An Unusual Oxygen-sensitive Lactate Dehydrogenase Isozyme Associated with Kirsten Murine Sarcoma Virus in Human Serum


ABSTRACT

An unusual isozyme of lactate dehydrogenase, lactate dehydrogenase associated with Kirsten murine sarcoma virus (LDHk) was found in the sera of many patients with malignant tumors, while the sera of healthy persons had little or no such activity. This isozyme was detectable only when assayed in a nitrogen atmosphere, and its activity showed little or no relationship to the total lactate dehydrogenase activity as measured by a standard clinical assay. The activity of serum LDHk appeared to be correlated with the presence of known metastases. Increased serum LDHk appeared in a wide variety of patients with cancer, although it appeared to be more common in certain types of cancer. Increased serum LDHk activity was also found in the sera of some patients with nonmalignant disease. The activity of serum LDHk may be useful to monitor recurrence or response to therapy in certain types of cancer.

INTRODUCTION

In 1979, Anderson et al. (3) described an antigen common to anaerobically shocked cells and cells transformed by Kirsten murine sarcoma virus. This protein was shown to have a LDHk (EC 1.1.1.27) activity and was therefore called LDHk. It may be related to the uncharacterized LDH VI originally noted by Markert and Moller (10) in their seminal paper on the separation of LDH isozymes. Several characteristics distinguish LDHk from the other LDH isozymes, which we will call the standard LDH isozymes. LDHk is a basic protein which is reversibly inhibited by oxygen and by certain dinucleotides (4), and, as mentioned above, it can be induced in untransformed cells by anaerobic shock (2).

Genetic evidence suggests that an oncogene of Kirsten transforming virus encodes LDHk. That is, a mutant of Kirsten sarcoma virus which is temperature sensitive for transformation produces an LDHk activity which is similarly temperature sensitive when the purified enzyme is assayed in vitro (2). This association with malignant transformation was reinforced by the finding that most tumors tested show increased activity of LDHk when compared to adjoining normal tissue (1, 4). This finding prompted us to test sera of patients having malignant disease for the presence of LDHk.

A summary of some of the data in this study has been published previously (5).
LDH<sub>k</sub> in Serum of Patients with Cancer

**Chart 1.** Serum LDH<sub>k</sub> activity in patients with cancer and in healthy individuals. Patients were classified according to the site of the primary tumor or the type of neoplastic disease; points, one patient; •, patients with known metastatic disease; O, patients with nonmetastatic disease and healthy individuals. u, units; AML, acute myelocytic leukemia.

A cutoff between normal and elevated levels. About one-half of the patients had LDH<sub>k</sub> activity above this level.

**Serum LDH<sub>k</sub> in Sera of Patients with Nonmalignant Disease.**
The control sera in Chart 1 were obtained from healthy donors (for plasmapheresis). To examine serum LDH<sub>k</sub> in patients with nonmalignant diseases, we obtained sera from patients admitted at a general hospital, assayed them for LDH<sub>k</sub> in a blind fashion, and then consulted their hospital records for diagnostic information. The patients whose diagnoses did not include cancer are summarized in Table 1. Of 78 patients, 13 (17%) had values greater than 3 units, and 22 (28%) had values greater than 2. These groups could still have included patients with undetected cancer; i.e., the diagnostic work-up could have been incomplete.

Two of these patients with the most increased serum LDH<sub>k</sub> were older than any of the cancer patients studied. We speculate that advanced age may affect serum LDH<sub>k</sub>, but we do not presently have enough data to test that speculation. As mentioned above, there was no apparent connection between age and serum LDH<sub>k</sub> within the population of cancer patients.

**LDH<sub>k</sub> in Sera of Patients with Metastatic Malignant Disease.**
The data in Chart 1 seemed to show a correlation between the serum LDH<sub>k</sub> activity and the presence of metastases. To test this possibility, we performed a one-way analysis of variance to compare patients with metastatic disease to those with primary tumors only. The difference between these groups was significant (p = 0.018).

