Natural Resistance to Tumors and Viruses

Edited by Otto Haller

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Dr. Otto Haller
Institut für Immunologie und Virologie
der Universität Zürich, Gloriastraße 32 B
CH-8028 Zürich, Switzerland

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Preface

Natural resistance is now coming to be recognized as a potentially important phenomenon in host defense against infection and malignancy. Genetically controlled resistance mechanisms are usually effective early in infection and before conventional immune responses are generated. Comparisons of experimental systems where natural resistance plays a prominent role demonstrate the complexities of the host defense mechanisms involved, as evidenced in the present volume. Nevertheless, some common components of genetic resistance are discernible and largely comprise natural killer cells, macrophages, and interferon. These and additional factors would seem to constitute a first line of defense in host resistance against both viruses and tumors. It is evident that considerable variation in the relative importance of distinct mechanisms may be found among various resistance systems and that, most likely, additional effector functions will be discovered.

Resistance to tumors and most viruses is under polygenic control, has a complex mode of inheritance, and depends on appropriately complex effector mechanisms. Instances, however, where a single gene locus determines resistance or susceptibility to a virus, as in the case of resistance to flaviviruses or influenza viruses, would seem to offer good prospects for elucidating the basic factors involved. Resistance to influenza virus would indeed seem to represent a comparatively simple situation: resistance is expressed at the host cell level, and interferon is its main mediator.

The present volume provides insight into current concepts of such resistance mechanisms. It contains contributions from distinguished laboratories presently engaged in relevant research in this field. A variety of experimental systems are analyzed covering genetic resistance in mice to flaviviruses, herpes simplex virus, lactate dehydrogenase elevating virus, influenza viruses and mouse hepatitis virus. Other chapters deal with interesting aspects of resistance to leukemogenesis and immunosuppression by Friend virus, with the biological significance of natural cell mediated immunity in viral infection and with tumor resistance and immune regulation by natural killer cells.

Zürich, April 1981 Otto Haller

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MARGO A. BRINTON*

1 Introduction

The first demonstrations that a host gene could control resistance to disease induced by an animal virus were reported independently by *Lynch* and *Hughes* (1936) and *Webster* and *Clow* (1936). Subsequently this resistance was found to be specifically directed against flaviviruses. A number of other genes which confer resistance to other types of virus infections have since been identified (*Pincus* and *Snyder* 1975; *Bang* 1978). Different classes of viruses vary greatly in their mode and site of replication, and it would be expected that the mechanisms of action of various resistance gene products would also differ significantly. The strict virus specificity of host genetically controlled resistance indicates that the resistance gene products interact with unique molecular events characteristic of only one type of virus. Such a specific resistance mechanism acting at the cellular level constitutes a first-line host defense mechanism. However, the phenotypic expression of resistance genes on the whole-animal level can certainly be modified by the degree of functioning of other types of host defense mechanisms.

2 Genetically Controlled Resistance to Flaviviruses

More than 50 different flaviviruses have been identified to date by serologic means, and several of these are the cause of significant human morbidity and mortality worldwide. However, many of the molecular details of the flavivirus replication cycle are still poorly understood. Flaviviruses belong to the togavirus family, which also consists of alphaviruses, pestiviruses, rubivirus, and several unclassified viruses, including lactate-dehydrogenase-elevating virus (*Schlesinger* 1980). In general, togaviruses are characterized by a lipid envelope and an infectious single-stranded RNA genome. The various genera represented within the togavirus family are distinguished by differences in their modes of replication, by their fine morphological detail, and by their interaction with host resistance genes.

*The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

Webster (1923), working with *Bacillus enteriditis* infection in a stock of randomly bred Swiss mice found that susceptibility varied greatly among individual mice. By selection and inbreeding, *Webster* (1933) developed bacteria-resistant (BR) and bacteria-susceptible (BS) strains. Subsequent studies indicated that mouse strains resistant (VR) or susceptible (VS) to Louping ill virus could also be selected, but that virus and bacterial resistance were inherited independently (*Webster* and *Fite* 1933, 1934). Mice resistant to Louping ill virus were also resistant to St. Louis encephalitis virus (*Webster* 1937) and Russian spring-summer encephalitis virus (*Casals* and *Schneider* 1943). Since at the time of *Webster's* studies these viruses had not yet been classified (*Casals* and *Brown* 1954), the flavivirus-specific nature of the observed host-controlled resistance was not at first realized (*Sabin* 1953).

Genetically controlled resistance to yellow fever virus (YFV), another flavivirus, was independently observed by *Sawyer* and *Lloyd* (1931) among randomly bred Rockefeller Institute mice, by *Lynch* and *Hughes* (1936) among randomly bred mice of the "Det" strain, and by *Sabin* (1952a, b, 1954) in Princeton Rockefeller mice (PRI).

Within all known resistant mouse strains, "Det", BRVR, BSVR, and PRI, the flavivirus resistance is inherited as a simple autosomal dominant allele (*Lynch* and *Hughes* 1936; *Webster* 1937; *Sabin* 1952b). No other inbred mouse strains commonly used in laboratories have been found to possess the flavivirus resistance gene (*Darnell* et al. 1974). Using PRI mice as a source for the gene, another inbred resistant strain, C3H/RV, was created congenic to C3H/HE (*Groschel* and *Koprowski* 1965). C3H/RV and C3H/HE mice share common red blood cell antigens, and skin grafts are interchangeable between them. The development of the congenic C3H strains has allowed comparative studies of genetically controlled resistance to flaviviruses to be carried out against a low background of unrelated variables. A study of wild mice caught in California and Maryland demonstrated the presence of the flavivirus resistance gene among wild mouse populations (*Darnell* et al. 1974) (Table 1). The finding that this resistance gene has continued to segregate within wild mice populations indicates that the gene may actually convey a selective advantage under natural conditions. However, which flaviviruses exert selective pressure on wild *Mus musculus* populations, other than possibly Powassan, is not yet known.

