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Lymphatic Leukemia and Retrieval of Defective Friend Virus in Athymic Nude Rats[1] (41350)

A. HOWARD FIELDSTEEL,* M. JOSEPH COLSTON,* AND PETER J. DAWSON†

Life Sciences Division, SRI International, Menlo Park, California 94025, and †Laboratory of Surgical Pathology, University of Chicago, Chicago, Illinois 60637

Abstract. We have investigated the ability of Friend virus (FV) and the associated lymphatic leukemia virus (LLV) to replicate in athymic rats. We have also explored the ability of the LLV to act as helper in the retrieval of the defective FV genome from a virus-free, FV-induced tumor of BALB/c mice xenografted to this host. Athymic rats were found to be resistant to Friend disease. However, they did develop lymphatic leukemia when inoculated with FV. Following inoculation of LLV into newborn athymic rats, viremia was detected as early as 20 days and was followed by death from lymphatic leukemia approximately 100 days later. Athymic rats inoculated with LLV when newborn were later xenografted with cells from the virus-free, FV-induced tumor grown *in vitro*. Cell-free extracts of the tumors that developed induced typical Friend disease in newborn mice. Because the rat apparently lacks target cells for FV replication, the entire output of virus must have originated in the tumor itself, a situation in marked contrast to earlier experiments in BALB/c mice.

The role of the thymus in virus-induced leukemia appears to differ in the various model systems studied. For example, both Gross virus and Moloney virus will replicate in the absence of the thymus, but the thymus itself is necessary for the induction of leukemia (1, 2). Friend virus (FV) is not thymus dependent. FV actually consists of two components (3, 4): lymphatic leukemia virus (LLV), which is replication competent and induces lymphatic leukemia in both rats and mice; and one that is replication defective which, with an associated LLV, acting as a helper, produces erythroid leukemia in many strains of mice. The athymic nude rat appeared to be an interesting tool for exploring the biologic relationship between these two viruses because it is known that this animal will accept xenografts of mouse tumors (5) and conceivably would accept grafts of the FVTCT (6). The latter is an FV-induced tumor of BALB/c mice that is free from infectious virus, but that contains the FV genome, which can be rescued with certain LLVs (7). To our knowledge, neither FV nor LLV has been studied in athymic rats, but it might be predicted that they—like euthymic rats—would be resistant to erythroid disease caused by FV. However, their response to LLV could not be anticipated. We report here the induction of lymphatic leukemia, the transplantation of FVTCT, and the retrieval of the Friend virus genome from the latter in athymic nude rats.

Materials and Methods. *Animals.* Athymic and euthymic rats came from our colony, which was established from a small breeding nucleus of rats heterozygous for the *rnu* gene, obtained from the Laboratory Animal Centre, Carshalton, England. The breeding system employed was that commonly used for the breeding of nude mice, i.e., the homozygous recessive male is crossed with the heterozygous female. Athymic rats could be distinguished at birth by their lack of vibrissae; euthymic rats were heterozygous litter mates.

BALB/c mice were also obtained from our colony.

Viruses. Extracts of Friend virus were prepared from infected spleens and LLV was prepared from spleen, thymus, and lymph nodes of leukemic mice by methods previously described by us (3, 8).

Tumor cell culture and inoculation. FVTCT was grown in Eagle's minimal essential medium (MEM) containing 10% fetal bovine serum. For inoculation into

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athymic rats, FVTCT cells were counted and the percentage viable was determined by the trypan blue dye exclusion test. The cells were then centrifuged and resuspended in MEM to give a cell concentration of 5×10^7 viable per milliliter. Rats were inoculated between the scapulae with 0.2 ml of this suspension.

Preparation of tumor cell extracts. Tumors were excised from athymic rats and a 10% homogenate was made using a Virtis homogenizer. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was quick-frozen in a dry ice–alcohol bath and stored at −70°. Before inoculation into newborn BALB/c mice for recovery of retrieved FV, it was quick-thawed in a 37° water bath.

Results. To determine whether LLV could replicate in athymic rats, newborn athymic and euthymic rats were inoculated ip with LLV. All six of the athymic rats died of lymphatic leukemia 90 to 151 days (mean = 127.7 ± 20.8) after inoculation. Eight of the nine euthymic rats died of lymphatic leukemia after 92 to 103 days (mean = 98 ± 6). Inoculation of FV into newborn athymic rats resulted in the development of lymphatic leukemia in two of four recipients at 98 and 135 days; the other two were aleukemic when the experiment was terminated at 135 days. All seven young adult BALB/c mice inoculated with the same preparation of FV developed histologically confirmed Friend disease, with the characteristic short latent period and massively enlarged spleens.

