IMMUNE RESPONSES IN EC PATIENTS

S. Hashemi *
B. Parhami *
M. S. Enayat
K. Dowlatshahi **
N.E. Day *** and
N. Mohagheghpour *

ABSTRACT

General immune responses were studied in patients with esophageal carcinoma (EC) and were compared to those in normal individuals of similar age, sex and ethnic origin. There was a significant increase in the titer of “background” antibodies in the sera of cancer patients; however, this elevation was not associated with an increase in the levels of serum immunoglobulins. EC patients had a diminished number of E rosetting lymphocytes but normal proportion of EAC rosetting cells. Lymphocytes from a small but significant number of patients showed decreased responses to PHA when cultured in medium containing fetal calf serum. Plasma samples from these patients were capable of inhibiting the in vitro proliferative responses of lymphocytes to PPD.

* Department of Pathobiology, School of Public Health, and
** Khomeini Hospital, University of Teheran, Iran
*** Epidemiology and Biostatistics Unit, International Agency for Research on Cancer, Lyon, France.
INTRODUCTION

A number of studies have revealed varying levels of immunoincompetence in patients with malignant diseases of nonlymphoid tissue (Garrioch et al., 1970; Ducos et al., 1970; Whittaker et al., 1971; Catalana et al., 1973; Knight and Davidson, 1975; Chan et al., 1976; Cochran et al., 1976). In this paper, we present information on the immunological state of patients with esophageal carcinoma (EC). The immunological competence of the patients was assessed prior to any therapy by determination of serum immunoglobulin levels, titers of "background" antibodies to ubiquitous antigens, levels of E and EAC rosetting lymphocytes in the peripheral blood and by the in vitro lymphocytes responsiveness to phytohemagglutinin (PHA). In addition, the effect of plasma from these patients on the in vitro reactivity of normal lymphocytes to PPD was determined.

MATERIALS AND METHODS

Patients

The present study is one of a series characterizing the esophageal cancer patients and the population at high risk. One hundred and ten esophageal cancer patients (76 male and 34 females) seen at Pahlavi Hospital in Gorgan as well as those who came to Teheran for treatment were studied preoperatively. They ranged in age from 29 to 76 years, and all had a positive diagnosis of cancer based on clinical criteria as well as radiology, endoscopy and biopsy.

Control data were obtained by examining 137 healthy individuals (94 males, 43 females) from Gorgan or the surrounding villages. The age distribution of the controls ranged from 20 to 77 years. Based on the availability of samples, some or all of the tests were performed on each individual.

Assessment of general immunological competence

The level of immunoglobulins (IgM, IgG, and IgA) was estimated using single radial immunodiffusion (Mancini et al., 1965) on commercially prepared plates (Hoechst Pharmaceuticals). Titer of "background" antibodies to bovine serum albumin (BSA), egg albumin (EA), keyhole limpet hemocyanin (KLH), Entameba histolytica antigen (EH) and sheep red blood cells (SRBC) was measured by
passive hemagglutination using chromic chloride-treated erythrocytes (Faulk and Houba, 1973) and by hemagglutination. Titers are expressed as the log₂ of the highest dilution showing agglutination.

The percentage of E and EAC rosetting lymphocytes in peripheral blood was estimated employing the method of Jondal et al. (1972). In each test 200 lymphocytes were scored and those cells binding three or more sheep red blood cells were considered rosettes.

