reduced or absent, but there is normal or increased concentration of a protein with antigenic determinants that react with a rabbit antibody to AHF (*10, 16, 17*). In von Willebrand's disease, AHF procoagulant activity and AHF antigen are proportionally decreased (*10, 16*). In studies of AHF procoagulant activity, VWF activity, and AHF antigen, we observed a high degree of correlation ($r > .80$) between pairs of these variables in normal plasmas and in plasmas from patients with von Willebrand's disease (*13*). This correlation is further evidence that these three factors are either located on the same molecule in plasma or on separate but closely associated molecules.

The dissociation of AHF procoagulant activity from VWF activity and AHF antigen by chromatographic separations, as well as the selective effect of thrombin on AHF procoagulant activity, indicates that two separate components—one associated with AHF activity and the other with VWF activity—are present in the plasma complex that has both activities. It remains to be determined whether these components are separate molecules or whether they are subunits of a complex macromolecule. Immunologic studies demonstrated that there are common antigenic determinants on the factor VIII components of high molecular weight (VWF and AHF antigen) and low molecular weight (AHF activity) (*13*). This suggests that the intact complex is a polymer that includes repeating subunits. The VWF activity and AHF antigen (detected by immunoprecipitation by rabbit antibodies) appear to be found only in the polymeric form, whereas AHF procoagulant activity is retained by the dissociated smaller fragment. Additional studies are necessary to achieve a fuller characterization of this macromolecule.

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18. We thank J. Rogers, H. Brand, C. Church, and M. Van Why for technical assistance. Supported in part by PHS grant HL 14595 and by a research grant from the Veterans Administration.

30 July 1973; revised 14 September 1973 ■

Xenotropic Viruses: Murine Leukemia Viruses Associated with NIH Swiss, NZB, and Other Mouse Strains

Abstract. *Murine leukemia virus activity is present in tissues from NIH Swiss and other mouse strains after cocultivation with nonvirus-yielding rat cells transformed by Harvey sarcoma virus. The resulting pseudotype sarcoma virus has the same type-specific coat as the virus previously isolated from New Zealand black (NZB) mice, and, like the NZB virus, it is unable to infect mouse cells. The results show that this NZB type virus is endogenous in other strains of mice and is xenotropic; that is, it grows only in cells foreign to the host. This is the first clear demonstration that NIH Swiss mice also carry indigenous infectious murine leukemia virus.*

A C-type virus which differs from standard murine leukemia viruses (MLV) has been isolated from New Zealand black (NZB) mice (*1*). This virus can be recovered from NZB embryo fibroblasts as well as from adult NZB tissues and does not propagate in mouse cells but does grow in rat and human cells. It rescues the murine sarcoma virus (MSV) genome from nonvirus-producing rat and hamster cells transformed by MSV and yields a pseudotype sarcoma virus that transforms rat and human cells (*1*). Although the NZB mouse cells contain the MLV group-specific (gs) antigen, the NZB virus added exogenously induces gs antigen only in rat and human cells; even at high titer, it does not produce MLV XC plaque formation (*2*) in these cells (*1*). The NZB virus contains reverse transcriptase but is not significantly neutralized by antiserums prepared against the standard murine C-type viruses [FMR (Friend, Moloney, Rauscher) and Gross AKR]; it is only neutralized by certain serums from NZB mice and by antiserums prepared by hyperimmunization of rabbits with concentrates of the NZB virus (*1*).

NIH Swiss are a random-bred colony of mice that have been used for studies with murine C-type viruses. One reason for their general use has been the failure to demonstrate the presence of an infectious indigenous C-type virus in these animals. Despite this fact, MLV gs antigen (*3*) and C-type particles (*4*) have been detected at times in their tissues. By using techniques which revealed the NZB virus, we have been able to demonstrate the presence of infectious MLV similar to the NZB virus in tissues from NIH Swiss as well as other mouse strains.

