LAMA Tumor in the Rat as an Experimental Model for Pre-B-Cell Leukemia

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ABSTRACT

A late pre-B-cell leukemia model in the rat, the LAMA tumor, is described. A mouse monoclonal antibody (HIS30) was developed against LAMA cells. HIS30 reacts with a membrane antigen in tumor tissue, whereas its reactivity with normal tissues is limited to the zona glomerulosa of the adrenal cortex and to the adrenal medulla. HIS30 was used for both the immunohistological detection of tumor cells in tissue sections and the immunolocalization of tumor cells in vivo.

To enable in vitro studies with the LAMA model, an in vitro growing cell line (LAMA-K1) was established from the LAMA tumor. LAMA-K1 is immunophenotypically similar to the original tumor.

Two tumor transplantation models were characterized. In the first model LAMA was implanted s.c., and local tumor growth occurred at the injection site, which was then followed by lymphatogenic and subsequent hematogenic tumor dissemination. In both models early dissemination was especially prominent to the bone marrow, spleen, and liver. Later in the disease most visceral organs became involved, and partial paralysis of the animal was observed in the end stage of the disease.

In combination with HIS30, the LAMA pre-B-cell tumor offers a model for both the investigation of in vivo transplanted tumor cells and disease.

INTRODUCTION

ALL2 is the most common malignant disease in childhood, which, using immunological criteria, can be further subdivided in several subtypes. Despite major refinements in therapeutical modalities, some subtypes of ALL still have a bad prognosis (1–3). For instance, pre-B ALL has a shorter remission duration after chemotherapy than standard (common) ALL (4, 5). New methods of treatment are still needed, and immunotherapy might provide such a new treatment modality.

To design and study new therapeutical procedures, a well-defined tumor model in an immunocompetent animal might be very useful. We selected a rat model for such a purpose, because the rat is a very accessible experimental animal, and a lot is known about the immune system of the rat. However, in the rat only a few B-cell leukemias have been described (e.g., Ref. 6), whereas, except for the LAMA tumor, no pre-B-cell leukemias are available.

The LAMA pre-B-cell leukemia has arisen spontaneously in 1978 in a male AO × BN F1 rat (7, 8). The LAMA tumor was detected in this animal as a malignant outgrowth of the spleen. Histological examination revealed lymphoid tumor cells. In honor of the students who made this observation (Laura Faber and Magda van Oven), the tumor was called LAMA. Since then the tumor has been serially transplanted in syngenic AO × BN F1 rats by s.c. injection of tumor cells isolated from the blood taken from end-stage animals. Alternatively the tumor was regrown in AO × BN F1 rats after cryopreservation. In this paper a further characterization of the LAMA tumor is given. It is indicated that the LAMA tumor, together with the (almost completely) LAMA-specific Moab HIS30 and an in vitro established LAMA-K1 cell line, offers a suitable model for pre-B ALL in the rat.

MATERIALS AND METHODS

Rats. Male AO/G × BN/G F1, hybrid (RT1+/+) rats at the age of 3 to 5 mo were used for all experiments. The animals, caged in groups of two or three, were fed and given water ad libitum.

Preparation of LAMA Cells. The LAMA tumor was routinely transplanted from s.c. growing tumors. Cells used for tumor transfer were prepared by mincing an extirpated tumor. Subsequently the thus obtained cell suspension was purified from dead cells and erythrocytes by Percoll density gradient centrifugation (Pharmacia, Uppsala, Sweden), at 600 × g for 30 min, using a gradient of 35 to 75% Percoll. Viable cells were collected from the gradient at a density of d = 1.070 to 1.085 g/liter, pelleted, and washed with PBS containing 2.5% newborn calf serum and 0.5% Dulbecco’s B (Oxoid, England). The percentage of viable tumor cells was determined by the trypan blue exclusion test and was always more than 85%. Tumors were transferred by injecting 1 × 104 viable cells s.c. in the left flank of an animal. After about 6 wk a s.c. growing tumor with a diameter of approximately 5 to 7 cm had developed. The animal was sacrificed then, and tumor cells were isolated and used for further transplantation or experiments.

