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Virus Expression in Various Tissues of Mice Inoculated with Variants of Gross Leukemia Virus*

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Abstract—*Evolution of virus expression in various lymphoid tissues of C3H/Fe mice, inoculated with 2 variants of tissue culture adapted Gross Passage-A virus, was studied with the aid of parallel* in vitro *XC co-culture technique and electron microscopic examination. When the mice were neonatally inoculated with a highly leukemogenic variant (TGV virus), 3 phases of virus expression could be distinguished during the average* 2 months *of latent period. In the early phase (for the first* 3 weeks *after virus inoculation) as well as in the later phase (after the* 50*th* day), *the virus could be detected abundantly in bone marrows and spleens, moderately in thymuses and slightly or not at all in lymph nodes and kidneys. In the intermediate phase (the* 20*th to the* 50*th* days), *the virus disappeared completely or decreased significantly from all tissues tested. In the case of mice similarly inoculated with a non-leukemogenic variant (N1 virus), no virus could be detected in all tissues during the* 2 months *period after virus inoculation. The virus recovered from* in vitro *explanted and cultured kidney cells, taken from mice inoculated neonatally with TGV virus, induced typical Gross type lymphoid leukemia in all C3HeB/Fe mice inoculated as newborns. However,* in vitro *cellular tropism of this virus was revealed as B tropic while that of the original TGV virus was N tropic. Frequent differentiation to heterologous tissues was observed by electron microscope in the thymuses of mice inoculated as newborns with TGV virus.*

INTRODUCTION

THE STUDY on the spread and replication of the murine leukemia virus (MuLV) in susceptible hosts was hampered because of the lack of rapid and quantitative procedures for detection of the virus. Recently developed techniques, such as XC test [1] and radioimmunoassay [2, 3], made it possible to approach the problem more rapidly and quantitatively with regard to the expression of infectious viruses or their related antigens in various tissues of normal or virus-inoculated animals. One of these studies was recently reported in our laboratory using a sensitive *in vitro* XC test performed directly with tissue suspensions obtained from either normal or syngeneic tumor-bearing mice inoculated with a variant of Rauscher leukemia virus [4].

In this paper, we describe similar studies carried out in another virus-host system: Gross Passage-A leukemia virus and C3HeB/Fe mice. The viruses used in these experiments were two homologous strains of long-term *in vitro* tissue culture adapted viruses: TGV and N1. The TGV virus was highly leukemogenic inducing typical Gross type lymphoid leukemia when inoculated into susceptible newborn mice, while N1 virus was completely devoid of such leukemogenic capacity regardless of its perfect *in vitro* infectivity and its antigen related to Gross virus.

The purpose of this study is to give answers to the following questions: (1) What are the principal tissue sites for localization and replication of inoculated virus? (2) What are the evolutionary processes of viral expression in such tissues as a function of time during a long lasting latent period? (3) Are there any differences in tissue affinity between highly leukemogenic TGV virus and homologous non-leukemogenic N1 virus? (4) Have the viruses recovered from the tissues of infected

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mice the same biological characteristics as the original inoculated viruses?

MATERIALS AND METHODS

Mice

New-born or 5–6 week old, male and female C3HeB/Fe mice were used for the experiments. They were supplied by the Animal Selection Center, Orléans, France.

Virus

The TGV virus was produced by an *in vitro* cell line developed in our laboratory from the fragments of hypertrophied thymus of a leukemic C3HeB/Fe mouse following neonatal inoculation of Gross Passage-A virus [5]. This cell line, maintained *in vitro* since its establishment in 1971, produced constantly a large amount of virus in culture supernatants. The virus suspension was prepared with 48 hr culture supernatant of a subconfluent culture, clarified by centrifugation (2500 *g* for 10 min) and kept at −70°C in standard 1 ml aliquots until use. This virus stock contained average 10^8 virus particles per ml estimated by Millipore® filter-counting technique [6] and its reverse transcriptase activity was approximately 36,100 cpm/ml. The TGV virus was N-tropic and its infectivity titer, measured *in vitro* on CFT2 cells by the end point dilution technique with the aid of XC assay, was 10^{-5}/ml. Intraperitoneal (i.p.) inoculation of newborn C3HeB/Fe mice within 48 hr after birth with 0.2 ml of the virus suspension induced in all inoculated mice typical lymphoid leukemia characterized by generalized lymph node enlargement, hypertrophied thymus and moderate spleno-hepatomegaly after average 65 days of latent period. The mean survival time of these leukemic mice was 110 days.