Of special interest was the question of whether FVTCT would continuously support the replication of FV in a resistant host such as the athymic rat. All of our earlier retrieval experiments had been carried out in BALB/c mice, which are highly susceptible to Friend disease. Thus, it would be necessary for only minute quantities of FV to be produced by the FVTCT in order for further replication to occur outside of the tumor in the target cells of the BALB/c mice. If the athymic rat accepted grafts of the FVTCT, it would be worthwhile to attempt retrieval of the FV genome from the virus-free FVTCT in an animal in which Friend disease does not develop.

In a preliminary experiment, 10^7 FVTCT cells were inoculated sc into five athymic rats. All developed palpable tumors in 7 days. In two of the rats, the tumors regressed after 21 days. Tumors in the remaining rats grew progressively, and these animals were moribund by the 84th day. Necropsy showed peritoneal metastases in one animal.

Two retrieval experiments were carried out (Table I). In the first experiment, four athymic rats were inoculated with LLV as newborns. Twenty days later the rats were bled and the pooled whole blood was inoculated into four newborn euthymic rats, all of which subsequently developed lymphatic leukemia. The day after being bled, the athymic rats were inoculated sc with 10^7 FVTCT cells. Three weeks later the rats were sacrificed. Weight of the tumors ranged from 30 to 38 g. Grossly and histologically, none of the organs showed evidence of leukemia or metastases. Equal portions of the tumors were pooled and a 10% cell-free extract was made. This extract was inoculated into nine newborn BALB/c mice, all of which subsequently developed Friend disease, which was confirmed histologically.

In the second experiment, a single athymic rat inoculated 112 days earlier (when newborn) with LLV was also inoculated sc with 10^7 FVTCT. Nine days later the animal was found to be moribund and to have a subcutaneous tumor approximately 1 cm in diameter. It was sacrificed, and a 10% cell-free suspension of its tumor was inoculated into seven newborn mice, all of which developed histologically confirmed Friend disease.

None of nine mice inoculated with a 10% cell-free extract of the FVTCT grown in athymic rats, which had not previously been inoculated with LLV, developed Friend disease.

Discussion. The results of this study raise several interesting questions. The first of these is the nature of lymphatic leukemia in the athymic nude rat. In extensive earlier

TABLE I. *In Vivo* Retrieval of Infectious FV from a Noninfectious FV-Induced Mouse Tumor Xenotransplanted to rnu/rnu Rats Using LLV as Helper

Retrieval procedure	Results in newborn BALB/c mice	
	Day of sacrifice	No. with FD[a] total inoculated
Four rnu/rnu rats inoculated when newborn with LLV and given 10^7 FVTCT cells 21 days later. Tumors harvested 21 days later, and cell-free extracts inoculated into newborn mice	35	9/9
One rnu/rnu rat inoculated when newborn with LLV followed by 10^7 FVTCT cells 112 days later. Tumor harvested 9 days later, and cell-free extracts inoculated into newborn mice	40	7/7
Rnu/rnu rats inoculated with FVTCT cells only. Tumors harvested 20 days later, and cell-free extracts inoculated into newborn mice	232	0/9

[a] Friend disease, confirmed histologically.

studies (9), we found that leukemias induced by LLV consisted of either T cells or null cells or a mixture of the two, but never B cells. In the absence of a thymus, it is likely that the leukemia in athymic rats involves prethymic T cells. Presumably, these cells or their precursors in the bone marrow are the sites of viral and/or cell multiplication. The occurrence of early viremia before the onset of clinical leukemia is well known, and in this study we were able to demonstrate viremia 20 days postinoculation, which was approximately 90 days earlier than the development of overt disease. Viremia was obviously still present 112 days after inoculation, since retrieval of FV could not have occurred in its absence.

The retrieval of FV from the xenografted FVTCT is not unexpected per se. What is remarkable is that suspensions of the tumors contained at least $10^{2.5}$ ID_{50} of Friend virus per gram of wet tissue. This is the first time that such large amounts of virus have been associated directly with this tumor. In all earlier studies involving the BALB/c mouse, FV would have been replicating in the host animal as well as in the tumor. As a result, the FV recovered from the tumor would be the product of (a) virus initially retrieved when the tumor first formed, (b) FV that had subsequently replicated in the target cells of the host and would then be present in the blood of the highly vascularized tumor, and/or (c) reinfection of tumor cells with FV itself, which we have previously shown can occur (10). Since we have shown that the athymic rat is resistant to Friend disease, all virus retrieved from the tumor must have been the product of continual helper-assisted FV replication in the tumor cells occurring in a host totally resistant to FV infection.

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