Relation of Mastocytoma to Mast Cell Leukemia, and of
Heparin, Histamine and Serotonin to Mast Cells

By SABURO ONO, LENNA ZOMPETTI, PAUL HAGEN AND JACOB FURTH

ALTHOUGH much information about mast cells has become available in recent years (reviews by Padawer,¹ Uvnäs,² Riley³), little is known about the function of these cells. The relation of tissue mast cells to blood basophil leukocytes has been debated since their discovery. Because of their similar staining properties, Ehrlich⁴ regarded the latter as blood mast cells. The availability of a readily transplantable, well differentiated, slow-growing mast cell tumor containing large quantities of heparin, histamine and serotonin⁵ has made possible studies of the biosynthesis, storage and liberation of these pharmacologically active substances and of the growth of mast cells both in vivo and in vitro.

This paper describes some characteristics of the tumor, some properties of mast cells grown in tissue culture and the experimental production of mast cell leukemia in mice by the introduction of isolated tumor mast cells into the circulation. A preliminary report of these experiments has already been given.⁶

MATERIALS AND METHODS

Routine transplantation.—The origin of the tumor has been described.⁵ Transplants were made in the strain of origin (LAF₁ mice) by mincing the tumor tissue and injecting the fragmented material into the thigh muscle.

Preparation of tumor tissue for intraperitoneal or intravenous injection.—Fragments of minced tumor were stirred in 0.25 per cent trypsin in Hank’s saline (minus calcium, magnesium and phosphate)⁷ for 20 minutes at 36°C, centrifuged at 1,000 rpm for 10 minutes and resuspended in Eagle’s basal medium.

Histamine and serotonin determinations in tumor, liver tissue, blood and urine.—Blood was obtained by cardiac puncture from animals under deep ether anesthesia. An equal volume of normal hydrochloric acid was added to the sample of oxalated plasma, serum or urine, neutralized with sodium hydroxide and diluted with sea water for serotonin assay on the isolated clam heart, or Locke’s solution for histamine assay on the guinea pig ileum.

A one in ten homogenate of liver or tumor tissue was made in normal hydrochloric acid using a glass tissue grinder. After centrifugation, the supernatant was neutralized with sodium hydroxide and assayed as above.

Heparin determination in liver, tumor tissue and plasma.—One part of liver was homogenized in 3 parts of 0.1 M phosphate buffer of pH 8. To this homogenate or to oxalated plasma was added one part of 2.5 per cent trypsin in phosphate buffer of pH 8. After incubation for 17 to 19 hours at 37°C, 10 N sodium hydroxide was added to destroy the trypsin, and the solution was neutralized and assayed. The assay procedure was

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based on the ability of heparin to inhibit clot formation, and differed from that previously used only in that bovine fibrinogen was used instead of dog fibrinogen.

RESULTS

Character of the tumor with repeated passages.—Table 1 presents the latency period and duration of our mastocytoma in mice following intramuscular grafts. In the course of passages, the latency period of the tumor (time after inoculation required for the tumor to become apparent) has decreased, and now the strain tends to give rise to rapidly growing variants.

Mastocytomas (fig. 4a) attained size of 20 to 24 grams in the first few passage generations. The cells were well granulated (fig. 3) even in the fast-growing line. No tendency to metastasize was observed in the course of a number of passages. Although mitotic figures were scarce in any one section, the growth of the tumor indicated that there must have been considerable cell division. Figure 1 shows the mast cells in a preparation stained with hematoxylin. Note the uniformity of the size and shape of the cells, which could be mistaken for either a monocytoma or an epithelial tumor of low-grade malignancy. The character of the cells is disclosed in imprints (fig. 3) or sections stained with Wright-Giemsa or toluidine blue in which all cells are loaded with the characteristic granules.

Production of mast cell leukemia.—Although Maximow (1910) had observed the development of basophil leukocytes (Blutmastzellen) in the bone marrow, mast cells are rare in the normal hemopoietic tissues of the mouse. Experiments have been carried out to determine whether mast cells derived from solid tumors could multiply in the hemopoietic tissues of the mouse and give rise to blood basophils.

Isolated mast cells, obtained from trypsinized mastocytomas, were injected intravenously in normal LAF1 mice. A leukemia developed, after a latency period of about two to three months, characterized by the presence of numerous mast cells in the blood, hepatomegaly and splenomegaly (fig. 4b), without enlargement of lymph nodes. Lung metastases were rare. Infiltration of the spinal cord and ganglia frequently caused paralysis of the hind legs, as is not uncommon in the lymphatic leukemias of mice. In a few cases the skull was infiltrated. Small tumor nodules were commonly formed in the subcutaneous tissues.