The N1 virus was produced spontaneously by an *in vitro* cell line, NCTC2472, a clone isolated from a long-term culture of fibroblasts originating from the subcutaneous tissue of a normal C3H mouse [7]. The virus suspension was prepared similarly with 48 hr culture supernatant of a subconfluent culture and kept at −70°C until use. This virus stock contained approximately $10^{9.5}$ virus particles per ml and 65,500 cpm/ml of reverse transcriptase activity. Its infectivity titer was 10^{-6}/ml as determined by the same method used for TGV virus. This virus shared an antigen common to Gross leukemia virus and it was devoid of oncogenic capacity when checked by inoculation of susceptible mice (newborns of both C3HeB/Fe and BALB/c strains) with a concentrated (50 ×) virus preparation [8].

Cells

The XC cells [1] kindly supplied by Dr. J. W. Hartley (Viral Carcinogenesis Branch, National Cancer Institute, National Institute of Health, Bethesda, Md., U.S.A.), were used for the detection of infectious virus by the *in vitro* direct assay as described below. The CFT2 cells, developed in our laboratory from an embryo of C3HeB/Fe strain, were fully susceptible to the infection with N tropic MuLV and used for the determination of viral tropism in parallel with SIMR cells (B type). The CFT2 cells were also used for the infection with blood samples. The SIMR cells [9] were kindly provided by Dr. A. A. Axelrad (Division of Biological Research, Ontario, Cancer Institute, Toronto, Canada). The S^+L^- cells [10], kindly provided by R. H. Bassin (Viral Leukemia and Lymphoma Branch, National Cancer Institute, National Institute of Health, Bethesda, Md., U.S.A.), were used to check the helper activity by superinfecting the virus to be tested.

Direct XC test

Virus expressions in various tissues of normal or virus inoculated mice were assayed by *in vitro* direct XC test as described previously [4]. Periodically, 3–5 mice in each experimental group were sacrificed by cervical dislocation and femur bones, spleens, thymuses, inguinal and mesenteric lymph nodes and kidneys were removed aseptically. In most experiments, pooled cell suspensions were prepared with each tissue of these mice. Bone marrows were mechanically dispersed with the aid of syringe in phosphate-buffered saline (PBS). Spleens were perfused with 10 ml of PBS to eliminate erythrocytes, minced with curved scissors into fine pieces and the upper two-third of the supernatant containing mostly monodispersed cells was taken after decantation for 1 min. Lymph nodes and thymuses were minced in the same manner and filtered through sterilized metal meshes. Kidneys were minced into small fragments and trypsinized. These suspensions were washed twice with PBS and the pellets were suspended in culture medium. Ten million cells of each pooled cell suspension were cocultured with 10^6 XC cells in flattened tubes containing an adapted coverslip. The culture medium (Minimal essential medium of Eagle

plus 10% heat-inactivated fetal calf serum, 100 μ/ml penicillin, 100 μg/ml streptomycin and 15 μg/ml aureomycin) was renewed the next day, and 24 hr later the cultures were fixed with methanol and stained with Giemsa. The number of foci of polycaryocytes was counted under a microscope and graded as follows: −, no foci; +, 1–10 foci; + +, 11–30 foci; + + +, 31–70 foci and + + + +, over 71 foci per coverslip (32 × 12 mm).

Electron microscopy

Fragments (about 1 mm^3 size) of each tissue were fixed at 4°C for 1 hr with 1.5% glutaraldehyde (W/V) in Sörensen 0.1 M phosphate buffer (pH 7.4), washed for 2 hr in the same buffer and then postfixed at 4°C for 1 hr in 1% osmium tetroxide in the buffer solution. Dehydration and Epon inclusion were performed in the usual manner. Ultrathin sections were obtained with a LKB ultramicrotome with a glass knife, placed on 300 mesh grids and double stained with uranyle acetate and lead citrate. For the detection of virus particles in tissues, 3 samples were made from each tissue. With one of these samples taken at random, 2 grids covered with about 15 sections were prepared. About 100 squares were systematically explored for each grid. If the virus was not detected, further detection was carried out using another sample in the same manner.

RESULTS

Attempts at virus detection in various tissues of normal C3HeB/Fe mice

At first approach, 12 new-born C3HeB/Fe mice from 3 litters were used for this experiment. Three age-matched mice were sacrificed on the 13th, 31st, 45th and 60th days after birth and bone marrows, spleens, thymuses, inguinal-mesenteric lymph nodes and kidneys were removed aseptically. Tiny fragments of each tissue were immediately fixed with 1.5% glutaraldehyde solution for electron microscopy. Pooled cell suspensions of each of these tissues from the 3 mice were prepared and checked for the presence of infectious virus by the *in vitro* direct XC tests as described in Materials and Methods.

