Histaminase and Other Tumor Markers in Malignant Effusion Fluids

Chi-Wei Lin, Norma R. Inglis, Allyn H. Rule, R. Nuran Turksoy, Clifford M. Chapman, Sandra D. Kirley, and Leo L. Stolbach

Departments of Pathology [C-W. L., N. R. I., C. M. C., S. D. K.], Medicine [C-W. L., A. H. R., L. L. S.], and Obstetrics and Gynecology [R. N. T.], Tufts University School of Medicine, Boston, Massachusetts 02111, and Medical Service, Pondville Hospital, Massachusetts Department of Public Health, Walpole, Massachusetts 02081 [L. L. S., N. R. I.]

ABSTRACT

The association of histaminase with human cancer has been evaluated in this study by its presence at elevated levels in malignant effusion fluids. Over 400 fluid samples collected from 162 cancer patients of different primaries were analyzed in this study. Incidence of elevation of histaminase in the fluids were compared with that of three other tumor markers: carcinoembryonic antigens (CEA); β-subunit of human chorionic gonadotropin; and Reagan isoenzyme of alkaline phosphatase, all measured in the same fluids. High levels of histaminase were found in 44% (72 of 162) of all cases examined compared with 43% for carcinoembryonic antigens, 35% for β-subunit of human chorionic gonadotropin, and 23% for Regan isoenzyme. Except in lymphoma, elevations of this enzyme were found in all major types of cancer with varying degree of frequency. A high percentage of elevated histaminase was found in the effusion fluids of patients with cancers of the ovary (87%), colon (73%), and stomach (88%).

Examination of results of analyses of the four tumor markers in each individual patient reveals an immense diversity in the pattern of increase of these markers. Different patterns of elevation of these markers were observed among patients of different tumor primaries as well as patients with the same tumor primaries. In patients with cancer of the colon and stomach, the elevation of histaminase was found to correlate with the production of carcinoembryonic antigens in the majority of the cases; whereas among those with cancer of the ovary, the elevation of histaminase tended to concur with the production of β-subunit of human chorionic gonadotropin.

Results of this study also demonstrated that histaminase in the malignant effusion fluids was immunologically identical to that of the placenta; thus, this study provides the basis for using the placental histaminase as an antigen for the detection and quantitation of this enzyme by immunological methods in tumor cells and in the circulation of cancer patients. Positive immunohistochemical staining of this enzyme in tumor cells collected from effusion fluids has provided supportive evidence for the tumor origin of the enzyme in the effusion fluids. Overall results of this study therefore indicate that histaminase is associated with a wide variety of human cancers and suggest that this enzyme could be a biochemical marker for some of the cancers.

INTRODUCTION

Early studies have shown that elevation of histaminase (diamine oxidase, EC 1.4.3.6) activity occurs in a number of human cancers (11, 12), and results of more recent studies have established an association of this enzyme with medullary carcinoma of the thyroid. In the later studies (7, 8), high levels of serum histaminase activity were found in 50% of patients with thyroid tumor and in 70% of patients in whom metastases were present. The enzyme activity in the thyroid tumors was found to be 15 to 1500 times higher than that in the adjacent, noncancerous thyroid tissue (3). Since the enzyme is present at very low levels in the serum and in most tissues of normal individuals, it was suggested that the elevated histaminase in serum had originated from the tumor and that histaminase could be used as a biochemical marker for the detection of medullary thyroid carcinoma (8).

A large quantity of histaminase is produced by the placenta (16, 17, 22) and resulted in high histaminase levels in the pregnancy serum, ranging from several hundred- to several thousand-fold of that of the nonpregnancy serum (10, 34, 39).

In an earlier study (25), we have detected high levels of histaminase in a large percentage of malignant effusion fluids of patients with ovarian cancer. In a significant number of these cases, the elevation of histaminase coincided with the presence of Regan isoenzyme, a placental-type alkaline phosphatase isoenzyme detected in a number of human cancers. A later study further showed that histaminase present in the malignant effusion fluids was biochemically and immunologically identical to the enzyme of the placenta, thus suggesting the possibility of using the placental histaminase as an antigen for the study of this enzyme in cancer (24).

We now report the results of a more extensive survey where the activity of histaminase was measured, together with 3 other tumor markers, CEA, β-HCG, and Regan isoenzyme, in 414 malignant effusion fluids obtained from 162 cancer patients with various primaries.

MATERIALS AND METHODS

Malignant Effusion Fluids. The malignant effusion fluids (pleural, peritoneal, or pericardial) used in this study were obtained from Pondville Hospital (Walpole, Mass.) from patients with histologically proven carcinomas of various primaries. The fluids were collected as part of the clinical procedures, either by paracentesis or thoracentesis, depending upon the location of the fluid accumulation.

