.

In another experiment, the effect of heat on F.Ab in normal human serum was determined as follows. F.Ab was titrated using the EDTA method in sera with and without heat inactivation. A reduction of F.Ab activity by 20 or 30% was observed with 4 different heated normal human sera when compared with unheated corresponding sera, suggesting that by heat inactivation of serum activation of complement may occur.

Titration of F.Ab in Normal and Patient Sera Using 2 Methods. Fifteen sera from healthy donors and 33 sera from patients with lung cancer were assayed for F.Ab by the 2 methods described above. Two titers from each sample were plotted, and these findings are given in Chart 2. Statistical analysis revealed that the 2 titers correlated well when compared with each other using normal (r = 0.83) and lung cancer sera (r = 0.86). However, with heat-inactivated serum, the titer was always significantly lower than when the EDTA method was used, and in some instances, the titers were reduced to zero.

F.Ab Levels in Sera of Healthy Adults of Different Ages and Various Blood Groups. Sera of 116 normal subjects were assayed for F.Ab using the EDTA method. The tested sera were divided into 4 groups, i.e., into Blood Groups A and AB, Blood Groups O and B, (young normal, 20 to 45 years of age) age group, and old (old normals, 60 to 80 years of age) age group. One of the most striking features was that the titers were distributed widely in all groups. For example, in the young A and B groups, the lowest titer was 26 while the highest was 2200 AB.Hse units/ml. The distribution of these groups is shown in Chart 3. Statistical analysis revealed that the levels of F.Ab in sera of young individuals with Blood Groups O and B were significantly higher than those in sera of young individuals with Blood Groups A and AB (p < 0.03). However, in comparing levels of F.Ab in older individuals with any of the blood groups the difference was not seen.

When levels of F.Ab were compared between the 2 groups of individuals, i.e., young and old, the median of the distribution was lower in the old group than in the young group, and no significant difference was observed between the 2 groups when the blood groups were A and AB. However, there was a highly significant difference in F.Ab levels between young and old (p < 0.0001) when the blood groups were O and B.

F.Ab Levels in Sera of Lung Cancer Patients. Forty-one lung cancer sera were tested. These patients were also divided into 4 groups according to blood group and age of the patient (Chart 3). The distribution of each group was compared with the corresponding normal group. When comparison of F.Ab levels was made between young normal and age-matched lung
cancer patients of O and B or A and AB blood groups, no
difference was seen. This finding may be due to the small
patient population tested because lung cancer usually occurs
in older persons. However, differences in F.Ab levels between
old normal persons and age-matched lung cancer patients
were significant in cases of Blood Groups O and B (p < 0.001).
Table 1 shows the ratio of low F.Ab levels with the number of
tested sera, and again a significant difference was seen be-
tween the old-normal individuals and age-matched lung cancer
patients.

Correlation of F.Ab Levels with Levels of Circulating Im-
mune Complexes and Total Hemolytic Complement. Sera
from 32 lung cancer patients, 19 colon cancer patients, and 8
neuroblastoma patients were assayed for F.Ab by the EDTA
method and for circulating immune complexes by the Raji cell
radioimmunoassay. Sera from 52 lung cancer patients were
assayed for F.Ab by the EDTA method and for hemolytic activity
of total complement. Charts 4 and 5 show that no correlation
was found between F.Ab levels and immune complexes or
between F.Ab levels and total hemolytic complement.

DISCUSSION

In 1935, Buchbinder (2) reviewed studies of lytic activity by
human serum and indicated that not all human serums contain
lysins for sheep cells (13, 14). In Blood Group A the lowest
incidence of sheep lysins occurred, in Group B a high incidence
occurred, and in Group O an intermediate value incidence
occurred. Recently, Levine (10), using a standard method,
showed that the incidence of F.Ab is lower in cancer patients
than in normal individuals. By this microtube method, dilutions
of heat-inactivated serum followed by the addition of guinea
pig complement and sheep RBC were used. Because some
heated sera have anticomplementary activity or activate exog-
enuous complement and since the sera of patients tested by
Levine also included patients under treatment, we studied a
group of patients with lung cancer who had not been previously
treated and used a macrotube method with EDTA to inactivate
the complement in the test serum. In addition, we used a 2-
step incubation method which allowed any extraneous material
other than F.Ab to be removed by washing. Here we show
clearly that heating sera not only activates the exogenous
complement but also decreases F.Ab activity. Comparative
studies between the heat inactivation and EDTA methods
showed that the titers by the 2 methods correlated well. How-
ever, a significant drop of lytic activity occurred by the heat-
inactivation method.