As shown in Table 1, no virus could be detected in any tissue tested throughout the experiment by the direct XC tests. The kidney cells, explanted *in vitro* on the 31st day and cultured for 1 month, also gave negative result in the same test. The electron microscopic examinations revealed equally negative results with all tissues examined except for the thymus of a 60-day-old mouse, in which the presence of virus-like particles was noted in the vacuoles of thymic epithelial cells.

Virus detection in various tissues of C3HeB/Fe mice inoculated as newborns with TGV virus

A group of 30 new-born C3HeB/Fe mice were inoculated i.p. with 0.2 ml of TGV virus suspension within 48 hr after birth. Starting from the 15th day after the virus inoculation, 3 mice were sacrificed weekly and processed as in the previous experiment. Trypsinized kidney cells as well as CFT2 cells on which 0.1 ml of heparinized whole blood samples were overlaid, were cultivated *in vitro* for 1 month and submitted to the direct XC tests.

The results are presented in Table 2. On the 15th and 22nd days, the virus could be detected by the XC test abundantly (+ + + or + + + +) in bone marrows and spleens, moderately in thymuses and slightly (+) in lymph nodes. This "early" phase was then followed by the "intermediate" phase, from

Table 1. *Virus detection in various tissues of normal C3HeB/Fe mice**

	Age of mice (days)							
	13		31		45		60	
Tissue	XC†	EM†	XC	EM	XC	EM	XC	EM
Bone marrow	−	−	−	−	−	−	−	−
Spleen	−	−	−	−	−	−	−	−
Thymus	−	−	−	−	−	−	−	+‡
Lymph node	−	±	±	−	−	−	−	−
Kidney	−	−	−	−	−	−	−	−
Kidney culture			−					

*Results obtained with pooled cell suspensions of each tissue from 3 mice.
†XC = Direct XC test. EM = Electron microscopic examination.
‡Virus-like particles observed in the vacuoles of thymic epithelial cells.

*Table 2. Virus detection in various tissues of C3HeB/Fe mice inoculated as newborns with TGV virus**

Tissue	Days after inoculation of TGV virus																			
	15		22		31		38		44		53		60		67		74			
	XC†	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM
Bone marrow	+ + + +	+	+ + + +		−	−	−		+ + +	+	+ + +		+ + +	+	+ + +		+ + +		+ + +	+
Spleen	+ + +	−	+ + + +		−	−	−		+ + +	+	+ + +		+ + +	+	+ + +		+ + +		+ + +	+
Thymus	+ +		+ + + +		−	−	−		+ + +	+	+		+ + +	+		+	+ + +		+ + +	+
Lymph node	+	−	+		−	−	−			+	−		+	+	+			−		+
Kidney	+	−			−	−	−			−			−	−	−					−
Kidney culture			+ + + +				+ + + +		+ + +		+ + +		+ + +		+ + +		+ + +			
Blood culture	+ + + +		+ + + +		+ + + +		+		+ + +		+ + +		+ + +		+ + +		+ + +			

*Results obtained with pooled cell suspensions of each tissue from 3 mice.
†Results of direct XC tests were graded as follows: − no foci, + 1–10 foci, + + 11–30 foci, + + + 31–70 foci, + + + + over 71 foci per coverslip (32 × 12 mm).

the 31st to the 38th days during which no virus could be detected in all tissues tested by both direct XC test and electron microscopic examinations. After the 44th day up to the end of the experiment, the virus reappeared in bone marrows, spleens and thymuses as abundantly as in the early phase. During this later phase, on average 60 days after virus inoculation, most of the inoculated mice began to show clinically apparent leukemic symptoms such as splenomegaly and inguinal lymph node enlargement. Lymph node cell suspension gave, in general, negative or slightly (+) positive results throughout the experiment even the donor mice showed clinically apparent leukemic symptoms. With kidneys, the results of both direct XC tests and electron microscopic examinations were also negative throughout the experiment except for that of the mice killed on the 15th day after virus inoculation. However, the same kidney cells, cultured *in vitro* for 1 month, gave systematically positive results in XC tests. Similarly, blood cultures on CFT2 cells gave also positive results in all tests during the whole period of the experiment.

These results obtained by the *in vitro* direct XC tests generally agreed well with those obtained by electron microscopic observations, except for several cases of lymph node samples in which the presence of virus was noted by electron microscope while the XC tests with the same tissue were negative.

Electron microscopy of thymuses from mice in the early and later phases (Figs. 3–5) shows frequent tissular rearrangements and differentiations as compared to control thymuses of uninoculated mice (Figs. 1 and 2). Figure 3 illustrates the inclusion of thymic lymphoid tissue in an important mass of mitochondria-rich oncocyte-type cells (Fig. 4). Virus particles are present not only in the vacuoles of the reticulo-epithelial cells, but also frequently associated to the differentiated heterologous tissue structures (Fig. 5).

