A Cancer-associated, Fast, Homoarginine-sensitive Electrophoretic Form of Serum Alkaline Phosphatase

Sharon L. Ehrmeyer,1 Brian L. Joiner, Lawrence Kahan,2 Frank C. Larson, and Robert L. Metzenberg

INTRODUCTION

The presence of neoplastic tissue occasionally results in the release of tumor-associated proteins into the blood, e.g., carcinoembryonic antigen, α-fetoprotein, placental lactogen, chorionic gonadotropin, and placental alkaline phosphatase isoenzymes. We have identified an electrophoretic form of alkaline phosphatase (FHAP) which is present in the blood of a high percentage of cancer patients and lower percentages of other hospital patients and healthy individuals (9). In contrast to other cancer-related proteins, FHAP is associated with neoplasms of a variety of organ sites and cell types.

Preliminary experiments and theoretical considerations suggested the following set of hypotheses: (a) FHAP is more common in cancer, diabetes, hepatitis, and renal failure patients than in all other patients; (b) among cancer patients, FHAP is more frequently present in those with tumor present than in those who have been successfully treated (free of neoplastic tissue for more than 5 years); (c) the amount of FHAP in the blood correlates positively with the probability of the presence of neoplastic tissue.

For determination of the prevalence of FHAP in blood of cancer patients and other hospital patients, the alkaline phosphatase electrophoretic patterns of serum samples from 533 consecutively admitted hospital patients were analyzed.

MATERIALS AND METHODS

Serum Samples. Blood was collected by venipuncture in red stopper (untreated) Vacutainer tubes. Blood was allowed to clot, and the serum was separated by centrifugation. Samples were analyzed the same day as collected.

Electrophoretic Separation. Separation was accomplished using cellulose polyan acetate strips (Gelman Sephr Phore III) equilibrated with 50 mm triethanolamine acetate buffer, pH 7.0, at 250 V (approximately 20 V/cm) for 1 hr in a Gelman deluxe electrophoresis chamber (7). The starting temperature of the buffer was 5 to 10°C and the run was conducted without refrigeration. Subsequent experiments have indicated that cooling during the run or electrophoresis at 200 V for 1.5 hr gives better separations and prevents loss of enzyme activity due to heat inactivation.

Detection of FHAP. Alkaline phosphatase was visualized by placing the strips in contact with 1.2% agar containing 0.75 μ 2-amino-2-ethyl-1,3-propanediol, pH 9.8 (when measured at 50 mm concentrations), and 0.85 mm naphthol AS-MX phosphate (1). After 0.5 hr at room temperature, the fluorescent product was observed under a short-wavelength UV lamp. (Serum samples did not contain interfering fluorescent substances as shown by the absence of fluorescent bands at the beginning of the incubation period.) All sera contained alkaline phosphatase that migrated with a mobility of 0.74 relative to albumin. FHAP migrated very slightly faster than albumin under these conditions (see Fig. 1). The FHAP band in each strip was scored on a scale of 0 (absent), 1 (questionable), 2 (definitely present but faint), or 3 (strong) by 3 independent observers. A sample was considered to contain FHAP if the average score was greater than 1.

Clinical Evaluation. The patients’ records were examined by a fourth person, and relevant clinical information was abstracted. The fourth person had no knowledge of the scores that had been assigned by the first 3 observers.

RESULTS

As shown in Table 1, the prevalence of FHAP was significantly greater (p < 0.005) (13) in cancer patients than in noncancer patients. In contrast, the prevalence of FHAP in patients who had had cancer but had been free of detectable neoplastic tissue for more than 5 years ("Presumed cured" in Table 1) did not differ from that in noncancer patients. The frequency of FHAP was elevated in cancers of a variety of organ sites, inter alia lung, colon, and breast, as well as in cancers of a variety of cell types, e.g., adenocarcinoma, squamous cell carcinoma, and leukemia. Patients with cancers of sites other than those listed above had a significantly increased prevalence of FHAP when taken as a group; however, the small number of patients with cancers of any single site and the relatively high prevalence of FHAP in noncancer hospitalized patients precluded further specific conclusions.
As shown in Chart 1, the prevalence of cancer in patients who had high levels of FHAP (p = 0.66% for average FHAP scores >2) was significantly higher (p < 0.025) than in patients having average FHAP scores between 1.3 and 2 (p = 0.48%), particularly when patients with diabetes, hepatitis, and chronic renal failure were excluded (p = 0.80% for average FHAP scores >2; p = 0.53% for average FHAP scores between 1.3 and 2.0; p < 0.005). This observation suggests that the amount of FHAP in the blood is a better indicator of the probability of the presence of malignant tissue than is the mere presence of FHAP. This result has been confirmed using a quantitative assay for FHAP.

We also determined the prevalence of FHAP in presumably healthy individuals. FHAP was present in the blood of 24 (6.7%) of 630 presumably healthy blood donors. Women that were 20 to 29 years old were found to have an anomalously high prevalence of FHAP (9.3%; n = 151) when compared with males that were 20 to 29 years old (2.7%; n = 111). FHAP is significantly less prevalent in healthy individuals than in any group of hospital patients examined in this study.