Isolation of MoAb HIS30. Female BALB/c mice were immunized by i.p. injection of 1 × 107 LAMA cells suspended in complete Freund’s adjuvant. A second i.p. injection was given with 1 × 107 LAMA cells in incomplete Freund’s adjuvant 1 mo later. After an additional month an i.v. booster with 1 × 107 LAMA cells in PBS was given. Subsequently after 3 additional days the spleen was removed, and isolated spleen cells were fused with X63/Ag8 myeloma cells as described before (9). Reactivity of hybridoma culture supernatants for LAMA tumor cells was screened on frozen tissue sections of s.c. grown LAMA tumor by an indirect immunoperoxidase staining technique (10). A monoclonal antibody with high specificity for the LAMA tumor was selected and subcloned. This mouse antibody, called HIS30 (isotype IgG2a) was further assayed for reactivity toward a large panel of normal tissues and PBMC (as indicated in the text) by indirect immunoperoxidase staining on acetone-fixed preparations.

Rat Tumors. The Roser tumor (11) was obtained from the late Dr. W. L. Ford, Department of Pathology, Manchester, United Kingdom. The Y3 tumor (myeloma) was a kind gift from Dr. H. Bazin, University of Louvain, Brussels, Belgium. The Jobo tumor (fibrosarcoma) was isolated in the Department of Histology and Cell Biology.

Detection of LAMA Cells in Cytological and Histological Preparations. Heparinized blood was collected by cardiac puncture, and the PBMC fraction (containing LAMA cells if present in the blood) was isolated by Ficoll-Isoaque density centrifugation (d = 1.079 g/liter). BMC, obtained by flushing the left upper femur, were layered on newborn calf serum at 4°C for 5 min, to remove bone fragments, after which the BMC were collected by centrifugation through filtered newborn calf serum at 200 × g for 10 min. For cytological examination of
PBMC and BMC, cytospin preparations were made on glass slides. These were air dried and subsequently fixed with acetone/0.3% H2O2 at room temperature for 10 min. LAMA cells were specifically detected using Moab HIS30 (see above) and HRPO-conjugated rabbit antibodies directed against mouse immunoglobulin (P260: Dakopatts, Glostrup, Denmark; diluted 1:40 in PBS containing 1% normal rat serum) as a second step reagent. Peroxidase activity was demonstrated by using 0.5 mg of diaminobenzidine (Sigma Chemical Co., St. Louis, MO) per ml of 0.05 M Tris-HCl, pH 7.6, containing 0.01% H2O2 as a substrate. In addition one cytospin preparation was routinely stained according to MGG to allow optimal morphological evaluation of the cells.

For histological and immunohistological detection of disseminated LAMA cells, several lymphoid and nonlymphoid tissues (as indicated in the text) were used for immunohistology. Immunostaining was done as given above for the cytological preparations. For conventional histological assessment, frozen tissue sections of lymphoid tissues were formaldehyde fixed in Baker’s solution and stained according to Brachet (12), whereas sections prepared from nonlymphoid tissues were histochemically stained with Mayer’s hematoxylin-eosin.

Establishment of a LAMA Tumor Cell-derived, In Vitro Growing Cell Line, LAMA-K1. The LAMA-K1 cell line was established from tumor derived cells from a s.c. grown LAMA tumor. For this purpose LAMA cells were put in RPMI-1640 medium, supplemented with 10% fetal calf serum and antibiotics, on a feeder layer consisting of rat fibroblasts, which had been cultured from tumor stromal tissue. Initially the LAMA cells only grew attached to the fibroblasts. Subsequently, cells were adapted to feeder layer-free culture conditions by selectively culturing those LAMA cells which grew in suspension. The thus established LAMA cell line was designated LAMA-K1 and has been in culture for more than 6 mo (67 passages).