<table>
<thead>
<tr>
<th>Table 1.—Latency and Duration of Mast Cell Tumor Grafts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passage</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>No. of Mice</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Latency,* main line</td>
</tr>
<tr>
<td>Latency, fast line</td>
</tr>
<tr>
<td>Duration,* main line</td>
</tr>
<tr>
<td>Duration, fast line</td>
</tr>
</tbody>
</table>

*Days.
The animals here listed either died or were sacrificed with large tumors. All inoculations were intramuscular.
Fig. 1 (at top).—Microscopic appearance of a mast cell tumor grafted in thigh. Note the uniformity of size and shape of the cells. The tumor was well localized and measured about 2.0 cm. across. (H&E 350×).

Fig. 2 (bottom, left).—Mast cells in ascitic fluid. They are round, unlike those in tumors or those grown on glass which are polygonal with processes. (Wright-Giemsa 600×).

Fig. 3 (bottom, right).—Imprint of mast cells from a mastocytoma. (Wright-Giemsa 1250×).

rapidly than leukemias in mice that had been injected intravenously with the same number of cells. Cells from the intramuscular graft, however, spread from the site of injection, producing disseminated tumor nodules in the liver, spleen, kidney, subcutaneous tissues and bone marrow. Blood mast cell counts
Fig. 4 (at top).—Mouse a has a grafted mast cell tumor in the right thigh. Mouse b has mast cell leukemia with hepatomegaly and splenomegaly. Arrow points to paravertebral infiltration. Mouse c has innumerable tumor nodules in the lung following intravenous injection of trypsinized cells of mammary carcinoma.

Fig. 5 (at bottom).—Smear of blood from a mouse with mast cell leukemia. (Wright-Giemsa 600×).

were elevated. This reminds one of earlier experiences in establishing solid tumors with blood of mice with lymphoid leukemia.

Salient blood findings are shown in table 2, and the leukemic picture is illustrated in figure 5. Mice with mastocytomas rarely contained mast cells in the blood, in contrast to those with mast cell leukemia, in which mast cell...
Table 2.—Blood Changes in Mice with Mast Cell Tumors and Leukemia

<table>
<thead>
<tr>
<th></th>
<th>Normal Mast cell tumor</th>
<th>Mast cell leukemia</th>
<th>Mast cell leukemia line C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WBC (cu.mm.)</strong></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>7,110</td>
<td>6,100-8,200</td>
<td>11,300</td>
</tr>
<tr>
<td><strong>Hematocrit</strong></td>
<td>53</td>
<td>53-55</td>
<td>58</td>
</tr>
<tr>
<td><strong>Differential count per 100 cells:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neutrophiles</td>
<td>9</td>
<td>6-13</td>
<td>45</td>
</tr>
<tr>
<td>Lymphocytes, large</td>
<td>4</td>
<td>3-5</td>
<td>4</td>
</tr>
<tr>
<td>Lymphocytes, small</td>
<td>81</td>
<td>78-85</td>
<td>43</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Monocytes</td>
<td>6</td>
<td>2-5</td>
<td>7</td>
</tr>
</tbody>
</table>

*Four cases. 14-14 cases.

...cells averaged 13 per cent in the main line and 23 per cent in subline C. Hematocrit values were normal in mast cell tumor-bearing mice, but much decreased in mast cell leukemic hosts. This is readily explained by the extensive diffuse infiltration of the bone marrow.

The circulating mast cells appear to be somewhat smaller and less granulated than the tissue mast cells. A valid comparison is difficult because blood mast cells are examined in smears, and tumor mast cells in imprints. By way of comparison, in fluid tissue cultures the mast cells floating in the fluid are spherical and much smaller than the polygonal cells which adhere to the glass.

Examples of the microscopic picture of the hemopoietic organs of mice with mast cell leukemia are seen in figure 6 (spleen) and figure 7 (liver). In the liver there is a massive diffuse intrasinusoidal accumulation of mast cells with occasional small tumor-like nodules. The splenic pulp is densely infiltrated with neoplastic mast cells. The follicles are preserved, although small, being encroached upon by the mass of mast cells in the pulp. The bone marrow is diffusely infiltrated (fig. 8), and mast cell infiltration extends to the meninges, spinal cord and ganglia.

The intraperitoneal injection of tumor cells produced an ascitic type of neoplasm with diffuse infiltration of the mesentery and structures around the peritoneal lining. Small tumor masses were present on the surfaces of the viscera. Figure 2 shows a smear from ascitic fluid.