The above results have been confirmed in a blind analysis of 200 coded serum samples. The prevalence of FHAP in sera was 3% (n = 30) in normal controls, 35% (n = 40) in gastrointestinal benign disease, 78% (n = 40) in gastrointestinal cancer, 14% (n = 21) in gynecological benign disease, and 53% (n = 49) in gynecological cancer.

We have separated FHAP from other alkaline phosphatase isoenzymes by chromatography on DEAE-Sephadex A-50 in 50 mM triethanolamine buffer (pH 7.4) using a 0.15 M to 0.5 M sodium chloride gradient. FHAP differs from 3 previously reported cancer-associated alkaline phosphatases, the Regan (3), Nagao (10), and Regan-variant or Kasahara (15) isoenzymes and most closely resembles liver alkaline phosphatase, the "a," serum alkaline phosphatase observed in patients with biliary obstruction (8, 12), and the recently reported chorionic band A alkaline phosphatase in inhibitor sensitivity (20% inhibition by 10^{-2} M L-phenylalanine, 70% inhibition by 10^{-5} M L-homoarginine) and heat inactivation (85% inactivated by incubation for 5 min at 65°C) (2, 4). Like the "biliary" phosphatase, FHAP has α₁-globulin electrophoretic mobility at pH 8.6 in cellulose polyan acetate.

The presence of the biliary form in serum of cancer patients has been attributed to biliary obstruction due to hepatic metastases. However, Nordentoft-Jensen (11) observed an increase in serum α₁ alkaline phosphatase activity in 13 of 19 patients with pulmonary disease (including some lung cancer patients not having hepatic metastases). Recently, Hägerstrandet al. (5) confirmed the presence of the biliary isoenzyme in 22 of 34 cancer patients not having hepatic metastases.

Table 1

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>% prevalence</th>
<th>p a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>209</td>
<td>56</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Tumor present</td>
<td>148</td>
<td>64</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Breast</td>
<td>26</td>
<td>54</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Colon</td>
<td>17</td>
<td>82</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Leukemia b</td>
<td>14</td>
<td>64</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Lung</td>
<td>11</td>
<td>82</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>11</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Cervix</td>
<td>7</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>6</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>5</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Mouth</td>
<td>5</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>All others</td>
<td>48</td>
<td>65</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Possible cure c</td>
<td>36</td>
<td>53</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Presumed cure d</td>
<td>11</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>45</td>
<td>55</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Diabetes mellitus and cancer</td>
<td>14</td>
<td>93</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>8</td>
<td>75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Renal failure</td>
<td>10</td>
<td>70</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>All other patients</td>
<td>264</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

a p values (4) are given for groups that significantly differed from the "All other patients" group.

b Included 1 patient with a benign tumor (villous adenoma) and 1 patient who had had breast cancer but was presumed cured.

c Cancer patients who had completed a course of treatment which is frequently associated with total eradication of the disease.

d Cancer patients who had had commonly curable lesions completely removed by surgery, and were without recurrence for more than 5 years.


Cancer Research Vol. 38, No. 6, 1978
involvement. Like the chorionic band A phosphatase, FHAP is immunochemically distinguishable from both the mature placental (Regan) and the liver alkaline phosphatases (L. Kahan, F. Larson, and H. Sussman, unpublished observations). We have found that at pH 7.0 FHAP is identical in electrophoretic mobility with the fastest of the alkaline phosphatases extracted from approximately 10-week placenta; however, the identity of FHAP and chorionic band A has not yet been established. Fishman et al. (2) have reported that an alkaline phosphatase indistinguishable from the chorionic band A alkaline phosphatase may be isolated from some human neoplasms, testicular teratomas, and lung tumors. Thus, it seems possible that the FHAP and chorionic band A are identical.

**DISCUSSION**

The high frequency of occurrence of FHAP in a wide variety of cancers, including some (e.g., leukemia) for which no enzymatic or antigenic markers have been described, and the lower frequency of occurrence of FHAP in patients thought to be cured of cancer suggests that FHAP may prove to be useful in the estimation of the amount of tumor present during the course of therapy.

The sensitivity of FHAP as a diagnostic marker for cancer appears to be at least comparable to that of carcinoembryonic antigen, the placental alkaline phosphatase isoenzymes, and chorionic gonadotropin (6, 14). The low prevalence of cancer in the healthy population, coupled with the incomplete specificity of all of these assays, including the FHAP assay, unfortunately precludes their use as screening tests. However, FHAP, alone or in combination with other markers, may be useful in the detection of cancer in populations having a high prevalence of cancer, e.g., treated cancer patients.

**ACKNOWLEDGMENTS**

We thank Dr. W. H. Wolberg and Dr. R. O. Johnson for helpful discussions, Marilyn Stewart for technical assistance, Cathy Campbell for assistance with data analysis, and Dee Frana for her secretarial assistance.

**REFERENCES**

A Cancer-associated, Fast, Homoarginine-sensitive Electrophoretic Form of Serum Alkaline Phosphatase

Sharon L. Ehrmeyer, Brian L. Joiner, Lawrence Kahan, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/3/599

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.