Phenotyping of LAMA and LAMA-K1. The HIS Moabs used for immunophenotyping (HIS8, HIS15, HIS19, HIS22, HIS24, HIS25, HIS30, and HIS40) were developed in our own laboratory (see Table 3 for specificity of the Moabs listed). The MRC OX-8, OX-18, and OX-19 were kindly provided by Dr. A. F. Williams, Sir William Dunn School of Pathology, Oxford, United Kingdom; ER-2 and ER-4 were a generous gift of Dr. J. Roosing, Institute for Experimental Gerontology, Division for Health Research TNO, Rijswijk, The Netherlands; and ED2 was obtained from Dr. C. D. Dijkstra, Department of Cell Biology, Free University, Amsterdam, The Netherlands. Viable LAMA cells were incubated with Moabs (0°C, 45 min), followed by washing with PBS (3 times). Cell-bound Moabs were subsequently detected by indirect immunofluorescence with FITC-conjugated rabbit antibodies directed against mouse immunoglobulin (F261; Dakopatts, Glostrup, Denmark; diluted 1:40 in PBS), and after washing (3 times) with PBS, the cells were analyzed both by fluorescence microscopy (Orthoplan; Leitz Wetzlar, West Germany) and quantitative flow cytometry (FACS 440; Becton and Dickinson, Mountain View, California). In the flow cytometry experiments viable cells were selected by propidium iodide exclusion. FITC-derived fluorescence was measured on an arbitrary scale and expressed as MFU. Control incubations were performed by applying second step FITC-conjugated antibodies only. The obtained (control) MFU values were subtracted from the results obtained with the Moab-treated samples.

Detection of HIS30 IgG Injected i.p. into LAMA Tumor-bearing Rats. Purified LAMA tumor cells (1 × 10⁶) were injected i.v. in AO X BN F₁ rats. Thirteen days later, 2 mg (4 mg/ml in PBS) of HIS30 IgG (purified from mouse ascites fluid by Protein A-Sepharose column chromatography) were injected i.p., and rats were sacrificed 1, 2, 4, 8, or 16 h later. Control tumor-bearing animals were sacrificed without receiving HIS30. The animals were screened for HIS30 which could be present both free in the blood and (LAMA) cell attached. To detect free HIS30 in the blood, sera were titrated on frozen tissue sections of (control) s.c. grown LAMA tumor. HIS30 was subsequently visualized in the sections by immunoperoxidase staining using HRPO-conjugated rabbit anti-mouse immunoglobulin. Peroxidase activity was visually scored, and the last serum dilution providing a positive reaction was determined. Using this method about 0.1 μg of HIS30/ml of serum was just detectable. LAMA cells present in blood or bone marrow cell suspensions and in tissue sections (liver, spleen, and kidney) were detected by immunostaining with HIS30 as described above. The presence of in vivo LAMA cell-associated HIS30 was assessed by incubation of subsequent tissue sections with peroxidase-conjugated rabbit antirat immunoglobulin only. Further incubations were as given for the indirect immunoperoxidase staining procedure.

RESULTS

Production of LAMA-specific Moab HIS30

A LAMA tumor cell-associated monoclonal antibody, HIS30 (IgG2a), was produced. HIS30 showed strong reactivity with all cells of the LAMA tumor (Fig. 1a) and no reactivity with virtually all normal AO X BN F₁ rat tissues; i.e., no reactivity was found in cryostat sections taken from a variety of both lymphoid (spleen, Peyer’s patches, thymus, and several lymph nodes) and nonlymphoid (liver, kidney, lung, heart, skin, striated muscle, thyroid, tongue, brain tissue, salivary gland, bladder, pancreas, esophagus, testicle, epididymus, and prostate gland) organs. HIS30 was also unreactive with peripheral blood lymphocytes and bone marrow cells. Reactivity of HIS30, however, was seen in the adrenal glands, i.e., in the zona glomerulosa and medulla (Fig. 1b). With regard to other assessed rat tumors, a partly positive staining (5 to 15%) was observed with rat leukemia cells called Roser (mixed lymphoid lineage) (13), whereas Y3 (rat myeloma) and Jobo (rat fibrosarcoma) were...
unreactive. HIS30 detects a membrane antigen as was shown by an indirect immunofluorescence staining technique using viable LAMA cells. HIS30 reacted strongly with LAMA in acetone-fixed cell preparations as well as in frozen tissue sections. A weak (but clear) reactivity was seen in formol-fixed, paraffin-embedded tissues.