Using the EDTA method, we studied F.Ab levels in sera from
116 normal subjects divided into 4 groups, i.e., into Blood
Groups A and AB, Blood Groups O and B, young group (20 to
45 years), and old group (60 to 80 years). It was revealed that
although the titers were widely distributed in each group, those
of the young group were significantly lower in Blood Groups A
and AB than in Blood Groups O and B (p < 0.03). This may be
due to the cross-reactivity between F.Ag and Blood Group A
substance. However, the difference was not evident in the F.Ab
titers of the older persons. When levels of F.Ab were compared
between the 2 groups, i.e., young and old, the median of
distribution was lower in the old group than in the young group

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Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Total no. of sera tested</th>
<th>No. of sera with anti-Fs levels 100 Ab.Hso</th>
<th>No. of sera with anti-Fs levels 200 Ab.Hso</th>
</tr>
</thead>
<tbody>
<tr>
<td>A or AB</td>
<td>Young normal</td>
<td>25</td>
<td>2 (8.0)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td></td>
<td>Old normal</td>
<td>29</td>
<td>5 (17.2)</td>
<td>12 (41.4)</td>
</tr>
<tr>
<td></td>
<td>Young lung cancer</td>
<td>5</td>
<td>1 (20.0)</td>
<td>3 (60.0)</td>
</tr>
<tr>
<td></td>
<td>Old lung cancer</td>
<td>17</td>
<td>9 (52.9)</td>
<td>10 (56.8)</td>
</tr>
<tr>
<td>O or B</td>
<td>Young normal</td>
<td>22</td>
<td>0 (00.0)</td>
<td>0 (00.0)</td>
</tr>
<tr>
<td></td>
<td>Old normal</td>
<td>40</td>
<td>6 (15.0)</td>
<td>18 (45.0)</td>
</tr>
<tr>
<td></td>
<td>Young lung cancer</td>
<td>6</td>
<td>1 (16.7)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td></td>
<td>Old lung cancer</td>
<td>13</td>
<td>8 (61.5)</td>
<td>12 (92.3)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of total number of sera tested.
as was also observed in the study of Levine, et al. (11), and although no significant difference was observed between those with Blood Groups A and AB, a highly significant difference between young and old of Blood Groups O and B (p < 0.0001) was evident.

Lung cancer patients who were untreated prior to blood sampling for this study were also divided into 4 groups according to blood group and age of patient. F.Ab levels of old patients were significantly lower than age-matched normal individuals regardless of whether their blood group was A and AB (p < 0.05) or O and B (p < 0.001).

This suggests that in older lung cancer patients, irrespective of blood group, F.Ab levels drop even more than when cancer is not present. Such a conclusion could not be reached in the young group, probably because the sample size was so small.

Extensive studies in our laboratories in the past six years have shown that over 50% of cancer sera have circulating immune complexes (1, 4, 14). The observations by Hakimori et al. (6) that F.Ag was present in human gastric and colonic tissue prompted us to speculate that if F.Ag was present on lung tissue of F.Ab-negative individuals, then F.Ab will be either released in the circulation or be absorbed to the cancerous tissue. Circulating immune complexes were detectable in many of the sera of our patients, but we could find no correlation between levels of F.Ab and circulating immune complexes with our method.

The finding that F.Ab levels are clearly lower in sera of untreated lung cancer patients is provocative. Our studies suggest that the lower levels are not caused by a nonspecific decrease of antibody-producing activity because although

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REFERENCES


Forssman-like Antibody Levels in Sera of Patients with Lung Cancer

Hajime Kitamura, Philip Levine, Po-Jun Cheng, et al.


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