Heparin, histamine, and serotonin levels of tumors, blood and liver.—In the original description it was pointed out that the mast cell tumors contained large quantities of histamine, heparin and serotonin. Tumors of the first few generations contained 240 to 1,540 units/gram of heparin, 0.85 to 4.2 mg. histamine/gram and 8 to 140 μg./gram of serotonin. Despite the presence of such large quantities of such pharmacologically active materials these animals exhibited no sign of hemorrhage or shock, indicating that these materials were held within the mast cells.

In table 3 are given the values for histamine, heparin and serotonin in mast cell tumors, in the livers of normal mice and those with mast cell tumors and mast cell leukemia. The values in livers of mice with transplantable leukemias are also included as controls. In general, the liver histamine, heparin...
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and serotonin content appear to parallel the degree of mast cell infiltration.

The plasma and urine values of these active materials are given in tables 4 and 5. The results indicate that in animals with large localized mastocytomas, the active materials are retained by the mast cells, and only small quantities are present in the blood, even though significant amounts are excreted in the urine.
### Table 3.—Histamine, Heparin and Serotonin Content of Mastocytomas and Livers

<table>
<thead>
<tr>
<th>Types of Neoplasms</th>
<th>Histamine equiv. γ/g.</th>
<th>Heparin units/g.</th>
<th>Serotonin γ/g.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Mastocytoma*</td>
<td>5</td>
<td>2982</td>
<td>850-4200</td>
</tr>
<tr>
<td>Liver of hosts:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>0.07</td>
<td>0.01-0.1</td>
</tr>
<tr>
<td>Mastocytoma</td>
<td>13</td>
<td>1.56</td>
<td>0.97-7.8</td>
</tr>
<tr>
<td>Mast cell leukemia</td>
<td>8</td>
<td>304.0</td>
<td>30-560</td>
</tr>
<tr>
<td>Mast cell leukemia (line C)</td>
<td>8</td>
<td>395.0</td>
<td>30-560</td>
</tr>
<tr>
<td>Viral leukemia†</td>
<td>4</td>
<td>0.5</td>
<td>0.5-0.5</td>
</tr>
<tr>
<td>Reticulum sarcoma</td>
<td>1</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

Each assay of the same kind was made on a different animal.

*Some of these values were included in the preliminary report.†Virus of Friend, causing reticulum cell sarcoma.

### Table 4.—Histamine, Heparin and Serotonin Values of Plasma

<table>
<thead>
<tr>
<th>Host mice</th>
<th>Histamine equiv. γ/ml.</th>
<th>Heparin units/ml.</th>
<th>Serotonin* γ/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Normal</td>
<td>5</td>
<td>0.4</td>
<td>0.04-0.73</td>
</tr>
<tr>
<td>Mast cell tumor-bearing</td>
<td>5</td>
<td>0.74</td>
<td>0.3-1.82</td>
</tr>
<tr>
<td>Friend leukemia</td>
<td>4</td>
<td>0.5</td>
<td>0.5-0.5</td>
</tr>
</tbody>
</table>

See footnote to table 3.

*Serum values.

### Table 5.—Histamine Values in Urine

<table>
<thead>
<tr>
<th>Host</th>
<th>Histamine equiv. γ/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
</tr>
<tr>
<td>Mast cell tumor bearing</td>
<td>4</td>
</tr>
<tr>
<td>Mast cell leukemic</td>
<td>4</td>
</tr>
</tbody>
</table>

See footnote to table 3.

In animals with mast cell leukemia, plasma histamine and heparin values are greatly elevated, and the urinary histamine levels are on the average eight times greater than in animals bearing mast cell tumors. This suggests that the mast cells in the blood release their active materials. The lack of a hemorrhagic state in the presence of raised plasma heparin values may be due to a binding of heparin with proteins. The heparin assays were carried out on trypsinized plasma.

**Mast cells grown in tissue culture.**—Tissue cultures were prepared from mast cell tumor cells using Eagle's medium. Without supplements, this medium supported the growth of mast cells with the formation of granules (figs. 9 and 10). The cells retained their capacity to grow and form tumors; when injected into mice 35 days after growth in vitro, typical mastocytomas with a latency of 138 days developed. However, assays of tissue cultures did not show the presence of histamine in the tissue culture fluid at this time. Apparently the basophilic granules are only a matrix, probably of protein,
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Fig. 9 (at top).—Mast cells grown on glass in liquid medium stained with Wright-Giemsa. The cell in the center is a fibroblast. (625X).

Fig. 10 (at bottom).—Unstained mast cells grown on glass in liquid medium. Note the numerous pseudopodia and the characteristic granularity of the cells. (Phase contrast; 600X).