To allow a visual comparison of serum LDH<sub>k</sub> activity among healthy individuals, patients with nonmalignant disease, patients with primary malignant disease, and patients with metastatic malignant disease, we combined the data of Chart 1 and Table 1. The cases were grouped according to the activity of serum LDH<sub>k</sub> as shown in Chart 2. The distribution of cases among the
Table 1

LDH<sub>k</sub> in patients without known cancer

Sera from 96 patients were assayed in a blind fashion for LDH<sub>k</sub>, and medical records were then consulted for diagnostic information. Patients with cancer were omitted from the table; 7 had LDH<sub>k</sub> activity greater than 2 units and 4 had activities of 2 units or less.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>LDH&lt;sub&gt;k&lt;/sub&gt; (units/0.1 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes, arteriosclerotic heart disease, anemia, small bowel obstruction (cancer not ruled out)</td>
<td>F</td>
<td>81</td>
<td>26</td>
</tr>
<tr>
<td>Cholangitis, perforation of gall bladder</td>
<td>F</td>
<td>70</td>
<td>17</td>
</tr>
<tr>
<td>Back pain, no cancer</td>
<td>F</td>
<td>73</td>
<td>14</td>
</tr>
<tr>
<td>Lumbar disc protrusion, no cancer</td>
<td>M</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>Leg ulcers, bedsores</td>
<td>F</td>
<td>90</td>
<td>11</td>
</tr>
<tr>
<td>Carcinact</td>
<td>F</td>
<td>65</td>
<td>11</td>
</tr>
<tr>
<td>Pneumonitis of leg</td>
<td>F</td>
<td>73</td>
<td>11</td>
</tr>
<tr>
<td>Diabetes, hypergastrinemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior septal myocardiial infarction, left ventricular aneurysm, coronary artery disease</td>
<td>M</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Coronary spasm, mitral valve prolapse, cholecystitis</td>
<td>F</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td>Right tympanoplasty</td>
<td>F</td>
<td>52</td>
<td>7</td>
</tr>
<tr>
<td>Common duct cyst</td>
<td>F</td>
<td>71</td>
<td>6</td>
</tr>
<tr>
<td>Inflammatory perinetal cyst</td>
<td>F</td>
<td>56</td>
<td>6</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>M</td>
<td>63</td>
<td>6</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>F</td>
<td>71</td>
<td>5</td>
</tr>
<tr>
<td>Hematoma</td>
<td>F</td>
<td>74</td>
<td>5</td>
</tr>
<tr>
<td>Umbilical hernia</td>
<td>F</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>Primary malignancy</td>
<td>M</td>
<td>65</td>
<td>4</td>
</tr>
<tr>
<td>9 patients without cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57 patients without cancer</td>
<td></td>
<td></td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

3, there was little or no correlation between standard LDH activity and LDH<sub>k</sub> activity. The correlation coefficient between log(LDH + 1) and log(LDH<sub>k</sub> + 1) was 0.44, confirming that the 2 parameters were not correlated. This was consistent with previous work which showed that LDH<sub>k</sub> differs from the standard isozymes both biochemically (2) and in its expression during the cell cycle (4).

Electrophoretic Variants of Serum LDH<sub>k</sub>. In a few cases, we detected electrophoretic variants of serum LDH<sub>k</sub>. Fig. 1 shows a section of one electrophoretogram which contained 3 variants, all of which migrated less rapidly than the usual form of serum LDH<sub>k</sub>. The patients with these apparent variants had different types of malignant tumors: colon; pharyngeal; and testicular. The serum enzyme in all except these 3 cases co-migrated with tumor LDH<sub>k</sub> but in no cases did we have tumor and serum enzyme from the same patient.

Inhibition by Diadenosine Tetrathosphate. One unusual characteristic of LDH<sub>k</sub> is that its activity is inhibited by diadenosine tetrathosphate and diguanosine tetrathosphate (4). The K<sub>i</sub> for these inhibitors is about 100 µM, and they are not competitive with NAD<sup>+</sup>. This property is shared by LDH<sub>k</sub> activities from cells groups of serum LDH<sub>k</sub> activity is shown in Chart 2, bottom. The ratio of metastatic cases to total malignant cases in each of the activity classes is shown in Chart 2, top.