Next, experiments were undertaken using a similar experimental protocol with individual mice in order to check eventual variation in virus expressions among mice. A group of newborn mice were inoculated with TGV virus as with the previous experiment. Beginning from the 8th day, 4 mice were weekly sacrificed and virus assays were performed with each tissue of each individual mouse, instead of pooled cell suspension of each tissue from these mice. Highly homogenous results were obtained with a given tissue of an individual mouse at a given time

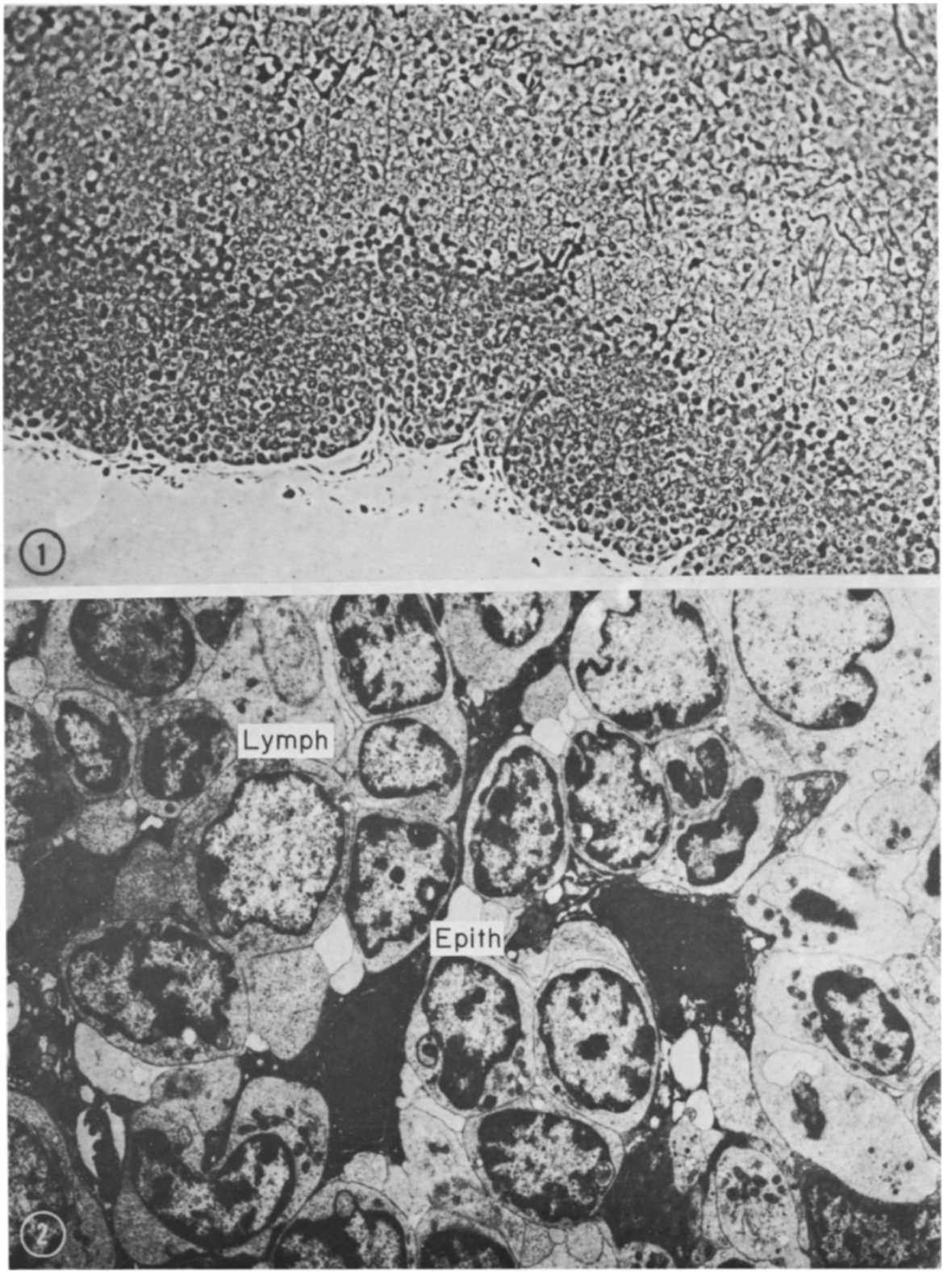

Fig. 1. *Phase contrast optical microscopic aspect of the thymus of a* 60-day-old *control C3HeB/Fe mouse. The cortical and medullary (top) zones of the thymus are clearly distinguishable* (× 288).

