Lymphosarcoma: Virus-induced Thymic-independent Disease in Mice

Herbert T. Abelson and Louise S. Rabstein

SUMMARY

A new murine oncogenic virus has been isolated which induces solid lymphoid tumors within a short latent period. A unique feature of this disease is the lack of thymic involvement. Thymic-independent tumor induction distinguishes this virus from the other experimental murine lymphoid leukemia viruses. In addition, massive meningeal tumors, a myelocytic leukemoid reaction, and no evidence of a disseminated leukemia constitute this disease syndrome.

INTRODUCTION

Thymic-independent lymphatic neoplasms are infrequently observed in mice. Lymphomas not involving the thymus have been reported to occur spontaneously in C58 mice (14), after topical application of various carcinogens (3, 9), and as spontaneous reticulum cell sarcomas in aging SJL/J mice (13). Recently, we reported the experimental virus induction of lymphosarcomas that arise without thymic involvement (1). The disease process is unique and is characterized by the rapid induction of solid lymphoid tumors, which does not involve the thymus. There is also massive tumor involvement of the meninges, a polymorphonuclear leukemoid reaction in the peripheral blood, and a lack of diffuse lymphocytic infiltration of organs.

The lymphosarcoma virus was isolated from a tumor which developed in a BALB/c mouse treated with a high dose of prednisolone from birth and inoculated with MLV at 28 days of age (2).

This report concerns the pathological and biological response of various strains of rodents to this lymphosarcoma-producing virus. The short latent period, meningeal tumors, and lack of thymic involvement make this new experimental virus disease an important multipotential model.

MATERIALS AND METHODS

Source of the Lymphosarcoma Virus. The tumor from which all subsequent lymphosarcoma virus originated occurred in a 93-day-old BALB/c mouse which had received 0.1 ml of a 1:100 dilution of MLV (Pfizer Lot 3042-278) i.p. at 28 days of age. The mouse had been treated with 0.05 mg of prednisolone s.c., starting less than 24 hr after birth, and continued twice a week until it was sacrificed. The tumor was detected 65 days after virus inoculation (2). The tumor was frozen for 166 days at -70°. A 10% extract of the original tumor was made by 2 low-speed (2400 X g) spins in an International centrifuge. A 1:10 dilution of the clarified extract was then inoculated i.p. into newborn (<1-day-old) and weanling (28- to 30-day-old) BALB/c mice. One-half of each group of mice received prednisolone acetate (Rugby Laboratories, Inc.) 0.05 mg s.c., twice a week from birth until termination of the experiment.

From this and subsequent passages of the lymphosarcoma virus, tumor or plasma concentrates were prepared by differential centrifugation according to the method of Moloney (10). In addition, several passages were made after filtering the lymphosarcoma virus concentrates through a 0.45-μ Milipore filter which, in each case, was impervious to Serratia marcescens. A pooled plasma concentrate was also filtered prior to inoculation. Prior to the preparation of the lymphosarcoma virus concentrates, the tumors or plasma were stored at -70°. The standard lymphosarcoma virus preparations were serially diluted 1:10, 1:100, 1:1,000, and 1:10,000 with phosphate-buffered saline, and 0.1 ml was inoculated i.p. Uninfected control mice were inoculated i.p. with 0.1 ml of phosphate-buffered saline.

Three other comparably induced lymphosarcomas (2) were also prepared as 10% extracts and passaged simultaneously with the original extract described above.

Mice. All inbred strains of mice and rats were bred and maintained at Microbiological Associates, Inc., Walkersville, Md. Those strains used were BALB/c, DBA/2, C3H, C57BL/6, and NIH Swiss mice, as well as Fischer rats. All animals were given inoculations of lymphosarcoma virus within 36 hr after birth. The mice were observed and palpated daily for tumor formation from 14 days postinoculation until tumor detection or termination of the experiment. The animals were housed without segregation by sex. Both experimental and control animals were fed Purina laboratory chow and water ad libitum.
Preparation of Tissues. Peripheral smears were routinely made from tail vein blood. Animals were sacrificed by exsanguination from the left brachial artery after ether anesthesia. Blood was collected in an equal volume of 0.153 M potassium citrate. After centrifugation, the plasma was separated and stored at −70°. Tissues taken at autopsy or necropsy were fixed in either 10% buffered formalin or Zenker formol and routinely stained with hematoxylin and eosin. Touch preparations of tumors were made on glass slides, air dried, and stained with Giemsa, as were the peripheral smears.