Comparison with Standard LDH Isozymes. The activity of standard LDH isozymes was also sometimes increased in sera of patients with neoplastic disease. However, as shown in Chart Fig. 1. Electrophoretic variants of serum LDH<sub>k</sub>. The LDH bands in Lanes A, C, and L are variant; these were obtained with sera from patients with colon, pharyngeal, and testicular cancer, respectively.
transformed by Kirsten murine sarcoma virus, from human tumors, from human liver, and from rat retina (11). Human serum LDH₄ was, by contrast, not inhibited by diadenosine tetraphosphate (Chart 4). This property may reflect a unique source for the serum enzyme, or it may reflect modification of the enzyme by serum components.

**DISCUSSION**

In this study, we examined the presence of LDH₄ in the serum of a wide variety of patients. The variety of diseases in the patient population limits our conclusions. Nevertheless, serum LDH₄ seemed to be correlated with cancer and especially with the presence of metastases. The observation does not necessarily imply that metastatic disease leads to production of serum LDH₄. Perhaps, for example, the types of tumor which were most likely to metastasize in our population also tended to be LDH₄ producing. However, we feel that the probability is a relationship between the extent of metastases and the level of serum LDH₄ for at least some types of cancer. We have recently observed such a correlation in some patients by following serum LDH₄ levels during disease progression or during treatment.

It also seems that different types of cancer may have different effects on the serum LDH₄ of the patient. Among the 23 groups of cancer presented in Chart 1, 8 were represented by samples from at least 10 patients. These groups were: colon; breast; ovarian; lung; oral and pharyngeal neoplasms; melanoma; Hodgkin’s disease and malignant lymphomas; and acute myeloblastic leukemia. Of these groups, patients with colon cancer had the highest mean serum LDH₄, and patients with oral and pharyngeal tumors had the lowest. The difference between these 2 groups was significant at the 5% level when tested by the Wilcoxon 2-sample test (9).

Serum LDH₄ may also appear in patients with nonmalignant disease. The interpretation of our data on this point is somewhat limited by the fact that some patients may have had undocumented malignant disease. It will be important to define nonmalignant conditions which cause the appearance of serum LDH₄ and to establish the origin of serum LDH₄ in such patients.

Two previous reports also describe a cathodal LDH activity in the serum of some patients with nonmalignant disease (6, 7). This basic LDH could have been LDH₄. Although LDH assays in these cases were presumably performed under aerobic conditions, LDH₄ does have some residual aerobic activity, and thus it might have been detected in some patients with a very high level of this enzyme in their sera. Since many of these patients had severe hypoxia, an extreme level of LDH₄ might not be surprising.

We do not know the source of serum LDH₄ in the patients studied. Since many tumors have high LDH₄ activity, it is reasonable to assume that they may secrete this enzyme or release it during necrosis. On the other hand, since anoxia can induce LDH₄ in rat cells and rat muscle (4), LDH₄ could be produced in nontumorous tissue as a result of circulatory disturbance caused by the tumor mass. The source of serum LDH₄ could be investigated by correlating the levels of LDH₄ in tumor and serum of individual patients or by correlating the presence of LDH₄ variants in the tumor and serum.

Further definition of the occurrence and origin of serum LDH₄ will show whether this is a valuable marker for any aspect of malignant disease. If serum LDH₄ were characteristic of metastatic disease, e.g., it might be useful to estimate the extent of metastatic disease and to monitor the response to treatment.

**ACKNOWLEDGMENTS**

The authors thank V. Onorato and W. Kovacik for technical assistance; B. Britten, A. Mittelman, N. Petrelli, and P. Reinagel for stimulating discussion; M. Held for preparation of the manuscript; and L. Enrich for advice on statistical tests and for performing the analysis of variance.

**REFERENCES**

An Unusual Oxygen-sensitive Lactate Dehydrogenase Isozyme Associated with Kirsten Murine Sarcoma Virus in Human Serum


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/5/2236

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.