Fig. 2. *Electron microscopic representative view of normal structure of the thymus from a C3HeB/Fe mouse: groups of lymphoid cells (Lymph) are separated by electron dense reticular epithelial cells (Epith) which are connected to each other making a network* (× 3960).

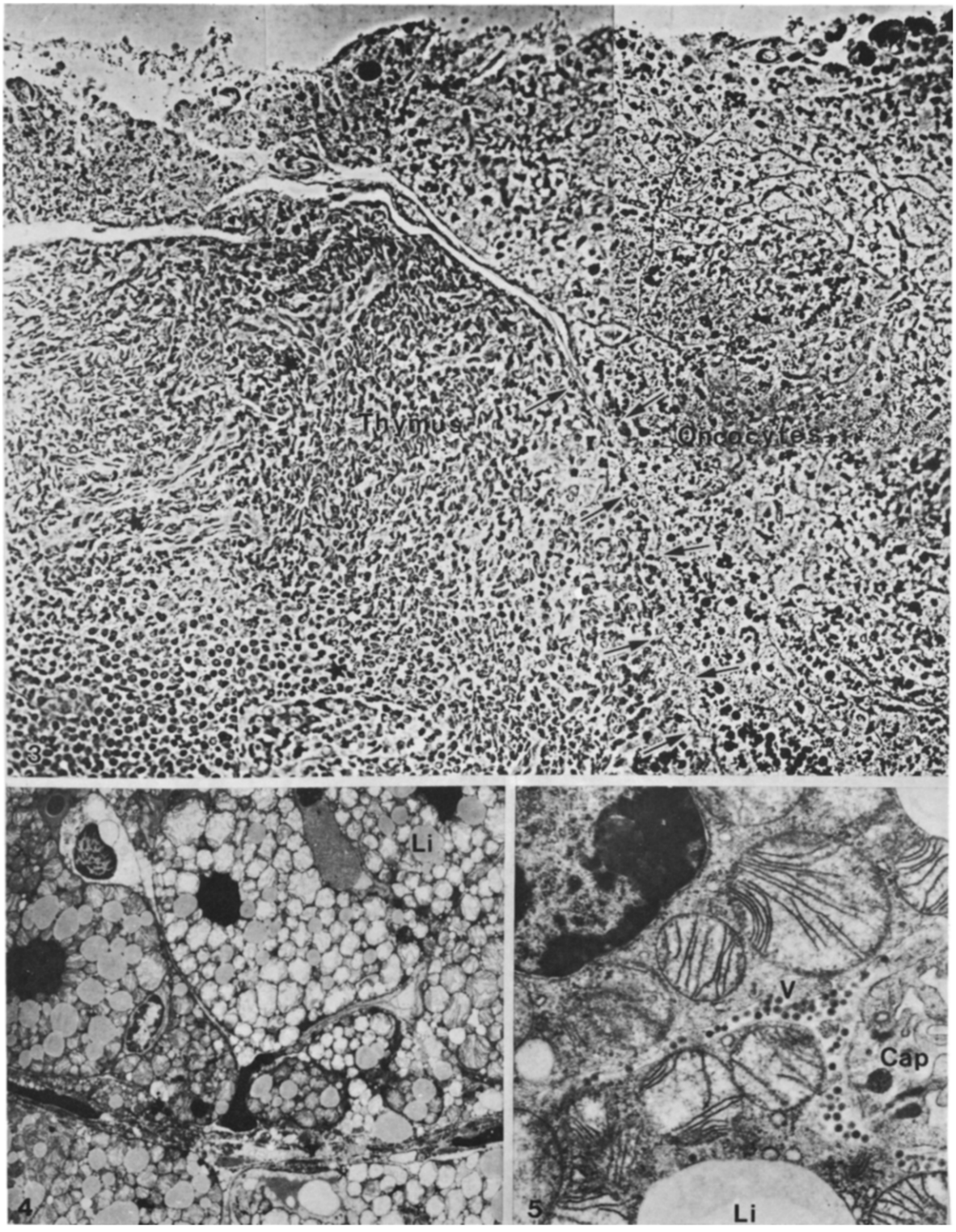

Fig. 3. *Phase contrast optical microscopic view of the thymus of a* 29-day-old *mouse inoculated as new-born with TGV virus. Some typical thymus tissue (left bottom) is surrounded by a large zone of oncocyte cells (top and right). Close contact between the two cellular types is indicated by arrows. Several zones of modified lymphoid tissue are labeled by stars* (× 270, *photograph mounting*).