For storage of the pharmacologically active compounds when formed. Experiments to determine the formation of histamine and serotonin by increasing the level of the corresponding amino acids are in progress. Recently Schindler, Day and Fischer9 have shown the ability of mast cells of the Dunn and Potter mastocytoma10 to produce serotonin in tissue culture.
Two transplantable strains of mast cell neoplasm are known. Both originated in mice as solid tumors and are transplantable in the strain of origin. That described by Dunn and Potter is invasive and grows relatively fast; that isolated by us grows slowly, does not metastasize readily and is highly functional, producing as much as, or more, heparin, histamine and serotonin as any known tumor or other mammalian tissue.

Experiments reported elsewhere indicate that heparin, histamine and serotonin are contained in mast cell granules which are separable from mitochondria and other cytoplasmic particles by centrifugation in a sucrose density gradient. The mast cells contain 5-hydroxytryptophane decarboxylase and histidine decarboxylase and are capable of synthesizing serotonin and histamine from the corresponding amino acids. That mast cells also synthesize heparin in vitro has recently been demonstrated.

Mast cell leukemia is rare in man. The relation of blood mast cells to tissue mast cells has thus far been largely conjectural. The present experiments indicate their essential identity. In two decades of studies with diverse types of leukemias of mice and in studies of changes in the hemopoietic system of the rodent no one has indicated a developmental relation between mast cells and hemopoietic organs of mice. Present studies lead us to suppose that mast cells are an independent blood cell type possessing special functions with usual residence in connective tissues and occasionally entering the blood stream in very small numbers.

The first reported transplantable myeloid leukemia was characterized by basophilic granules in the cytoplasm of the myeloid cells. By subcutaneous injection of these basophilic granulocytes, localized tumors were produced. It was also noted that after reduction of host resistance by irradiation the tumor cells tended to spread and produce leukemia. The storage in mast cells of heparin, histamine and serotonin was not known at that time, so the character of those basophilic granulocytes remains unknown. They may have been of the premyelocyte type. Although it is believed that the active materials are specific to mast cells, their presence or absence in premyelocytes with basophile granules remains to be determined.

Mast cells are conceived as constituting an independent cell system with a primary habitat in connective tissue possessing the capacity to synthesize and store histamine, heparin and serotonin. These substances are stored in the cell in specific granules. The basic problems to be explored are: What regulates the production of these substances? What causes their release when needed? What are their functions when released?

**SUMMARY AND CONCLUSIONS**

A highly functional, transplantable neoplasm of mast cells is described. It causes solitary slowly growing tumors localized at the site of graft in muscles and subcutaneous tissues.

Mast cell leukemia results when isolated cells are injected intravenously.
The blood of mice with mast cell leukemia produces solid mastocytomas when injected intramuscularly.

These and other observations suggest the essential identity of blood and tissue mast cells and suggest that mast cells are an independent cell type with primary residence in tissues outside the hemopoietic organs.

In mastocytomas, heparin, histamine and serotonin are present in great quantities. Some histamine is released and partly excreted in the urine.

In the mast cell leukemias, histamine plasma levels are slightly raised and urine levels are highly elevated.

Plasma heparin values may be slightly raised in mice with mast cell tumors and are greatly increased in mast cell leukemias without a hemorrhagic state.

The liver histamine and heparin values appear to be related to the number of infiltrating mast cells.

SUMMARIO IN INTERLINGUA

Es describite un functionalissime neoplasma transplantabile. Illo causa tumores de lente crescentia que es localisate al sito del graffo in musculo e histos subcutanee.

Leucemia mastocytic resulta quando cellulas isolate es injicite p1' via intravenose. Le sanguine de muses con leucemia mastocytic produce solide mastocytomas quando injicite per via intramuscular.

Iste e alte observationes pare indicar que hematomastocytos e histomastocytos es essentialmente identic e que mastocytos es un typo independentemente de cellulas que reside primarimemente in histos foras del organos hemapoietico.

Grande quantitates de heparina, histamina, e serotonina es presente in mastocytomas. Un certe amonta de histamina es liberate e excernite in parte in le urina.

In le leucemias mastocytic, le nivellos de histamina in le plasma es levemente elevate. In le urina, illos es grandemente elevate.

Le valores pro heparina del plasma pote esser levemente elevate in muses con tumores mastocytic e grandemente elevate in leucemias mastocytic in le absentia de un stato hemorrhagic.

Le valores de histamina e heparina hepatic es apparentemente relationate al numero del infiltrate mastocytes.

REFERENCES

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