Serological Studies. A lymphosarcoma virus concentrate assayed by the mouse antibody production test was negative for the following antigens: PVM, Reovirus 3, GDVII, Sendai, K, Polyoma, MVM, mouse adenovirus, mouse hepatitis virus, and lymphocytic choriomeningitis virus. An aliquot of the lymphosarcoma virus was inoculated into weanling Swiss mice and produced an 11-fold increase in plasma lactic dehydrogenase levels after 72 hr. This indicated the presence of lactic dehydrogenase virus.4

Criteria for Diagnosis. The classification of murine reticular neoplastic diseases according to the method of Dunn (5) was used as a guide in arriving at diagnoses. Evaluations were made on the basis of combined gross and microscopic findings.

RESULTS

All strains of mice tested were susceptible to this new lymphosarcoma-producing virus. The disease was essentially identical in all strains and consisted of solid lymphoid tumors, meningeal tumors, and lack of thymic involvement. In addition, there was a polymorphonuclear leukemoid reaction and no lymphocytic invasion of organs.

Description of the Original Tumor. Gross examination at autopsy revealed moderate enlargement of the cervical and inguinal nodes and a 4-fold increase in size of the brachial nodes. The spleen was moderately enlarged, but the thymus was normal in size with no evidence of tumor. A large growth, which also invaded into the adjacent muscles, was found over the right hip. Similar tumor growth was found extending along the ventral surface of the thoracic, lumbar, and sacral vertebrae; it was invasive into the inferior vertebral muscles.

Microscopically, the tumor of the hip and those along the spine were composed of dense masses of quite uniform, immature lymphoid cells. Very fine strands of connective tissue were widely separated by the proliferating tumor cells, giving the appearance of irregular grouping of cells into small fascicles. No encapsulating membrane was present. The muscles adjacent to the solid growths were invaded by the neoplastic cells, masses of which extended between muscle bundles and between individual muscle fibers. The tumors were not well vascularized, and no necrosis or fibrosis was evident.

| 4Serological testing performed by Dr. John Parker, Microbiological Associates, Inc., Bethesda, Md. | Large; very immature cells were the predominant type. They were round or irregularly compressed, with large, round, angulated or indented nuclei and scant to moderate cytoplasm. Nuclear membranes were distinct, chromatin structure was relatively indented nuclei and scant to moderate cytoplasm. Nuclear membranes were distinct, chromatin structure was relatively coarse, and 1 or more nucleoli were usually present. Some nucleoli contained large vacuoles. There was a high mitotic index. The most mature forms appeared to be lymphoblasts, although most of the cells were less well differentiated. No giant cells were seen. | The parietal and interparietal bones of the skull were displaced due to the bulk of the tumor, with the neoplastic growth extending between the bony junctions and proliferating on the surface of the skull. The marrow spaces within the calvaria were filled with abnormal cells, although a few myelocytes and megakaryocytes were still identifiable. Extensive tumor growth filled the cranial cavity within all layers of the meninges, but did not invade the brain. Histologically, the tumor was identical to that already described.

Normal components of the lymph nodes were replaced by tumor cells. The sinuses were dilated and contained moderate numbers of abnormal lymphocytes, and there was extensive extracapsular proliferation of tumor cells.

In the spleen, the red pulp was partially replaced with tumor cells. The splenic follicles were evident, but their borders were indistinct, due to the encroachment of the neoplastic cells from the red pulp. Megakaryocytes were increased in number.

The thymus was not available for microscopic examination. All other organs examined appeared normal. In particular, there was no diffuse lymphocytic infiltration.