Fig. 4. *Electron micrograph of oncocytes from Fig.* 3. *These cells are characterized by their dark nucleus and a cytoplasm which is completely filled with lipid droplets (Li) and large spherical mitochondria. Oncocytes are surrounded by an important network of capillaries* (× 1980).

Fig. 5. *From the same sample represented on Fig.* 3, *this picture shows the presence of many virus particles (V) either mature or not, in the spaces surrounding oncocytes and capillary (Cap) (note the lipid droplet and the spherical mitochondria,* × 10,800).

after virus inoculation. The averaged results obtained from these individual mice are presented in Table 3. Abundant expression of virus in bone marrows and spleens, moderately in thymuses, could be observed in the early phase (the 8th to the 22nd day) as well as in the later phase (after the 54th day). Between these two phases, from the 25th to the 43rd days, the virus expressions were significantly decreased (− or +) in all examined tissues, The XC tests with lymph node cell suspensions were all negative except for that of the mice killed on the 74th day. These results, as a whole, confirm the results obtained in the previous experiments.

Virus detection in various tissues of C3HeB/Fe mice inoculated as adults with TGV virus

A group of 2-month-old C3HeB/Fe mice were inoculated i.p. with 0.5 ml of TGV virus suspension. Starting from the 7th day after inoculation, 3 mice were periodically sacrificed and direct XC tests were performed with pooled cell suspensions of each tissue from these mice. As shown in Table 4, the virus could be detected in a small amount (+) only on the 7th day in bone marrow, spleen and thymus and thereafter all tests were entirely negative up to the end of the experiment.

Virus detection in various tissues of C3HeB/Fe mice inoculated as newborn with non-leukemogenic N1 virus

Similar experiments were undertaken using N1 virus and C3HeB/Fe mice. A group of mice were inoculated i.p. within 48 hr after birth with 0.2 ml of N1 virus suspension. Starting from the 7th day after the virus inoculation, 3 mice were periodically killed and virus assays were performed as in the previous experiments.

The results are presented in Table 5. No virus could be detected in any tissue tested throughout the experiment. However, kidney cells cultured *in vitro* for 1 month gave in every instance positive results in XC tests.

Biological characteristics of virus recovered from in vitro *culture of kidneys from mice inoculated as newborn with TGV virus*

A cell line, designated as TGVR9, was developed from kidney tissue explanted *in vitro* on the 74th day from mice inoculated neonatally with TGV virus. As mentioned above, fresh kidney cell suspensions gave systematically negative results in direct XC tests, however, *in vitro* cultured cells of the same kidney tissues showed highly positive results in

*Table 3. Virus detection in various tissues of individual C3HeB/Fe mice inoculated as newborns with TGV virus**

Tissue	Days after inoculation of TGV virus																			
	8		15		22		25		32		36		43		54		66		74	
	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM
Bone marrow	+ + + +		+ + + +		+ + +		+		+		+ + +		+ + +		+ + +		+ + +	
Spleen	+ + + +		+ + + +	+ +	+ + +		+	+ +	+ + +		+ + +	+ +	+ + +	+ +	+ + +	+ +	+ + +	+ +
Thymus	−		+ + + +	+ +	+ + +	+ +	−	+ +	+ + +		+ + +	+ +	+ + +	+ +	+ + +	+ +	+ + +	+ +
Lymph node	−		−		−		−		−		−		−		−		+			

*Average results obtained from those with individual assays of each tissue from 4 mice.

*Table 4. Results of direct XC tests performed with tissues from C3HeB/Fe mice inoculated as adults with TGV virus**

Tissue	Days after inoculation of TGV virus				
	7	23	36	51	73
Bone marrow	+	−	−	−	−
Spleen	+	−	−	−	−
Thymus	+	−	−	−	−
Lymph node	−	−	−	−	−

*Results obtained with pooled cell suspensions of each tissue from 3 mice.

XC tests, and abundant C-type virus particles were observed by electron microscopy. The culture supernatant of this cell line was collected 48 hr after renewal of medium from a subconfluent culture, clarified by centrifugation (2500 ***g*** for 10 min) and kept at −70°C until use. As shown in Table 6, leukemogenic capacity of this virus suspension, determined by inoculation (0.2 ml, i.p.) of new-born C3HeB/Fe mice, was as high as the original TGV virus. It induced typical lymphoid leukemia in all inoculated mice (30/30) after an average latent period of 88 days. The tropisms of both TGV and TGVR9 viruses were determined comparatively by parallel *in vitro* infections of CFT2 (N-type) and SIMR cells (B-type) with serial dilutions of the viruses and checked the end-point dilutions with the aid of XC assay. By this technique, it was revealed that the TGV virus was N tropic since it was 100 times more infectious for CFT2 cells than SIMR cells, while TGVR9 virus was proven to be B tropic as it infected preferentially SIMR cells rather than CFT2 cells. However, both TGV and TGVR9 viruses shared other common biological activities such as positive helper activities tested on S^+L^- cells and an antigen common to Gross virus, as judged by *in vitro* seroneutralization tests using goat anti-Gross leukemia virus serum (supplied by The Office of Program Resources and Logistics, Viral Oncology, National Cancer Institute, Bethesda, MD., 20014, U.S.A.).