Assay of Histaminase and Regan Isoenzyme. Histaminase activity was assayed by the method of Okuyama and Kobayashi (28), using [14C]putrescine as a substrate. In this procedure, the assay was carried out in 37.5 mM sodium phosphate buffer...
Histaminase in Malignant Effusion Fluids

Measurement of CEA$^3$ and β-HCG. The CEA concentrations of the fluids were measured by a direct radioimmunoassay procedure without extraction of the sample by perchloric acid (26). CEA assay kits provided by Hoffman-LaRoche Inc. (Nutley, N. J.) were used. As reported earlier (33, 36), values are considered as positive if higher than 0.4 placental isoenzyme unit per ml, which is twice the mean value of normal serum, and confirmed to be of placental type by microzone electrophoresis in the presence of antiserum against placental alkaline phosphatase (18).

Immuno blotting. Immunoblotting was carried out on agarose plates by using the procedure of Ouchterlony (29). The antiserum against placental histaminase was prepared by giving injections to rabbits of placental histaminase purified by affinity chromatography, as described in an earlier publication (23). The antiserum obtained was further absorbed with glutaraldehyde polymerized normal human serum to remove minor contaminating antibody components. The specificity of the antiserum against histaminase has been verified by the results of immunodiffusion and immunelectrophoresis (22). The malignant effusion fluids used in this test were selected from fluids with the highest histaminase activity in cancers of the ovary, stomach, colon, and urinary bladder. These fluids were concentrated 5- to 10-fold by ultrafiltration before being applied for immunodiffusion. The visualization of the immunoprecipitin line was done by labeling the immunocomplex with peroxidase, using peroxidase-labeled anti-rabbit IgG (1:10 dilution; Cappel Laboratory, Cochranville, Pa.) and staining the peroxidase activity with 3,3′-diaminobenzidine and hydrogen peroxide (15).

RESULTS

Analyses of Histaminase, CEA, β-HCG, and Regan Isoenzyme on Malignant Effusion Fluids. Results of analyses of histaminase on effusion fluids obtained from 162 cancer patients of various primaries, 12 noncancerous patients, and 21 normal serum samples are presented on Chart 1. In about one-half of the cancer cases examined, multiple effusion fluids were obtained at different times during the course of the disease. All the fluid specimens were analyzed, and the highest value from each patient was used in the tabulation. The mean histaminase value, in units/ml, and S.D. of each cancer primary are: breast, 54 ± 105; lung, 59 ± 95; ovary, 517 ± 1096; colon, 1299 ± 2607; stomach, 1210 ± 2574; endometrium, 422 ± 93; lymphoma, 13 ± 8; and other types of cancer examined, 192 ± 472. Statistical analyses by t test indicates that the values of cancer of the breast, lung, colon, endometrium, and others are significantly higher than the normal serum value (16 ± 4) at the 0.05 level, whereas those of ovary and stomach are significantly higher at the 0.01 level. The activity of histaminase in fluids from noncancerous patients is 13.6 ± 8.6, which is not significantly different from that of normal serum.

The comparison of histaminase in the percentage of patients above the normal level in various cancer primaries with CEA, β-HCG, and Regan isoenzyme is summarized in Table 1. The result indicates that elevation of histaminase was detected in...
44% (72 of 162) of all cases examined. Elevation of this enzyme was observed in the high percentage of patients with cancer of the ovary (87%, 20 of 23), colon (73%, 8 of 11), and stomach (88%, 7 of 8). The overall percentage of elevation of histaminase in this study (44%) is similar to that of the CEA (43%) and is considerably higher than those of $\beta$-HCG (35%) and Regan isoenzyme of alkaline phosphatase (23%).

Patterns of Elevation of Histaminase and Other Tumor Markers. The results of analyses also indicate that histaminase activity varied widely among individual patients, ranging from under 10 to over 8000 units/ml. However, among multiple fluid samples collected during the course of the disease, the histaminase level usually fluctuated within a narrower range for each individual patient. A portion of this observation is shown on Chart 2 where the histaminase activities were plotted for multiple fluids taken over a 6-month period or longer from patients with ovarian cancer.

Results of analyses of the markers on the fluids also revealed that there was an immense diversity of patterns of increase of these markers among individual patients. Different patterns of tumor marker expression occurred among patients with different tumor primaries as well as those with the same tumor primary.

Concurrent Increases of Tumor Markers in Malignant Effusion Fluids. In comparing the results of analyses of different markers measured in the same fluid sample, it was found that there was concurrent production of both histaminase and CEA in the majority of patients with cancers of the stomach and colon. As illustrated in Chart 3, 14 of the 19 cases had elevated levels of both histaminase and CEA, 2 lacked both markers, and only 3 produced 1 of these 2 markers. However, this was not the case in cancer of the ovary where only 8 of 23 patients produced both histaminase and CEA, 12 produced histaminase alone, and 2 produced only CEA. Concurrent production of $\beta$-HCG and histaminase was observed in 16 of 23 patients with ovarian cancer, whereas 3 lacked both markers, and 4 produced only histaminase.

