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*Brief Communication*

# Transmission Experiments with Lymphocytic Sarcoma of the Mouse

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*Introduction.* The lymphocytic sarcoma of the mouse described here is noteworthy because of its peculiar selective affinity to the lymph nodes without involving the spleen or thymus. It may be of interest to workers in the leukemia-lymphoma field in connection with the puzzling problem of the individuality of different transplant lines as to their modes of dissemination and the organs affected. There are also some suggestive findings for the viral implication in the etiology of the tumor. This paper presents the results of our experiments on these points.

*Materials and Methods.* The donor mouse of the original tumor was a female of the ddN strain, about five months old, bearing one of those spontaneous "lymphomas" so familiar to workers on mouse tumors. As first observed, the tumor was represented by the greatly enlarged left axillary lymph node, measuring about 1 x 1.5 cm, accompanied to a much lesser extent by enlarged cervical and right inguinal nodes. The large axillary growth was surgically removed and fragments of it were used for transplantation. After three postoperative weeks, in very weak condition, the mouse was killed. Just prior to this, smears of peripheral blood showed apparent lymphocytosis, but the picture was not that of leukemia.

At autopsy all of the lymph nodes were found to be more or less enlarged, including a recurrence at the site of the operation. The peritoneal cavity was filled with hemorrhagic ascites and numerous tumor nodules; greatly enlarged mesenteric lymph nodes were very evident. The peritoneal tumor nodules weighed nearly 2 grams altogether. Liver and spleen were slightly enlarged, weighing 2.2 gm and 0.4 gm respectively. The lung showed no grossly recognizable metastasis. A large tumor weighing 0.2 gm, later verified to be of thoracic lymph node origin, occupied the region of the thymus.

Histologic examination revealed neoplasia of the lymph node system, consisting of uniformly small lymphocytic cells which actively invaded the surrounding tissue (Fig. 1), thus justifying the diagnosis of disseminating lymphocytic sarcoma. The absence of spleen, liver, and bone marrow involvement was noteworthy.

Young adult male and female mice of the ddN strain were used as the experimental animals, but newborn mice of the same strain were employed for testing transmissibility by cell-free filtrates.

Solid tumor tissue was transplanted subcutaneously through the thigh muscle into the right hind quarter of the mouse. The freshly removed tumor was minced sufficiently to be sucked up into a small injection syringe. The syringe was then fitted with a needle of a relatively large caliber and the tumor cells were injected in 0.05 ml doses. Transmission by intraperitoneal injection was done in the usual manner with homogenates of solid tumor, ascites, or pleural effusion in 0.2-ml amounts of physiologic salt solution.

For filtration experiments, the tumor tissue was homogenized with three times its weight of physiologic salt solution and centrifuged at 3,000 rpm for 10 minutes, and the supernatant was strained through either a standard Seitz filter (asbestos sterilizing film No. 85 of Toyo Roshi Co., Tokyo) or a Millipore filter of 0.45-$\mu$ pore size. Homogenization and filtration were carried out in an ice bath. Filtrates were injected intraperitoneally into newborn mice in 0.05 ml doses. The dose was increased to 0.1 ml for adults.

*Transplantation of Solid Grafts.* The first transplantation generation was started when the large axillary lymph node growth of the original mouse was transplanted into 6 normal mice. The grafts took in 3 mice. From these positive growths serial passages through generations of mice were started and they have yielded 100% takes, constantly, since the 13th generation. The tumor is presently in its 40th transplant generation and is available for distribution to workers in this field.

The growth of the solid grafts is very rapid and, currently, they are being transplanted weekly. The solid tumors produced show little necrosis and hardly any hemorrhage. They seldom penetrate through the skin, but spontaneous penetration into the peritoneal cavity is not uncommon in later stages of tumor growth, with the production of the usual tumor ascites and the formation of tumor nodules on the visceral peritoneum. Dissemination to various lymph nodes then takes place, sometimes to a very remarkable extent (Fig. 2). There is no notable enlargement of spleen and liver and no metastasis in the lung. The survival period of the mice is usually two to three weeks. When they lived four or five weeks there was sometimes very marked edema of the subcutaneous tissue.

*Transmission by an Intraperitoneal Route.* Intraperitoneal injections of tumor homogenates produced tumor nodules of varying sizes, which attached to the surface of visceral peritoneum, accompanied by highly hemorrhagic tumor ascites. The ascites was rich in tumor cells, and serial transmission could be made with it by intraperitoneal injections. The largest peritoneal growth was usually that of the mesenteric lymph node rather than tumor masses derived from the inocula.

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Other lymph nodes, such as pancreatic, renal, and retroperitoneal were also involved. These growths became confluent and finally produced extensive adhesion of the viscera. The liver and spleen did not become enlarged.

Very frequently hemorrhagic ascites was accompanied by pleural effusion, which was also highly hemorrhagic and full of sarcoma cells, and could be used to transmit the tumor. In such cases it was usual to find enlargement of the thoracic lymph node, sometimes so greatly enlarged as to press upon the lung. The lung itself was always free from macroscopically detectable tumor nodules.