DISCUSSION

This paper describes systematic studies on the virus expression in various tissues of mice inoculated with MuLV as a function of time after virus inoculation. The viruses used in these experiments were 2 tissue culture adapted variants of Gross virus: TGV and N1 viruses. TGV virus was highly leukemogenic while N1 virus was completely devoid of leukemogenic capacity when inoculated into new-born C3HeB/Fe mice known to be most susceptible to Gross Passage-A virus. Nevertheless, both viruses were ecotropic and fully infectious *in vitro* to mouse cells developed either from C3HeB/Fe or from other strains of mice. The *in vitro* assays with these viruses can be performed quantitatively by different biological and immunological procedures such as XC plaques, S^+L^- foci and radioimmunoassays.

When the TGV virus was inoculated into new-born C3HeB/Fe mice, 3 phases of virus expression could be recognized during the 2 months of average incubation period: early intermediate and later phases. In the early phase, the virus appeared abundantly in bone marrows, spleens, moderately in thymuses, and slightly or not at all in lymph nodes and kidneys. This phase was then followed by the intermediate phase during which the virus disappeared completely or decreased significantly in all tissues tested. The virus reappeared in the later phase in bone marrows, spleens and thymuses as abundantly as in the initial phase. A similar eclipse phenomena in the course of *in vivo* infection with MuLV were reported in Rauscher virus-BALB/c mice system [11], in Moloney virus-BALB/c system [12] and quite recently by ourselves in Rauscher virus-XLII mice system in which transitional decrease or disappearance of the virus was observed in the organs as well as in the tumors of tumor-bearing mice inoculated with a tissue culture adapted variant of Rauscher virus [4]. The mechanism for such viral eclipse or fluctuation in the target lymphoid organs is not clearly understood and it is difficult to determine at this time whether this phenomenon is due to a cyclic production of virus during infection process or to an immunological host response against the viral infection. Studies are currently underway in

Table 5. *Virus detection in various tissues of C3HeB/Fe mice inoculated as newborn with non-oncogenic N1 virus**

Tissue	Days after inoculation of N1 virus																	
	7		13		20		27		41		55		69					
	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM	XC	EM
Bone marrow	−		−	−	−		−		−	−					−		−	−
Spleen	−		−	−	−		+		−	−					−		−	−
Thymus	−		−	−	−		−		−	−					−		−	−
Lymph node	−		−	−	−		−		−	−					−		−	−
Kidney	−		−	−	−		−		−	−					−		−	−
Kidney culture	+	+	+		+		+	+	+		+	+	+		+	+	+	
Blood culture	−		−		−		−		−									

*Results obtained with pooled cell suspensions of each tissue from 3 mice.

our laboratory to further elucidate this problem.

The tissue affinity of TGV virus is mostly limited, in our experiments, to bone marrows and spleens, and less markedly to thymuses. Lymph nodes and kidneys showed, in general, very low or negative results in virus expression throughout the experiments. Feldman and Gross [13] reported in a study carried out by electron microscope on C3Hf mice with Gross Passage-A virus induced leukemia, that the highest numbers of virus particles appeared in thymuses and lymph nodes and less frequently in bone marrows and spleens. Recently, Kawamura [14] reported that rat-adapted Gross virus infectivity was detected by XC plaque assay only in thymuses of neonatally infected rats and that no other tissues showed virus infectivity until the development of thymic lymphoma. This discrepancy observed between their works and ours is most likely due to the differences in the materials and techniques used for virus detection, especially to the different biological characteristics between *in vivo* adapted virus that they used and *in vitro* long-term tissue culture adapted virus used in our experiments. In our previous works [4], a similar tissue affinity of the virus, limited only to bone marrow and spleen, was noted in mice inoculated as new-born with an *in vitro* tissue culture adapted variant of Rauscher virus. However, before a definite conclusion can be reached, additional studies will be required to determine whether different tissue affinity exist between *in vivo* adapted wild virus and homologous *in vitro* tissue culture adapted virus.