Immunological Identity of Histaminase in Malignant Effusion Fluids with Histaminase of the Placenta. As shown in Fig. 1A of the result of an immunodiffusion test, a single confluent precipitin line formed between various malignant effusion fluids and placental histaminase when reacted against the antisera of placental histaminase. This indicates the immunological identity of the enzyme in the fluids to that of the placenta. The immunological identity of histaminase in the fluids with a tumor extract of medullary thyroid carcinoma has also been demonstrated by immunodiffusion test (Fig. 1B).

Immunohistochemical Staining of Histaminase in Cells Collected from Malignant Effusion Fluids. Tumor cells were present in many of the malignant effusion fluids examined. Cell

**Table 1**

*Analyses of histaminase, CEA, $\beta$-HCG, and Regan isoenzyme in malignant fluids of 162 cancer patients of different primaries*

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Histaminase</th>
<th>CEA</th>
<th>$\beta$-HCG</th>
<th>Regan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>14/43 (33%)</td>
<td>20/43 (47)</td>
<td>6/41 (15)</td>
<td>4/43 (9)</td>
</tr>
<tr>
<td>Lung</td>
<td>13/45 (29)</td>
<td>19/45 (42)</td>
<td>17/45 (40)</td>
<td>6/45 (13)</td>
</tr>
<tr>
<td>Ovary</td>
<td>20/23 (87)</td>
<td>11/23 (48)</td>
<td>18/23 (70)</td>
<td>12/23 (52)</td>
</tr>
<tr>
<td>Colon</td>
<td>8/11 (73)</td>
<td>10/11 (91)</td>
<td>4/11 (36)</td>
<td>6/11 (56)</td>
</tr>
<tr>
<td>Stomach</td>
<td>7/8 (88)</td>
<td>6/8 (75)</td>
<td>5/8 (63)</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>Endometrium</td>
<td>4/8 (50)</td>
<td>2/8 (25)</td>
<td>3/8 (38)</td>
<td>4/8 (50)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0/6 (0)</td>
<td>0/6 (0)</td>
<td>0/6 (0)</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Others²</td>
<td>6/18 (33)</td>
<td>2/18 (11)</td>
<td>5/18 (28)</td>
<td>4/18 (22)</td>
</tr>
<tr>
<td>Total</td>
<td>72/162 (44)</td>
<td>70/162 (43)</td>
<td>65/162 (35)</td>
<td>38/162 (23)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of patients above the normal level.

² Includes cancers of: kidney, 5; pancreas, 4; gallbladder, 1; esophagus, 3; parotid, 1; prostate, 1; urinary bladder, 1; and skin, 2.
Histaminase in Malignant Effusion Fluids

Results of the present study indicate that elevation of histaminase occurs in a wide variety of human cancers. The elevation of this enzyme is not limited to tumors of the neural crest origin as has been suggested from results of studies in medullary thyroid carcinoma and small cell lung carcinoma (6, 7). Some degree of specificity related to the primary of cancer with the increase of this enzyme in the fluid has been observed. Relatively high incidences of elevation of histaminase were found in cancers of the ovary, stomach, and colon in comparison with other types of cancer examined. These data not only confirm our previous observation that a large percentage of ovarian cancer patients had elevated levels of histaminase in the effusion fluids (25) but also provide the first indication for the high association of this enzyme with cancers of the colon and stomach.

Results of the present study therefore suggest the possibility for histaminase as a biochemical marker for certain types of human cancer such as carcinomas of the ovary, stomach, and colon. Not every tumor, of even these types, exhibits an elevated level of histaminase. Also, in a number of cases where both serum and fluid samples from the same patient were assayed, the histaminase level in the serum is usually lower than that found in the fluid, and the overall percentage of elevation was also lower. Discrepancy between the histaminase levels of tumor tissue and plasma of the same patient has also been reported (6).

Results of the present study also show a lesser extent of association between the elevation of histaminase with the presence of Regan isoenzyme reported earlier (25). The specific association of the elevation of histaminase with small cell carcinoma of the lung, reported by Baylin et al. (6), was not observed in our study. We found that only 3 of 14 cases of small cell carcinoma of the lung that had high levels of histaminase, whereas 2 of 8 cases of large cell lung carcinoma had elevated levels of this enzyme in the effusion fluids.

There is an immense diversity of the production of different tumor markers among individual patients, even among the ones with the same tumor primary. This is to be expected if one recognizes the heterogeneous nature of tumors in terms of their differences in cell origins and stages of differentiation and tumor progression (30–32). The recognition of this variable and heterogeneous nature of the tumor in its biochemical expression therefore underscores the importance of measuring multiple markers rather than to rely on a single test for possible detection of the presence of certain cancers.