T-cell function was studied by assessing the lymphocyte response to phytohemagglutinin-P (PHA) (Difco Laboratories, Detroit, MI). Peripheral blood leukocytes were separated by Ficoll-Hypaque centrifugation as described by Boyum (1968), and cells from the interface layer were washed three times in TC 199 medium. Cells were then suspended in TC 199 supplemented with 10% fetal calf serum, 1% TC glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 100 μg/ml kanamycin. The pH of this medium was adjusted to 7.3 ± 0.2 using 7.5% sodium bicarbonate. Assessement of cell viability was performed by the trypan blue exclusion technique. By this criterion, 95-99% cells were viable. Cell concentration was then adjusted to 3 x 10⁵ per ml medium. For stimulation, 0.1 ml of a 1:64 or 1:128 dilution of PHA was added to each of the four culture tubes containing 0.9 ml cell suspension. For control purposes 0.1 ml of medium was added to another four tubes. The cells (1 ml/tube) were then cultured at 37°C for 72 hours in a humid atmosphere containing 5% CO₂. Sixteen hours before termination of the culture, 1 μCi-tritiated thymidine (specific activity 21.5 Ci/mM) (Radio Chemical Center, Amersham, England) was added to each culture tube. The cell pellets were collected by centrifugation at 700 xg for 10 min. Incorporation of ³H-thymidine was stopped by addition of 5 ml 0.14 M ice-cold PBS (pH = 7.2). The cells were then washed three times with phosphate buffered saline (PBS) and the acid-insoluble fraction was precipitated with 5 ml of ice-cold trichloroacetic acid at 4°C. After allowing 30 minutes for precipitation to take place, tubes were centrifuged at 700 xg. The precipitates were washed in 5 ml absolute methanol, then left to dry in inverted position at 37°C. When dried, precipitates were dissolved in 0.4 ml soluene-100 (Packard Instruments, Inc., Downers Grove, IL). Solubilization was achieved by incubating the tubes for 2 hours at 56°C. Each preparation was then transferred to scintillation vial containing 3 ml scintillation fluid (Packard Instruments). Radioactivity in each sample was counted in a Tri-Carb liquid scintillation spectrometer. The results are expressed by stimulation indices (SI), computed as:
SI = \frac{CPM \text{ of lymphocytes stimulated by PHA}}{CPM \text{ of unstimulated lymphocytes}}

CPM corresponds to the mean count per minute of each quadruplicate culture after correction for the CPM of medium control.

Effect of plasma for EC patients on the lymphoproliferative response was determined by comparing the response of lymphocytes to PPD in cultures containing 15% patient plasma with parallel cultures containing pooled normal AB plasma, kindly provided by the Iranian Blood Transfusion Service. For stimulation 4 x 10^5 cells from a single normal donor were cultured with 10 and 100 ug PPD (Statens Seruminstitut, Copenhagen, Denmark) for 72 hours. Control cultures were incubated in the absence of PPD. Cultures were then pulsed with 1 uCi of 3H-thymidine, incubated for an additional 18 hours, then harvested according to the procedure detailed above. The stimulation indices were calculated as follows:

\[ SI = \frac{CPM/\text{of cells cultured with PPD in the presence of patients or AB plasma}}{CPM/\text{of cells cultured in the absence of PPD}} \]

RESULTS

Serum levels of IgM, IgG, and IgA were similar in the esophageal cancer patients and the controls (Table I). Cancer patients, however, had significantly elevated levels of "background" antibodies with the exception of antibody to SRBC which was comparable in both groups.

The percentage of EAC rosetting lymphocytes was comparable in EC patients and normal individuals of similar age (33 ± 9%, vs 31 ± 8%) (Table III). Lymphocytes forming E rosettes, however, were marginally less frequent in peripheral blood of the cancer patients than in the controls (P<0.05 by "one-tailed" Student t-test).

The response of lymphocytes from 38 EC patients and 25 normal individuals of the same age range are compared in Table IV and graphically presented in Figure 1. Data presented here were obtained using a 1:128 dilution of PHA at which maximum stimulation occurred. Considerable variation in the range of SI was observed in both groups. However, a small but significant percentage (32%) of EC patients responded poorly to mitogen, producing low stimulation indices (values between 1.1-20). Such low values, however, were not encountered among the normal individuals, and this difference is significant at P = 0.0037 by Fishers exact test.

Table IV shows the effect of plasma from 17 EC patients on
the ability of lymphocytes from a single donor to produce a pro-
liferative response to PPD. Without exception, addition of patient
plasma to lymphocyte cultures resulted in a significant suppression
of PPD response as compared to cultures containing pooled normal AB
plasma.