Spleen and thymus from individual mice were removed, minced, and plated in 60-mm petri dishes. Kidneys were separated from their capsules, minced, trypsinized, and plated. The NIH Swiss mice were obtained from three separate sources: Microbiological Associates, Bethesda; Dr. Arthur Furst; and Dr. Paul Arnstein. The C57/B16J and (NZB × C57/B16J)F_1 were provided by Dr. Beatrice Mintz. The (NZB × NZW)F_1 mice were supplied by Dr. Norman Talal. Cell cultures were grown to confluency in Eagle's minimum essential medium supplemented with 10 percent unheated fetal calf serum and 1 percent antibiotics and glutamine. At this time most of the cells were fibroblastic stromal cells, although the kidney cultures also contained several islands of epithelial cells. Cultures were trypsinized, and the cells were plated at 50 percent confluency. Simultaneously or 24 hours later, 10,000 normal rat kidney (NRK) Harvey cells were added. The NRK

Harvey line is a nonvirus-producing cell line originally derived from NRK cells transformed by the Harvey strain of MSV (*5*). On day 7 after cocultivation, culture fluids were removed and stored at − 70°C. These supernatants were later thawed, filtered (Millipore, 0.45 nm), and assayed for focus formation on the following cell monolayers as described (*1, 5, 6*): NIH Swiss, NZB, and BALB/c mouse embryo cells, Fisher rat embryo cells, NRK cells (*7*), WI38 cells, and normal human foreskin cells.

After cocultivation with NRK Harvey cells, 9 out of 11 cultures of kidney cells from NIH Swiss mice of different ages produced a pseudotype sarcoma virus that could transform rat but not mouse cells (Table 1). NIH Swiss spleen and thymus cultures tested also demonstrated similar rescue ability. The titer of the rescued pseudotype sarcoma virus ranged in general from 2 to 100 focus-forming units (FFU) per milliliter of supernatant from the cocultivated cultures. The low titers may indicate the inefficiency of the rescue procedure or the small quantity of infectious particles present (or both). Spleen and kidney cultures from C57 mice, as well as from (NZB × NZW)F_1 and (NZB × C57/B16)F_1 mice, also produced the same tropic pseudotype sarcoma virus. The pseudotype virus also transforms and replicates in guinea pig, cat, rabbit, bovine, and human cells. In comparison with rat cells, focus formation was generally two to three times more efficient in human cells, and increasing titers of the pseudotype viruses could be obtained by passage through these cells. Particles resembling immature C-type viruses, budding from the surface of cultured NIH kidney cells, have been observed by electron microscopy, and filtered supernatants from these cells when added directly to NRK Harvey cells have yielded the pseudotype virus. Since the virus recovered by Todaro *et al.* (*8*) from the RD human tumor passed in an immunosuppressed NIH Swiss mouse resembled these other NIH isolates, we tested tissues from a similarly treated mouse not carrying a human tumor. After cocultivation with NRK Harvey cells, the tissues from this animal provided by Dr. Arnstein gave the highest titers of pseudotype virus (up to 100 FFU/ml) (Table 1). These results suggest the virus recovered from the passaged RD tumor was an indigenous virus of the NIH Swiss mouse and not a mouse-human hybrid.

NIH Swiss and BALB/c mouse embryo cells after cocultivation have not yielded a pseudotype virus. On the other hand, NZB mouse embryo including one-cell clones have readily produced high titers of the pseudotype virus after cocultivation with NRK Harvey cells (Table 1).

Attempts to demonstrate syncytial formation after addition of XC cells to the NIH Swiss and C57 kidney cell cultures have been unsuccessful. Similar results have been noted with NZB cell cultures even when they produced up to 10^7 C-type particles per milliliter. Concentrates (100-fold) of the NZB pseudotype virus have been added to NIH mouse embryo but no focus formation or gs antigen have been detected in these cells. This same preparation produced titers in rat embryo cells of 10^6 FFU/ml. To rule out the possibility that the pseudotype virus could infect the mouse cell but would not be detected because MSV-infected mouse embryo cells cannot propagate independently (*5, 6, 9, 10*), a "helper" concentration of Rauscher MLV was added after inoculation of the concentrated virus onto NIH mouse embryo cells. No foci were produced, and no Rauscher pseudotype sarcoma virus that was infectious for mouse or rat cells was recovered. These results indicate further the degree of resistance of mouse cells to infection by this virus.