LAMA Tumor Growth and Dissemination Pattern

LAMA Tumor Cell Spread after i.v. Inoculation. The LAMA tumor can be kept growing by serial transplantation, or, alternatively, cryopreserved. Fig. 2 shows the survival time of rats after i.v. injection of graded numbers of purified LAMA cells. Apparently the LAMA tumor grows aggressively with a mean tumor load doubling time of about 24 h as estimated on the basis of the data given in Fig. 2.

LAMA tumor spread was investigated after i.v. injection of $1 \times 10^6$ LAMA cells. Generally, three phases in the disease can be distinguished. In the first 9 days LAMA cells, single or present in small (2 to 10 cells) foci, were detectable immunohistologically in BM, spleen, liver, and lung. In the second phase, from about 10 to 15 days, tumor cells were also detectable in the blood (5% of PBMC) and kidney. In this phase a very high amount of LAMA cells was present in the bone marrow (90% of all bone marrow cells). In the last phase of the disease (15 to 19 days) the tumor load of particularly the spleen and liver increased. Also the blood became leukemic in this phase, since the total number of blood leukocytes increased to approximately 2 to 4 x $10^7$ cells/ml of blood. In addition some lymph nodes became involved, i.e., cervical, lower cervical, lumbar, and periaortic lymph nodes. The thymus was only occasionally involved. No LAMA cells were detected in mesenteric, caudal, and inguinal lymph nodes, nor in Peyer’s patches. In this terminal phase of the disease, the animals showed significant loss of weight (weight reductions of 10 to 20% of total body weight), and an involvement of the CNS was suggested by the observation that a paralysis of both hind limbs of the animals occurred in almost all cases. On sections made from a biopsy containing the spinal column of a paralyzed animal, an extradural outgrowth of LAMA tumor cells was found (Fig. 3). This growth appeared to proceed from vertebral bodies, which were destroyed by the tumor already. No intradural or intraspinal localization of tumor cells was observed, and brain biopsies taken from the same animal revealed no tumor localization. Thus there is no proof of tumor infiltration into the CNS.

Fig. 2. Survival time of male AO x BN F, rats after i.v. injection of LAMA cells. Line represents best-fitting curve drawn through mean values of the results of two separate experiments, each comprising a group of five animals (■ and ▼).

Dissemination of LAMA after s.c. Inoculation. To investigate the dissemination process after s.c. inoculation, $1 \times 10^6$ viable LAMA cells were injected in the left flank. As a result of this, the animals died about 49 days later. At different time intervals after injection, rats were sacrificed, and several tissues were investigated for the presence of LAMA cells. Starting from Day 28, when the s.c. growing tumor had reached a diameter of about 2 to 4 cm, the inguinal lymph node at the left side was found to be infiltrated with tumor cells. This was the first sign of metastasis. At Day 32 not only the left inguinal lymph node was greatly enlarged, but also the lumbar, periaortic, and axillary lymph nodes located at the left side of the animal were filled with tumor. The further dissemination was very similar to that observed after i.v. injection (see Table 1).

Around the time the LAMA tumor cells were detectable in the blood and BM (at Day 28), they were also found in several other tissues, e.g., spleen, liver, and lung (Table 2). Other tissues (like kidney, thymus, and thyroid) were not involved yet. In the spleen, initially only small scattered tumor foci were observed in the red pulp and to a lesser extent in the PALS. By Day 35 the metastatic foci in the red pulp increased in both size and number (Fig. 4). The white pulp remained tumor free until the terminal phase of the disease (after Day 42). Then the white pulp became involved too, and LAMA cells were detectable also in T-cell areas. At Day 45 normal spleen tissue was hardly detectable. In the liver, tumor infiltration started also at Day

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Table 1 Presence of LAMA tumor cells in the blood and bone marrow at different time points after s.c. injection of $1 \times 10^6$ cells in the left flank of AO x BN F, rats