1st Passage of the Original Tumor. Tumors occurred most rapidly in the suckling mice treated with only tumor extract (Table 1). Multiple sites of tumor in the same mouse were frequently seen, but there was generally 1 lesion which was much larger than the others (Figs. 1 and 2). Although the size of the tumors varied widely, their gross appearance and consistency were identical. They were creamy, flesh colored, firm, glistening, and nonhemorrhagic.

Microscopically, the tumors were identical regardless of their location. They closely resembled the parent tumor, but, in addition, macrophages containing ingested nuclear debris were a common finding throughout the tumors (Fig. 3).

Sternal bone marrow was generally replaced by sheets of tumor cells. Normal precursors of the cellular elements of the blood were rarely recognizable. Frequently, the tumor mass spilled out of the confines of the sternum and invaded the adjacent intercostal muscles. Invasion and proliferation of tumor into muscle with massive destruction was common from all tumor sites (Fig. 4).

Meningeal tumor was present in 28 of 34 mice (82%) in which heads were available for examination. There was often a characteristic bulging of the parietal and interparietal bones (Figs. 5 and 6). The degree of invasion and proliferation of the tumor was often of such great proportions that the parietal bones were displaced by the encroaching tumor mass. The tumor would often encompass the total extent of the meninges and along the base of the brain (Fig. 7). The tumor extended into the subarachnoid space, as tumor was
found in Virchow-Robin spaces, but the pia mater was never penetrated (Fig. 8). The marrow of the calvaria was also replaced with tumor.

The thymus was never involved. Grossly, the thymus often appeared enlarged at autopsy. When histological sections were prepared, the enlargement was due to extracapsular proliferation of tumor from the surrounding thoracic lymph nodes which were closely adherent to the capsule of the thymus (Fig. 9).

The lymph nodes, whether grossly enlarged or not, generally displayed the same histological pattern of replacement of normal components by tumor cells. Extracapsular proliferation of tumor was usually massive.

Spleens were moderately enlarged. They showed hyperplasia of most cellular elements, particularly erythroblasts and megakaryocytes. Lymphoid follicles showed some degree of stimulation, but were not enlarged.

In many mice, cells in the medullary cords of the lymph nodes were replaced with polymorphonuclear leukocytes (Fig. 10).

By electron microscopy, numerous budding and free C-type particles, indistinguishable from other murine type C particles, have been found in all tumors examined. The tumor cells often, but not invariably, had large numbers of polyribosomes.

Although direct leukocyte counts were not made, examination of peripheral blood smears indicated that moderate leukocytosis was present in about one-half of the cases. The remaining cases showed no increase in white blood cells. Polymorphonuclear leukocytes were the predominant cell type.

A touch preparation of tumor is illustrated in Fig. 11. The predominant cell type is an immature lymphoid cell, but reticulum cells, lymphocytes, and polymorphonuclear leukocytes are also seen.

In 1 mouse out of a total of 36 examined, a typical lymphoid tumor was found within 1 kidney. With this exception, all visceral organs were free of tumor or abnormal lymphatic infiltration.

In the suckling mice treated with prednisolone, and the weanling mice with and without prednisolone, the clinical and pathological findings were identical except for the latent period to tumor formation. These results are tabulated in Table 1.

Extracts from 3 other lymphosarcomas (2), inoculated in an identical manner, produced only typical murine lymphocytic leukemia with a pattern and latent period compatible with that described for murine lymphocytic leukemia (11).

Subsequent Cell-free Passage of the Lymphosarcoma Virus.
Tumors from both groups of suckling mice (Table 1), with and without prednisolone treatment, served as sources of the lymphosarcoma virus for further passages. Prednisolone treatment was not used in any of the subsequent passages. These results are given in Tables 2 and 3.