Table 1. Presence of X-tropic MLV in NIH Swiss and C57/B16J mice. Pseudotype sarcoma virus focus formation was measured after cocultivation of various mouse tissues in culture with nonvirus-yielding rat cells transformed by Harvey sarcoma virus (the NRK Harvey line) (*5*). The number of foci per milliliter of supernatant is given; NRK, normal rat kidney cells (*7*); ME, mouse embryo tissue culture cells.

Filtered supernatant from cocultivated cells*	Number of foci in	
	Mouse cells	Rat cells
NRK Harvey only	0	0
NRK Harvey (R MLV)†	>1000	>1000
NZB mouse embryo	0	0
NRK Harvey + NIH ME or BALB/c ME		
NRK Harvey + NZB ME	0	500‡
NRK Harvey + NIH		
2-month kidney (A) §	0	16
2-month kidney (B)	0	0
2-month kidney (C)	0	0
2-month kidney (D)	0	4‡
2-month kidney (E)	0	12
2-month kidney (F)	0	40‡
3-month kidney (G)	0	12‡
3-month kidney (H)	0	2
11-month kidney (I)	0	2
11-month kidney (J)	0	4
2-month spleen (E)	0	2
3-month thymus (K)	0	4
NRK Harvey + NIH (ATS treated)‖		
1-month kidney (L)	0	60‡
1-month spleen (L)	0	100‡
1-month thymus (L)	0	15‡
NRK Harvey + C57		
11-month kidney (M)	0	12‡
11-month spleen (M)	0	6‡
11-month kidney (N)	0	0

* Plus sign (+) denotes cocultivated cells. † Cells infected with Rauscher murine leukemia virus. ‡ Also assayed in human cells where two to three times as many foci were detected. § Letters in parentheses refer to individual animals. ‖ Animals treated with antiserum to thymocytes.

The NIH pseudotype virus has the NZB type-specific coat (Table 2). It was neutralized to the same extent as the NZB pseudotype virus by antiserums to the NZB virus and not by antiserums to FMR, Gross, or AKR type-specific antigens. Since pseudotype viruses have the same host range restrictions and neutralization patterns as their helper leukemia virus (*11, 12*), the NIH virus is most likely the same as the NZB virus.

The finding of a C-type virus associated with NZB mice which did not propagate in mouse cells demonstrated a new type of murine leukemia virus (*1*). The discovery now of similar viruses spontaneously produced by cells from other mice indicates that this NZB virus probably represents a common endogenous murine virus. Since these viruses have general characteristics, including the gs antigen, of MLV, and only grow in a variety of cells foreign to the host, we consider them xenotropic (X-tropic) type murine leukemia viruses. We refer to the NZB isolate as X-tropic NZB MLV, to the NIH virus isolate as X-tropic NIH MLV, and so forth. N- and B-tropic MLV (those that grow best in NIH or BALB/c type mouse cells, respectively) can be propagated to a limited degree in either cell (*11*); the X-tropic MLV are unable to infect either mouse cell type. The presence of another distinct C-type virus in mice modifies the view that only N or B types (or both) are indigenous to this animal. The murine cellular viral genome becomes more complex since, in the case of BALB/c mice for instance, different types of MLV may be present (*13, 14*). The existence of an X-tropic MLV explains the finding of "noninfectious" C-type viruses in NIH Swiss and other mice (*4*) and probably the rat-tropic virus activated from BALB/c cells, although this virus is neutralized effectively by AKR antiserum (*13*). Perhaps different subtypes of murine X-tropic viruses

Table 2. Type specific antigens of X-tropic viruses recovered from NIH Swiss and NZB mice. Neutralization tests were performed by exposing each virus to antiserum for 30 minutes at room temperature. The mixture was then diluted to give 30 to 90 MSV FFU or MLV XC plaque-forming units per plate and assayed on diethylaminoethyl-dextran treated NRK or NIH mouse embryo cells (*5, 9*). The number of foci or XC plaques were determined as described (*1, 2, 6*). Antiserums prepared in rats to the standard MLV were used at a final dilution of 1:40 (*18*). Rabbit antiserums to NZB MLV and NZB pseudotype virus were used at 1:10 and 1:40 dilutions, respectively.