<table>
<thead>
<tr>
<th>Time after injection (days)</th>
<th>Bone marrow* (%)</th>
<th>Blood* (%)</th>
</tr>
</thead>
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<tr>
<td>0</td>
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<td>0</td>
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<tr>
<td>7</td>
<td>0</td>
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</tr>
<tr>
<td>14</td>
<td>0</td>
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<tr>
<td>21</td>
<td>0</td>
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</tr>
<tr>
<td>28</td>
<td>&lt;0.1</td>
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<tr>
<td>32</td>
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<td>35</td>
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<tr>
<td>42</td>
<td>22</td>
<td>0.2</td>
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<tr>
<td>45</td>
<td>66</td>
<td>0.3</td>
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<tr>
<td>49</td>
<td>88</td>
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</tbody>
</table>

* LAMA cells were visualized immunocytologically staining with HIS30 and cytologically (staining with MGG). All morphologically recognizable tumor cells were HIS30 positive. The results are expressed as a percentage of the total number of nucleated cells.
Table 2  Detection of LAMA tumor cells in the spleen, liver, lung, and kidney at different time points after s.c. injection of $1 \times 10^6$ cells in the left flank of AO $\times$ BN F, rats

<table>
<thead>
<tr>
<th>Time after injection (days)</th>
<th>Spleen*</th>
<th>Liver</th>
<th>Lung</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
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<td>32</td>
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<td>35</td>
<td>+++</td>
<td>+++</td>
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<td>38</td>
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<td>+++</td>
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</table>

* Presence of tumor cells (using HIS30 as a marker) is scored as – (0%), + (0 to 5%), ++ (5 to 25%), +++ (25 to 50%), and ++++ (>50%) of the total nucleated cells present in a tissue section.

Fig. 4. Scattered metastases of LAMA tumor cells in the spleen 35 days after s.c. injection of $1 \times 10^6$ LAMA cells detected by HIS30 and immunoperoxidase staining. MZ, marginal zone; LC, lymphocytic corona; GC, germinal center; PALS, periarteriolar lymphocyte sheath; RP, red pulp. Most LAMA cells were present in the red pulp and T-cell areas (PALS), while the B-cell area (MZ, LC, and GC) remained relatively tumor free at this point of time. Arrow(s), LAMA cells in PALS. X 42.

BM, spleen, liver, and blood (see Tables 1 and 2). One h after i.p. injection, a small amount of HIS30 (approximately 1 µg/ml) was detectable in the serum, indicating that the antibody had reached the blood circulation. At all later time points, (free) HIS30 was present in high concentrations in serum (>30 µg/ml). HIS30 was clearly detectable on LAMA cells present in the bone marrow, spleen, and liver from 2 h after injection onward. A maximum of LAMA cell-bound HIS30 was found at 8 h after injection. Binding of HIS30 in vivo to LAMA cells present in the spleen and the liver turned out to be heterogeneous. Since all LAMA cells expressed the same amounts of HIS30 antigen, this indicated that not all areas of the tumor are equally well accessible for HIS30 (compare a and b of Fig. 5). Injected HIS30 also bound to adrenal gland tissue in a way similar to its reaction in vitro (not shown; compare Fig. 1b).

Phenotyping of an in Vitro Growing Cell Line: LAMA-K1

To enable in vitro studies using the LAMA model, a cell line was established from a s.c. growing LAMA tumor (see “Materials and Methods”). This cell line, designated LAMA-K1, showed a cell-doubling time of about 15 h. LAMA-K1 cells grown in vitro were highly tumorigenic when injected s.c. into AO $\times$ BN F, rats. The survival time of rats was significantly prolonged compared to those injected with LAMA tumor cells. In the terminal phase of the disease after s.c. injection of LAMA, frequently paralysis of the hind limbs was observed as was also seen after i.v. injection. Occasionally the forelegs were also involved.