In parallel experiments with non-oncogenic N1 virus, the virus expressions in various tissues of mice inoculated as new-born were markedly different from those observed in mice inoculated with highly leukemogenic TGV virus. No virus expressions were noted in all tissues tested from the beginning to the end of the experiments except for the kidney cell cultures. This is not unexpected if one considers the defective nature for oncogenesis of this virus when inoculated into new-born C3HeB/Fe mice with concentrated virus suspension. Nevertheless, this virus possesses a Gross virus related antigen and is fully infectious *in vitro* for mouse cells derived from different strains. Thus, the loss of oncogenic potential of the virus seems to be correlated with the absence of virus expression in different lymphoid tissues of the host inoculated with this virus. Similar results could be observed in the experiments in which highly

Table 6. Biological characteristics of TGV and TGVR9 viruses

Virus	Leukemogenic capacity: Number of mice* (%) leukemic/total	Average latent period (days)	Type of leukemia	Antigen†	Tropism‡	Helper activity on S^+L^- cells
TGV	48/48	(100%) 75	lymphoid	Gross	N	+
TGVR9	30/30	(100%) 88	lymphoid	Gross	B	+

*New-born C3HeB/Fe mice within 2 days after birth were inoculated i.p. with 0.2 ml of virus suspension.
†Determined by *in vitro* seroneutralization using goat anti-Gross virus serum with the aid of XC assay.
‡Determined by the end point dilution technique with the aid of XC assay following parallel infections of CFT2 and SIMR cells with serial dilutions of viruses.

leukemogenic TGV virus was inoculated into adult C3HeB/Fe mice. In this case, small quantities of virus could only be detected early (7 days after virus inoculation) in bone marrows and spleens and thereafter no virus could be found in any tissue throughout the experiments. It is well established that adult mice of various strains including C3HeB/Fe are entirely resistant to leukemogenesis by Gross Passage-A virus. So, the resistance of a host to the viral leukemogenesis may have brought to the negative virus expression in their lymphoid tissues after virus inoculation, probably through a different mechanism from that operated in the mice inoculated with N1 virus.

Fresh kidney tissues from mice inoculated as newborn either TGV or with N1 viruses gave systematically negative results both in direct XC tests and in electron microscopic examinations. However, *in vitro* cultured cells of the same kidney tissues showed invariably abundant virus expression in both tests. This may be explained by the fact that horizontal infection of virus could occur during the 1 month cultivation of the kidney cells, that had been infected with a subliminal dose of virus at the moment of explantation. Though kidneys are not the target organ for leukemogenesis by MuLV, the presence of the virus in this organ may be partly explained as non-specific trapping by their normal physiological secretary function.

In the thymuses of normal adult C3HeB/Fe mice, the presence of budding virus-like particles was occasionally noted in the vacuoles of reticulo-epithelial cells by electron microscopic examinations, as preceedingly described by de Harven [15]. It is noteworthy in our experiments by electron microscopy that thymus tissues from adult C3HeB/Fe mice inoculated neonatally with TGV virus showed not only abundant presence of virus particles in the reticulo-epithelial cells, but also frequent association of differentiated heterologous tissue structure such as oncocytes, striated muscle fibers and nerve tissues, etc. This phenomenon was rarely observed in the thymuses of normal uninoculated mice. However, the role of MuLV in such thymic tissue differentiation is obscure and needs certainly further investigation (the detailed analysis of these differentiations is in preparation for the next publication).

Biological characteristics of TGVR9 virus, recovered from an *in vitro* culture of kidney cells derived from mice inoculated as newborn with TGV virus, were compared with those of the original TGV virus. Both viruses shared Gross antigen evidenced by *in vitro* seroneutralization tests and showed positive helper activities to S^+L^- cells and highly leukemogenic potentials when inoculated into the susceptible new-born C3HeB/Fe mice. However, the tropisms of these viruses were different: TGV virus was N tropic while TGVR9 virus was revealed as B tropic when comparatively tested *in vitro* on CFT2 and SIMR cells. The tropism of an ecotropic MuLV is determined by a single genetic system with two alleles, $Fv\text{-}1^n$ and $Fv\text{-}1^b$ [16]. Recovery of N tropic viruses from BALB/c mice known to be B type was reported [17]. Inversely, recovery of B tropic virus from C3HeB/Fe mice known to be N type, as in our case, might be expected by the similar mechanism. Lieberman *et al.* recently reported that the viral isolate from radiation-induced lymphomas was apparently N tropic, different from RadLV which is the causative agent of radiation-induced lymphoma in C57BL/Ka mice and characterized as B tropic [18]. The shift of biological properties of a Rauscher virus after its *in vivo* or *in vitro* passages through homologous mouse tissues was reported in our previous works [4]. The me-

chanism operating such modification of viral properties is not known at present and it remains an open question meriting further study.

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