Antiserum against	Reduction (percent) in focus or plaque formation by these viruses:				
	Harvey MSV	AKR MLV	Gross MLV	NZB pseudotype	NIH pseudotype
Moloney MSV	100	18	10	28	27
AKR MLV	10	96	33	28	28
Gross MLV	0	97	100	10	15
NZB MLV	9	0	0	85	80
NZB pseudotype virus	8	10	0	90	90

exist. It militates against assuming that C-type particles observed in mice are the standard mouse-tropic types since either or both may be present. It encourages further virus assays with rat and human cells when mouse tissues containing gs antigen do not yield a mouse-tropic virus, and in those cases where both type viruses may be present.

The fact that this murine virus grows well in human cells offers a relatively easy method for its detection and isolation in preparations of other MLV and MSV, since passage through these cells would select for the X-tropic virus. Such a selection procedure probably accounts for the MSV described by Aaronson, which grows in rat and human cells (*15*), as well as the Rauscher MLV, which was "adapted" to propagate in human cells (*16*).

We have found the X-tropic virus in tissues from newborn NIH Swiss mice, but have not detected it in the embryos of NIH Swiss or other mice. However, its presence in every cell derived from NZB embryos may reflect a specific defect in NZB mice that has a relation to their development of autoimmune disease. In this regard, tissues from the immunosuppressed NIH Swiss mouse after cocultivation gave the highest titers of X-tropic pseudotype sarcoma virus (Table 1).

This unusual tropism of a mammalian C-type virus that we reported 3 years ago (*1*) is not now unique to mice. Certain feline C-type viruses propagate efficiently only in cells from animal species other than the host (*17*). It is likely that in many, if not all, animals, X-tropic viruses will be identified; and they must be true endogenous viruses of the host since horizontal infection is not likely. Similarly, in many different human tissues, C-type particles have been observed which have not been propagated. These may be X-tropic human viruses that await identification after successful growth in correctly selected cells from animal species other than the host.

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1 October 1973

Maternal Behavior in Wolf Spiders: The Role of Abdominal Hairs

Abstract. *Newly emerged, juvenile wolf spiders do not settle on clothed or shaved areas of their mother's abdomen until after a period of days. Spiny, knobbed hairs, peculiar to adult female lycosids, apparently provide the stimulus and means for attachment by the inner layer of spiderlings. Innervated long, smooth hairs are mechanoreceptors which probably serve in other aspects of brood care.*

After emerging from the egg sac, juvenile wolf spiders (Araneae: Lycosidae) immediately mass on the mother's abdomen (and posterior carapace, if a large brood) and are carried by her for a number of days. If brushed off, the spiderlings climb up the female's legs and settle again (*1*). Since coating the female's abdomen with certain chemicals did not repel the young whereas clothing it with various materials resulted in the young's settling on only nonclothed regions, Engelhardt (*2*) concluded that the spiderlings' aggregation resulted partly from tactile stimulation provided by the female's abdominal hairs. Graefe (*3*) has described knob-tipped abdominal hairs as being peculiar to adult female lycosids and serving to reduce the abrasion of the egg sac, which he claimed would result from pointed hairs. Using experimental and morphological approaches, we have found that the knobbed hairs probably trigger the young's attachment behavior and are likely important in providing a grasping surface for the inner layer of spiderlings (*4*).

In one series of tests we used peripherally placed dabs of paraffin to attach a rectangle of chiffon cloth to the female's dorsal abdomen. Unlike Engelhardt's (*2*) nearly complete covering, our treatment left the lateral and ven-