A significant thoracic finding was that the thymus clearly was not a primary seat of the sarcoma dissemination but became involved by the infiltration of sarcoma cells from the thoracic node. Fig. 3 represents an early stage of this process where the thymus, still intact, is surrounded by the extensively proliferating lymphocytic sarcoma cells which in later stages invade and finally replace the thymus.

Total white counts of the peripheral blood were as high as 10,000 per cu mm but no higher. The blood picture for indisputable leukemia was not found.

Tumor takes in mice injected intraperitoneally with tumor ascites or pleural effusion were about as constant as in the case of solid graft implantations, though the survival period was generally more variable. Acute dissemination to the thoracic node often shortened the survival period to 6 or 7 days, due probably to pressure on the lung by the greatly enlarged node and the accompanying profuse pleural effusion.

*Transmission by Filtrates.* By using 448 newborn mice, twelve Saitz filtrates were tested for their ability to transmit the sarcoma. Four of the filtrates produced a positive transmission in 63 of the mice (14.1%). Six other filtrates were injected into 43 young adults; three of these filtrates yielded 5 cases of positive transmission (11.6%). The details are tabulated in Table 1. Additional experiments using newborn mice were carried out with a Millipore filter of 0.45-$\mu$ pore size, but these consistently showed negative results. All of these filtration experiments were terminated 60 days after the injection.

The positive transmissions were represented by tumor ascites, tumors of mesenteric and other peritoneal lymph nodes, and, in some cases, by pleural effusion rich in tumor cells and dissemination to the thoracic lymph node. Pathologic pictures in these mice were in no way different from those already described as resulting from the usual intraperitoneal transmission.

*Discussion.* In diagnosing the neoplasm described above as lymphocytic sarcoma, we followed Dunn (1) who classified lymphocytic neoplasms of the mice as sarcomas, if localized, and leukemias, if generalized. This distinction is arbitrary since all are more or less disseminating and generally aleukemic. The prominent pathologic features of most of them are large thymic tumors and marked splenomegaly. Indeed, the lymphocytic leukemias of Gross (2), Lieberman and Kaplan (3), and Moloney (4), to cite a few well-known examples, are regarded as originating in the thymus. It may be significant that our lymphocytic sarcoma shows no enlargement of the spleen, no thymus tumor, and the sites of its dissemination are limited to the lymph nodes.

The possible viral nature of our lymphocytic sarcoma may be a question of some interest. Electron microscopic search for the virus particles has not yet yielded convincing findings, although the presence of particles, closely resembling the well-known murine leukemia virus, in the sarcoma tissue (Fig. 4) was established. The picture of the "budding" at the level of the cytoplasmic membrane was also seen (Fig. 5). Further work is required, however, to determine whether these virus-like particles are constantly present in the sarcoma and whether the particles are capable of transmitting the sarcoma without the aid of sarcoma cells. The results of the filtration experiments reported in this paper are not satisfactory, since they were terminated too soon to detect a virus, which had a very long latent period. This like that of Gross' lymphocytic leukemia, strongly indicates the need for long-term observations. Experiments are underway in this laboratory to clear up some of these points.

Table 1

| Filtrate | Mice | No. of litters | Total no. of mice | No. of mice with tumors |
|---|---|---|---|---|
| 1 | Newborn | 3 | 22 | 0 |
| | Adult | | 10 | 1 |
| 2 | Newborn | 5 | 32 | 0 |
| | Adult | | 9 | 0 |
| 3 | Newborn | 5 | 46 | 0 |
| 4 | Newborn | 5 | 47 | 0 |
| | Adult | | 7 | 0 |
| 5 | Newborn | 6 | 46 | 17 |
| 6 | Newborn | 11 | 76 | 39 |
| | Adult | | 7 | 3 |
| 7 | Newborn | 5 | 34 | 1 |
| 8 | Newborn | 5 | 32 | 6 |
| | Adult | | 8 | 1 |
| 9 | Newborn | 5 | 30 | 0 |
| | Adult | | 2 | 0 |
| 10 | Newborn | 5 | 30 | 0 |
| 11 | Newborn | 3 | 23 | 0 |
| 12 | Newborn | 3 | 30 | 0 |

Experiments for transmission of lymphocytic sarcoma using 12 Seitz filtrates.

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Fig. 1. Histologic section of the original tumor of the left axillary lymph node showing infiltration into the muscular tissue. H & E, × 700.

Fig. 2. Gross photograph of a mouse bearing a large subcutaneous graft and widespread dissemination to various lymph nodes.

Fig. 3. Histologic section of the thymus region showing intact thymus in midst of a profuse proliferation of sarcoma cells in surrounding tissue. H & E, × 350.

Fig. 4. Electron photomicrograph of the sarcoma tissue showing two bodies resembling the mouse leukemia virus in intercellular space. × 120,000.

Fig. 5. Electron photomicrograph of the sarcoma tissue showing "budding" of a virus-like body at the cell membrane level. × 120,000.