Although direct evidence has yet to be obtained, circumstantial evidence has suggested that the elevation of histaminase in the circulation of cancer patients has resulted from the production of this enzyme by the tumor. Production of histaminase by tumor cells has been demonstrated by biochemical and immunohistochemical studies which have shown the presence of this enzyme at high levels in tumor tissues of medullary thyroid carcinoma and in small cell lung carcinoma and also the absence of it in the surrounding noncancerous tissues (3, 9, 27). The positive immunohistochemical staining of this enzyme in tumor cells obtained from malignant effusion fluids can be considered as another supportive piece of evidence for this hypothesis.

The biological function of histaminase is not entirely known.

**DISCUSSION**

**Chart 3. Comparison of histaminase and CEA levels in effusion fluids of patients with cancer of colon (□) and stomach (○) showing the concurrent elevation of both markers in 14 of 19 cases. Both histaminase and CEA levels are plotted in logarithmic scale. — — — —, level of elevation for each marker.**

Smears were prepared from fluids obtained from 10 patients (6 with ovarian cancer, 2 with stomach cancer, 1 with breast cancer, and 1 with bladder cancer) and immunohistochemical staining of histaminase was performed. Detectable immunohistochemical staining of histaminase was found in 5 of the cases (3 from ovarian cancer and one each from cancer of the stomach and breast). Shown in Fig. 2, as an example, is the staining of histaminase in cell smears obtained from the fluid of an ovarian cancer patient. The enzyme stain usually appeared only in some of the cells and localized in the cytoplasm with varying intensity. In most cases, when clumps of cells were observed, cells within a single clump were either all positively stained or all negative. It was also observed that the morphology of the positively stained cells appeared to be that of typical tumor cells with enlarged nuclei.

**Fig. 1. Immunodiffusion of histaminase from different sources against antisera of placental histaminase. The antiserum (in central wells) was prepared from highly purified placental histaminase and has been absorbed with normal human serum to remove minor contaminating antibody components. The immunoprecipitin line was coupled with peroxidase-labeled anti-rabbit IgG and stained by 3,3'-diaminobenzidine and hydrogen peroxide. A, immunodiffusion of effusion fluids from patients with cancer of the ovary (OV), colon (CO), stomach (ST), and urinary bladder (UB) with a placental extract (PL) and purified placental histaminase (PE). The immunoidentity of the enzyme from fluids with the placental enzyme is indicated by the single confluent precipitin line formed. B, immunodiffusion of antiplacental histaminase against an effusion fluid from an ovarian cancer patient (OV), a tumor tissue extract from a medullary thyroid carcinoma (TE), an extract from placenta (PL), a highly purified placental histaminase (PE), and a normal human serum sample (NS). The immunoidentity of the enzyme from the fluid, the thyroid tumor, and the placenta is indicated by the resultant single confluent precipitin line.**
at the present time. Zeller (40) correlated the function of this enzyme with the general area of biogenic amine metabolism, due to the large number of biogenic amines that this enzyme can act upon in vitro, and suggested that it may be a potential regulator of the cellular concentrations of many of these amines. Baylin (5) pointed out that the ability of this enzyme to deaminate putrescine, a key component in the biosynthesis of polyamine, may indicate a functional role for this enzyme.

Another suggestion for the functional significance of this enzyme is that histamine of the placenta destroys excess histamine produced by the fetus during pregnancy, thereby preventing the return of histamine into the maternal circulation (4, 19, 20). Our recent finding, with immunofluorescent staining, that a significant amount of this enzyme is localized in the extracellular space surrounding the decidual cells in the placenta (22) suggests a possible functional secretion of the enzyme into the extracellular space and into the circulation by the decidual cells. This is consistent with the theory for the protective function of histaminase in destroying the excess histamine produced by the fetus. The same theory may be used as a basis for a hypothesis for the possible role of this enzyme in tumor biology; the expression of this enzyme by the tumor cell could provide a mechanism for the interference of the inflammatory process of the host during the invasive growth of the tumor. This speculation is consistent with the concept that tumors have certain abilities to escape the defensive mechanisms of the host, as exemplified by the well-recognized phenomenon of the existence of immunological escaping mechanisms in tumors (1, 21).

REFERENCES

Fig. 2. Immunoperoxidase staining of histaminase in tumor cells present in the effusion fluid of an ovarian cancer patient. Both histaminase-positive and -negative cells can be seen in A. At a higher magnification, it can be seen that histaminase-specific stain is localized in the cytoplasm of the cell (C). B and D, controls with normal rabbit serum substituted for anti-histaminase serum. A and B, × 300; C and D, × 2000.
Histaminase and Other Tumor Markers in Malignant Effusion Fluids

Chi-Wei Lin, Norma R. Inglis, Allyn H. Rule, et al.