DISCUSSION

The present study compared the general immune status of eso-
ophageal cancer patients with that of normal individuals having similar
age, sex and ethnic composition. In general, there was a significant
increase in the titer of "background" antibodies in the sera of EC
patients as compared to the normal controls. Included in these
studies of antibody were anti-BSA, anti-EA, anti-KLH, anti-EH and
anti SRBC. However, elevation in the titer of antibodies was not
associated with a significant increase in the levels of serum immu-
agoglobulins, or with a deviation from normal in the percentage
of EAC rosetting lymphocytes, comprising the B cell population.
EC patients, however, had a diminished number of T cells (E rosetting
lymphocytes), and lymphocytes from a significant proportion of the
patients (32%) showed decreased responses to PHA (a T cell mitogen)
when cultured in medium containing fetal calf serum. In addition,
plasma samples from EC patients were capable of inhibiting the
in vitro proliferative response of lymphocytes to PPD.

Our findings regarding low lymphocyte responsiveness to mitogen,
as well as reduction in the relative proportion of lymphocyte sub-
populations in patients with esophageal carcinoma, confirm a number
of prior observations (Ducos et al., 1970; Whittaker et al., 1971;
Knight and Davidson, 1975; Chan et al., 1976). Presence of in-
hibitor in the plasma of EC patients was not surprising, since sup-
pression of lymphocyte functions in vitro by humoral factors present
in the plasma of cancer patients has been repeatedly well-documented
(e.g., Sample et al., 1971; Succui Foca et al., 1973; Vankey et al.,
1971 and 1975). The diminution in lymphocyte responsiveness to
PHA noted in some EC patients could be the reflection of the
tumor burden or, alternatively, could be the consequence of poor
health and nutritional status of the patients.

Our findings concerning increased levels of "background" anti-
body in the EC patients are inconsistent with those of Parfentjiev
et al. (1951) who observed a decline in the incidence of natural
agglutinin for proteus in patients with untreated malignancies. A
possible link between depression of T cell function and high "background" antibody levels in the sera of patients with esophageal carcinoma remains to be established. It is possible that the tumor somehow commits B cells to nonspecific antibody production. Alternatively, the tumor may bring about an alteration in the control of B cell activity.

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LEGEND

Figure 1. Comparison of lymphocyte response to PHA (1:128) in EC patients and normal controls. Each point represents one individual.
TABLE I

COMPARISON OF IMMUNOGLOBULIN LEVELS
IN EC PATIENTS AND NORMAL CONTROLS

<table>
<thead>
<tr>
<th>Age</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG</th>
<th>EC</th>
<th>Non EC</th>
<th>t</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>200±83</td>
<td>416±96</td>
<td>1914±430</td>
<td>170±86</td>
<td>265±100</td>
<td>1.08</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>416±96</td>
<td>265±100</td>
<td>2023±365</td>
<td>1.57</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>180±80</td>
<td>279±95</td>
<td>1731±738</td>
<td>164±77</td>
<td>293±97</td>
<td>-0.82</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>164±77</td>
<td>293±97</td>
<td>1901±331</td>
<td>-0.53</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>170±95</td>
<td>290±92</td>
<td>1895±471</td>
<td>197±90</td>
<td>294±89</td>
<td>-1.14</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>197±90</td>
<td>294±89</td>
<td>1777±39</td>
<td>-1.17</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>161±97</td>
<td>336±102</td>
<td>2081±400</td>
<td>165±68</td>
<td>301±104</td>
<td>-0.12</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>165±68</td>
<td>301±104</td>
<td>1718±586</td>
<td>-0.94</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70+</td>
<td>177±57</td>
<td>428±252</td>
<td>2086±699</td>
<td>178±91</td>
<td>270±80</td>
<td>1.84</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>178±91</td>
<td>270±80</td>
<td>1616±259</td>
<td>1.84</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 t value
2 degree of freedom
The table gives the value in the table corresponding to the F statistic on 1 and n degrees of freedom.