Detection of HIS30 IgG Injected i.p. into LAMA Tumor-bearing Rats

To investigate the usefulness of HIS30 for tumor imaging and targeting in the LAMA model, HIS30 was injected i.p. in LAMA-bearing rats (13 days after i.v. injection of $1 \times 10^6$ LAMA cells). At that time LAMA cells are present in, e.g.,
shorter when the animals were given s.c. injections of LAMA-K1 as compared with LAMA (5 to 6 wk as compared to 7 wk, after s.c. injection of $1 \times 10^6$ cells). At obduction of the animals, the liver and the spleen showed an increase in weight, whereas no dissemination to the lymph nodes was detectable. The tumor morphology of LAMA-K1 was similar to that of LAMA. The cell morphology of LAMA cells and LAMA-K1 cells is shown in Fig. 6, a and b. LAMA appeared to be more heterogeneous in size, while LAMA-K1 cells were larger.

The (immuno)phenotype of LAMA and LAMA-K1 was assessed by flow cytometry analysis using a panel of Moabs (Table 3). Both cell types were reactive with HIS30, although LAMA bound 2 to 3 times more antibody than LAMA-K1 (Table 3). Apart from some minor differences, all assessed pre-B- and B-cell differentiation markers were similarly expressed in LAMA-K1 and LAMA cells, i.e., the markers detected by Moabs HIS14, HIS24, and HIS25. Also the (virtual) absence of binding of HIS22 was noted on both cell preparations. Thy-1, an antigen present in the rat on both stem cells and cells in the early phases of B-lineage, was strongly expressed by LAMA-K1 as well as LAMA cells. Flow cytometry analysis showed a heterogeneous staining pattern for HIS24 and HIS19 on LAMA (see Table 3). LAMA-K1 cells all were HIS24 and HIS19 positive. MHC Class I and II antigens were also present on both cell types. LAMA-K1, however, showed almost 50% more MHC Class I expression than LAMA cells. Antibodies specifically detecting macrophage-, natural killer-, or T-cell-associated antigens were essentially unreactive with both LAMA and LAMA-K1 cells, although a very weak reaction was noticed with OX-19.

With regard to immunoglobulin expression, LAMA-K1 was almost identical to LAMA: both cell types expressed $\mu$ (heavy) and $\kappa$ (light) chains in the cytoplasm and $\mu$ (heavy) chains only occasionally and to a variable extent on the cell surface (Table 3). LAMA-K1 showed a weak homogeneous expression of $\kappa$ (light) chains on the cell surface, while LAMA showed a variable expression. Surface $\delta$ or $\gamma$ chains were absent.

**DISCUSSION**

This paper describes a rat late-pre-B-cell tumor (LAMA) which can be used as an experimental model for pre-B ALL. A monoclonal antibody, HIS30, was developed and used for the investigation of LAMA tumor dissemination. This antibody may also be useful for future immunotherapeutical experiments. HIS30 reacts strongly with LAMA tumor cells (Fig. 1a; Table 3) and shows no reactivity with normal rat tissue except for a partial staining in adrenal gland tissue (Fig. 1b). HIS30 also reacts with a small percentage of the cells of the Roser rat leukemia. The origin of this last leukemia is unclear, since both T- and B-cell markers are expressed (13). The molecular nature of the antigen recognized by HIS30 is not determined yet, but its occurrence on restricted areas in the adrenal glands, on the one hand, and on LAMA and (a small percentage of) Roser leukemia, on the other, might be interesting and could relate to hormone-controlled functions, i.e., those involved in differentiation, proliferation, or homing of (pre-) B-cells.

The LAMA tumor cell dissemination was studied after both i.v. and s.c. injection. When LAMA cells were injected i.v., the disease had a very aggressive course (Fig. 2). BM, spleen, liver, and lungs became invaded quickly by LAMA cells, and the rats died within 3 wk.

When LAMA cells were injected s.c., it was shown that tumor growth first remained localized to the injection site, whereas subsequent dissemination occurred through the lymphatic system. Once disseminated into the draining lymph nodes, the further dissemination pattern is very similar to the tumor spread after i.v. injection. It appeared that LAMA cells preferentially homed in BM, a property compatible with the pre-B-cell nature of LAMA, and subsequently localize to, e.g., the spleen and liver by hematogenetic spread.