Cell-free Passage of Pooled Plasma. Pooled plasma from BALB/c mice with virus-induced lymphosarcomas was concentrated and filtered. The results of this passage into newborn BALB/c mice are given in Table 4. Lymphosarcomas were produced only in those mice inoculated with the 1:10 diluted virus filtrate. At that dilution, 13 of 16 mice developed lymphosarcomas. The remaining 3 mice at that dilution and the 29 mice examined from the higher dilutions all developed typical murine lymphocytic leukemia (11).
Table 2

*Cell-free passage of lymphosarcoma virus isolated from BALB/c mice without prednisolone treatment*

Inoculations of the filtered lymphosarcoma virus concentrate were made into newborn BALB/c mice.

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>Inoculated</th>
<th>Not available for examination</th>
<th>Alive at 6 mo.</th>
<th>No. with tumor/no. examined</th>
<th>No. with meningeval tumor/no. examined</th>
<th>Latent period to lymphosarcoma (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>5/5</td>
<td>3/5</td>
<td>17–21</td>
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<tr>
<td>$10^{-2}$</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>8/8</td>
<td>7/8</td>
<td>20–37</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>$7/10^a$</td>
<td>5/8</td>
<td>22–42</td>
</tr>
</tbody>
</table>

*aOne mouse had no evidence of tumor at 36 days; 1 mouse had a combination of lymphosarcoma and lymphocytic leukemia at 121 days, and 1 mouse had only lymphocytic leukemia at 178 days.*

Table 3

*Cell-free passage of lymphosarcoma virus isolated from BALB/c mice with prednisolone treatment*

Inoculations of the filtered lymphosarcoma virus concentrate were made into newborn BALB/c mice.

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>Inoculated</th>
<th>Not available for examination</th>
<th>Alive at 6 mo.</th>
<th>No. with tumor/no. examined</th>
<th>No. with meningeval tumor/no. examined</th>
<th>Latent period to lymphosarcoma (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10/10</td>
<td>9/10</td>
<td>21–34</td>
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<td>$10^{-2}$</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>9/11^a</td>
<td>7/8</td>
<td>23–41</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>14</td>
<td>3</td>
<td>4</td>
<td>$3/7^b$</td>
<td>3/3</td>
<td>26–36</td>
</tr>
</tbody>
</table>

*aOne mouse had a combination of lymphosarcoma and lymphocytic leukemia at 119 days, and 1 mouse had only lymphocytic leukemia at 153 days.*

*bThree mice had lymphocytic leukemia at 68 to 118 days, and 1 mouse had lymphosarcoma-lymphocytic leukemia at 112 days.*

Table 4

*Cell-free passage of lymphosarcoma virus prepared from pooled plasma of BALB/c mice with lymphosarcomas*

A filtered pooled plasma concentrate was used for all inoculations. The plasma was collected at the time of sacrifice from BALB/c mice with tumors.

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>Inoculated</th>
<th>Not available for examination</th>
<th>Alive at 6 mo.</th>
<th>No. with tumor/no. examined</th>
<th>Latent period to lymphosarcoma (days)</th>
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<tr>
<td>$10^{-1}$</td>
<td>21</td>
<td>2</td>
<td>3</td>
<td>13/16^a</td>
<td>26–113</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>18</td>
<td>0</td>
<td>9</td>
<td>$0/9^b$</td>
<td></td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>28</td>
<td>0</td>
<td>12</td>
<td>$0/16^b$</td>
<td></td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>$0/4^b$</td>
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</table>

*aLymphocytic leukemia occurred in the 3 remaining mice at 91 to 105 days.*

*bAll mice developed lymphocytic leukemia at 55 to 165 days.*
Table 5

**Incidence of lymphosarcoma in the various mouse strains tested**

All strains were inoculated from the same filtered lymphosarcoma virus concentrate.

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. with tumor/no. examined</th>
<th>No. with meningeal tumor/no. examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^1$</td>
<td>$10^2$</td>
</tr>
<tr>
<td>BALB/c</td>
<td>10/10</td>
<td>16/16</td>
</tr>
<tr>
<td>NIH Swiss</td>
<td>12/12</td>
<td>11/13</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>7/7</td>
<td>2/2</td>
</tr>
<tr>
<td>DBA/2(b)</td>
<td>14/14</td>
<td>9/9</td>
</tr>
</tbody>
</table>

*a* Those mice not developing solid lymphosarcomas developed lymphocytic leukemia with a latent period ranging from 72 to 175 days.