<table>
<thead>
<tr>
<th></th>
<th>100.0 &gt;</th>
<th>90.0 &gt;</th>
<th>80.0 &gt;</th>
<th>70.0 &gt;</th>
<th>60.0 &gt;</th>
<th>50.0 &gt;</th>
<th>40.0 &gt;</th>
<th>30.0 &gt;</th>
<th>20.0 &gt;</th>
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</thead>
<tbody>
<tr>
<td>Titer</td>
<td>9.79</td>
<td>2.10</td>
<td>1.18</td>
<td>5.52</td>
<td>5.74</td>
<td>0.75</td>
<td>1.27</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>Mean difference in titer</td>
<td>3.30 ± 0.36</td>
<td>3.53 ± 2.21</td>
<td>3.26 ± 0.90</td>
<td>3.95 ± 1.50</td>
<td>4.24 ± 0.79</td>
<td>4.56 ± 1.30</td>
<td>4.75 ± 1.10</td>
<td>4.64 ± 0.65</td>
<td>4.37 ± 1.08</td>
</tr>
</tbody>
</table>

Mean antibody titers (log2) ± S.E.

<table>
<thead>
<tr>
<th>Antibody Status</th>
<th>Anti-CH</th>
<th>Anti-ET</th>
<th>Anti-KLH</th>
<th>Anti-SEA</th>
<th>Anti-STRBC</th>
<th>Age</th>
</tr>
</thead>
</table>

Comparison of background antibody titers in EC patients and normal controls.

TABLE II
### TABLE III

**COMPARISON OF LYMPHOCYTE SUBPOPULATION IN EC PATIENTS AND NORMAL CONTROLS**

<table>
<thead>
<tr>
<th>Disease status</th>
<th>Number tested</th>
<th>% EAC rosetting lymphocytes</th>
<th>% E rosetting lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>28</td>
<td>33 ± 9</td>
<td>56 ± 11</td>
</tr>
<tr>
<td>Non EC</td>
<td>31</td>
<td>31 ± 8</td>
<td>60 ± 6</td>
</tr>
</tbody>
</table>

1. Results are expressed as means ± SD.

2. P < 0.05 by "one-tail" Student t test.

### TABLE IV

**COMPARISON OF LYMPHOCYTE RESPONSE TO PHA IN EC PATIENTS AND NORMAL CONTROLS**

<table>
<thead>
<tr>
<th>Disease Status</th>
<th>Stimulation Index</th>
<th>≥40</th>
<th>≤20 &lt; 40</th>
<th>&lt;20</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td></td>
<td>19</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Non EC</td>
<td></td>
<td>18</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

1. P = 0.0037 by Fishers exact test.
### TABLE V

**EFFECT OF PLASMA FROM EC PATIENTS ON THE IN VITRO PPD RESPONSE**

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Plasma Donor</th>
<th>Stimulation Index (\mu g) PPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>AB</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>P(_5)</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>P(_6)</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>P(_8)</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>P(_9)</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>P(_{10})</td>
<td>9.9</td>
</tr>
<tr>
<td>2</td>
<td>AB</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>P(_1)</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>P(_3)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>P(_{15})</td>
<td>1.7</td>
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<tr>
<td></td>
<td>P(_{16})</td>
<td>1.9</td>
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<td></td>
<td>P(_{17})</td>
<td>3.0</td>
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<tr>
<td></td>
<td>P(_{21})</td>
<td>3.0</td>
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<tr>
<td></td>
<td>P(_{22})</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>P(_{23})</td>
<td>2.6</td>
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<td>2.6</td>
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<tr>
<td></td>
<td>P(_{25})</td>
<td>1.6</td>
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<tr>
<td></td>
<td>P(_{26})</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>P(_{27})</td>
<td>2.7</td>
</tr>
</tbody>
</table>

1 15% plasma was used.

2 AB, normal pooled AB plasma; P, patient's plasma.

3 Cells from a single donor were used. SI values differ significantly from the control of each experiment at \(P < 0.01\).

4 \(P < 0.05\).
REFERENCES


Mancini, G., Carbonara, A.B. and Heremans, J.F. (1965) Immunoochemical quantitation of antigens by single radial immuno-