In the terminal phase of the disease, a paralysis of both hind limbs of the animals was almost always observed, which could indicate an apparent involvement of the CNS. However, microscopic evaluation of sections made from the CNS showed no LAMA cells, whereas a biopsy containing the spinal column showed only local extradural outgrowth of LAMA tumor cells (see Fig. 3). The extradural growth of tumor cells probably resulted in compression of the nerve roots, leading to paralysis. No signs of leptomeningeal or other CNS involvement was found in histological sections. In human ALL, leptomeningeal involvement is a frequently encountered complication, whereas leptomeningeal localizations are rare, and more often seen in lymphoma. Whether this difference in CNS involvement between human leukemia and the rat-derived LAMA is related to a difference in the degree of B-cell differentiation or not is a matter of speculation.

HIS30 can be used for in vivo experiments like imaging and immunotargeting, since (i.p. injected) HIS30 was shown to reach all LAMA cell-containing organs (see Fig. 5a). However, in vivo injected HIS30 was bound inhomogeneously, since not all possible binding sites, especially those present in the center of some tumor nodules, were found to be equally occupied. Apparently this is caused by a lack of penetration of the anti-
body, since HIS30 is present in excess in the blood stream. These results are compatible with observations made by others, who proposed that a heterogenous blood perfusion, a hindered diffusion, vascular permeability, and an elevated interstitial pressure account for a reduced uptake of MoAbs into tumors (14, 15). These findings illustrate the usefulness of an animal tumor model for the investigation of new (and existing) treatment modalities in which the treatment is dependent on delivery of the antitumor effect via the bloodstream.

During the establishment of the LAMA-K1 cell line, it was interesting to note that in vitro growth of LAMA cells was observed at first only when the cells were explanted on a feeder layer of (tumor-stromal tissue-derived) fibroblasts. Fibroblasts obtained from other sources were not suitable for such a purpose. This initial dependence on specific fibroblasts feeder layers is in agreement with the finding of others who studied mouse B-cell lymphopoiensis in vitro (16, 17). In these studies (bone marrow derived), stromal cells were used as a feeder layer for normal mouse-derived B-cells, and it was concluded that stromal cells can both provide a supportive framework and release soluble growth factors that are necessary for the in vitro growth and/or differentiation of precursor B-cells (16, 17). Apparently, in the early stages of adaptation to in vitro culture, LAMA cells needed specific cell-bound and/or soluble factors to sustain their in vitro growth. This dependency on additional factors might also be present in vivo and could be reflected in the disseminating behavior of LAMA cells. For instance, this might explain the preferential growth of LAMA in the BM.

The phenotype of the LAMA-K1 cell line was compatible with a cell in a late-pre-B-cell differentiation state (18) and related well to in vitro grown LAMA. As is shown (Table 3), all B- and pre-B-cell-specific antigens were less expressed on LAMA-K1 cells as compared to LAMA cells. The Thy-1 antigen is abundantly present on both LAMA and LAMA-K1 cells. In normal rat B-cell lymphopoiensis, this marker is strongly expressed on pre-B-cells and will diminish on surface immunoglobulin-positive cells.

Although most antigens are expressed at a lower level in LAMA-K1 as compared to LAMA, the opposite was noted for MHC Class I antigens (see Table 3). It has been suggested that increased MHC Class I expression relates to less sensitivity for lysis by natural killer cells (19, 20). Therefore it is interesting to note that LAMA-K1 cells were found to be more tumorigenic than LAMA cells, as deduced from the fact that the LAMA-K1-injected rats died earlier. A diminished sensitivity to natural killer cell lysis might have played a role in LAMA-K1 tumor metastasis and, therefore, in the survival time of rats receiving injections, although this point has to be investigated further yet. Despite the above indicated minor differences in phenotype, it seems that the LAMA-K1 cell line is a suitable in vitro counterpart of in vivo growing LAMA, which can be used for the determination of the sensitivity of LAMA-K1 cells for a number of (immuno)cytotoxic agents in vitro and for the study of the underlying (immuno)cytotoxic mechanisms.

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LAMA Tumor in the Rat as an Experimental Model for Pre-B-Cell Leukemia


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