*b* Dilutions were 1:5, rather than 1:10.

**Host Range of the Lymphosarcoma Virus.** The host range of the lymphosarcoma virus in mice was investigated by inoculating a cell-free lymphosarcoma virus concentrate into BALB/c, NIH Swiss, DBA/2, C3H, and C57BL/6 mice. All strains received the same virus preparation. These results are given in Table 5. All mouse strains inoculated were susceptible to tumor formation, although the latent period and end point titration varied between strains. The most susceptible strain was BALB/c and the least susceptible was C57BL/6. A comparison of latent period and median infective dose/0.1 ml for the various strains is given in Table 6.

Fischer rats were also given inoculations. All rats developed massive thymic tumors, most weighing 10 to 12 g. In addition to the thymic tumors, lymphoid tumors developed in the paravertebral region around, but not involving, the renal nodes. There was extensive organ invasion with tumor cells, which were also present in the peripheral blood. The latent period until the development of enlarged thymuses and respiratory distress was 81 to 139 days. This was consistent with the latent period described for MLV inoculation in rats (6).

**DISCUSSION**

Our results show that a lymphosarcoma-producing virus has been isolated which produces a unique disease syndrome. The disease process consists of the following components: rapid development of solid lymphoid tumors, massive meningeal tumors, no involvement of the thymus, a polymorphonuclear leukemoid reaction, and no diffuse organ invasion by tumor cells.

The disease process has been very similar in all of the mouse strains tested. Only the latent period and relative susceptibility to the lymphosarcoma virus have differed in the various strains.

Several features of this experimental virus-induced lymphosarcoma differ sharply from other virus-induced lymphocytic tumors. In other experimental virus-induced lymphoid neoplasms in mice inoculated as newborns, the thymus plays a central role and is the 1st site of neoplastic alteration (7, 8, 15, 16). From this primary site, there is subsequent invasion and proliferation of tumor cells throughout most of the organs and tissues of the mouse. In this new disease syndrome, lymphoid tissue (except the thymus) is susceptible to direct neoplastic transformation. This, then, would be contrary to other experimental virus-induced lymphoid neoplasms and provides a new model of murine oncogenic disease. The fact that the thymus is not involved in tumorigenesis and that solid tumors develop from a "peripheral" lymphoid tissue suggests that the viral genome may be different from that of other murine lymphocytic tumors.
tumor viruses. The possibility that this is due to an altered host response has been eliminated by demonstrating the production of the same disease with or without prednisolone treatment. Also, virus harvested from mice with or without prednisolone treatment produced the identical disease in similar groups of inoculated mice. The lack of thymic treatment. Also, virus harvested from mice with or without budding and free C-type particles associated with the tumor latent period compatible with MLV inoculation (11). The tumors developed with great rapidity. At comparable dilutions, MLV takes 2 to 4 times as long to induce lymphocytic leukemia (11). Tumor growth is exceptionally rapid once the process has been initiated. Mice frequently develop large palpable tumors and die, whereas 24 hr earlier they had appeared completely normal. No tumors have ever been observed to regress.

The massive degree of meningeal involvement is another characteristic of this disease syndrome. We have not determined whether the meningeal tumor arises from the meninges or by extension of the tumor from the bone marrow of the cranial flat bones. The early tumorous changes in the bone marrow would favor the latter. If the tumor is primary in the meninges, this could possibly serve as an excellent model for testing chemotherapeutic agents against meningeal lymphocytic leukemia.

There is no diffuse lymphocytic invasion of organs. The tumor does have a predilection for invading muscle, but this is by direct extension of the tumor from adjacent lymph nodes. The peripheral blood is also free of tumor cells. The predominant white blood cell type in the peripheral blood is a mature polymorphonuclear leukocyte. Leukocytosis is present in more than one-half of the mice with tumors. Since nests of mature polymorphonuclear leukocytes are also found in the bone marrow and in the medullary cords of the lymph nodes, the lymphosarcoma virus appears to induce a polymorphonuclear leukemoid reaction.

Assays of tumor material versus plasma for the lymphosarcoma virus showed that the tumor extracts had about 2.4 logs more activity than did plasma. This is consistent with a solid tumor system, rather than with leukemia (4).

In the higher dilutions of lymphosarcoma virus, a large proportion of the mice develop lymphocytic leukemia after a latent period compatible with MLV inoculation (11). Electron microscopy confirms the presence of many classical budding and free C-type particles associated with the tumor cells. These particles are indistinguishable from all other murine type C particles.

In secondary BALB/c and NIH Swiss mouse embryo tissue culture systems, 4.75 logs of complement-fixing activity have been obtained from an assay of tumor material, and 2.50 logs from an assay of plasma for virus producing the group-reactive murine leukemia antigen. When these assayed cells are sonically disrupted and injected back into newborn mice, the mice develop typical murine lymphocytic leukemia. There was no in vitro transformation of either the secondary BALB/c or NIH Swiss mouse embryo cell lines by the lymphosarcoma virus. (H. T. Abelson, L. S. Rabstein, R. L. Peters, and G. J. Spahn, unpublished data).

The preceding points suggest that MLV is present along with the lymphosarcoma virus in all inoculum. We have not determined what part, if any, the MLV plays in the development of lymphosarcomas. Since MLV is present along with the lymphosarcoma virus, it is also difficult to interpret the positive complement-fixation results for virus producing the group-reactive murine leukemia antigen.

There is no similarity between this disease syndrome and that produced by the Moloney sarcoma virus (12).

The clinical and pathological features of this new disease syndrome are so distinct from that produced by MLV that we consider this lymphosarcoma virus not as a variant of the MLV, but as a new and distinct entity. Its unique features should serve as a multipotential focus for further investigation.

ACKNOWLEDGMENTS

We thank Dr. Albert J. Dalton, Dr. John B. Moloney, and Dr. W. Ray Bryan for advice and encouragement during these studies.

REFERENCES

Virus-induced Thymic-independent Disease in Mice


Fig. 1. Tumor formation s.c. (arrows) in a 29-day-old BALB/c mouse.
Fig. 2. Multiple sites of paravertebral tumor (arrows) in a 34-day-old BALB/c mouse. The kidneys and adrenals were not affected.
Fig. 3. Retroperitoneal tumor illustrating the numerous debris-filled macrophages interspersed among very immature lymphoid cells. X 500.
Fig. 4. A tumorous inguinal lymph node has invaded the muscles of the thigh, destroying and replacing the muscle with proliferating tumor. X 125.
Fig. 5. Matched 55-day-old BALB/c mice. The top mouse, inoculated with the lymphosarcoma virus, has bulging and displacement of the cranial bones by meningeal tumor (arrow). The lower mouse, inoculated with MLV, had no meningeal involvement.
Fig. 6. The same 2 mice as in Fig. 5 with the skin removed from the heads to show the swelling and hemorrhage (arrow).
Fig. 7. Sagittal section through the skull from a mouse with a typical meningeal tumor. The tumor (dark area) surrounds the brain and is found through all layers of the meninges. X ~4.
Fig. 8. Higher power of the meningeal tumor. The parietal bone marrow (double arrow) is replaced with tumor. The brain (single arrow) is never invaded by the tumor. The meningeal tumor is indistinguishable from the tumors arising in lymph nodes. X 250.
Fig. 9. Extracapsular proliferation of the lymphosarcoma from a tumorous thoracic lymph node (double arrow) has encompassed the thymus (single arrow). The thymus remains uninvolved with tumor, although its capsule may be invaded. X 75.
Fig. 10. A single medullary cord from a tumorous lymph node is shown with its normal lymphoid cells almost completely replaced with polymorphonuclear leukocytes. X 500.
Fig. 11. Touch preparation of a typical lymphosarcoma stained with Giemsa, showing the predominant cell type to be an immature lymphoid cell. X 1250.
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