Factors such as the age of the host, its immune status, the degree of virulence of the infecting flavivirus, and the route of infection have been observed to influence the phenotypic expression of the flavivirus resistance gene. However, no evidence has been reported which indicates that these factors are involved in the specific mechanism of resistance mediated by the product of the gene.

Resistant mice do support the replication of flaviviruses, but virus yields are lower and the spread of the infection is slower and usually self limiting as compared to susceptible mice (*Goodman* and *Koprowski* 1962a; *Darnell* et al. 1974). For instance, C3H/RV mice survive intracerebral injection of undiluted 17D-YFV, whereas 100% of C3H/HE mice die. The phenotypic expression of resistance can be overwhelmed by large doses of a virulent flavivirus given by the intracerebral route (*Goodman* and *Koprowski* 1962a). West Nile virus (WNV) can kill resistant mice after being injected intracerebrally, but 100–1000 times more virus is required to produce disease and death in resistant mice as compared to susceptible controls (*Vanio* et al. 1961; *Hanson* and *Koprowski* 1969; *Darnell* et al. 1974). The day of onset of disease symptoms is delayed in resistant mice, and the maximum virus titer in the brain is 2 to 3 logs lower than in comparable susceptible mice (see Fig. 1).

Table 1. Inheritance of resistance to yellow fever virus (YFV, strain 17D) in wild *Mus Musculus*

Wild *(?)*[a] X C3H/He *(rr)* F_1		
Wild parent	Surviving:dead[b]	
$G3^{1}$ [c]	0:9[c]	
$G3^{2}$	0:14	
$G3^{3}$	9:2	
$G4^{1}$	13:0	
$G4^{2}$	4:6	
$G5^{1}$	7:10	
$G5^{2}$	17:0	
	F_1 Survivors ↓ Resistant F_1 *(Rr)* X Resistant F_1 *(Rr)*[d] ↓ F_2	
	–	
	–	
	7:3	
	9:1	
	7:2	
	5:1	
	6:0	
Total	34:7	

Reproduced with permission from *Darnell* et al. 1974

[a] Genotype

[b] Ratio of mice surviving challenge to those dying from it. Two litters of progeny from each parent were tested

[c] Two-month-old F_1 mice were given an intracerebral injection of 0.03 ml undiluted 17D-YFV

[d] Survivors were mated brother to sister approximately 2 months after their original infection

In susceptible animals the levels of neutralizing antibody and interferon which are produced in response to WNV infection are higher than in resistant ones; this corresponds to the higher titers of virus synthesized by susceptible animals (*Jacoby* et al. 1980; *Hanson* and *Koprowski* 1969; *Darnell* and *Koprowski* 1974). The in vivo expression of genetic resistance to flaviviruses requires an intact lymphoreticular system (*Goodman* and *Koprowski* 1962a; *Jacoby* and *Bhatt* 1976a, b; *Jacoby* et al. 1980). Immunosuppression of resistant animals with cyclophosphamide, sublethal X-ray irradiation, or thymus cell depletion converts a normally asymptomatic flavivirus infection into a lethal one. However, under such conditions the onset of sickness in resistant mice is delayed several days as compared to susceptible control mice, and the virus titers in moribund resistant brains are lower than in comparable susceptible brains. *Jacoby* and *Bhatt* (1976b) demonstrated that even though T-cell-depleted, flavivirus-infected resistant mice did produce detectable levels of hemagglutination-inhibiting antiviral antibody, they were not protected from lethal infections.

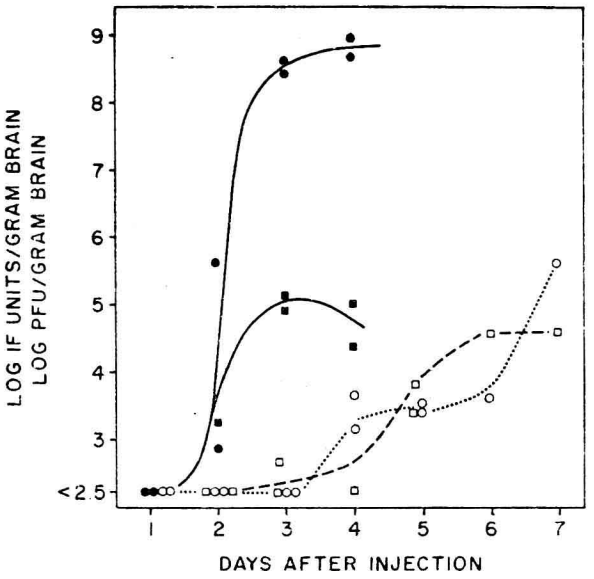

Fig. 1. Growth of West Nile virus (WNV) in the brains of susceptible C3H mice (●-●) and resistant C3H/RV mice (○-○). Interferon levels were also measured in brains of the C3H (■-■) and C3H/RV (□-□) mice. The intracerebral inoculum (10^5 PFU in 0.03 ml) was a WNV pool produced in MK_2 cells. (Reproduced with permission from *Hanson* and *Koprowski* 1969)

The clearance of WNV from the blood of resistant and susceptible mice has been found to be essentially complete by 10 to 12 h after an intraperitoneal injection of virus (*Goodman* and *Koprowski* 1962b). In susceptible mice, this clearance was immediately followed by a rise in the titer of infectious virus in the blood; virus levels in the blood remained high for at least 2 days. No such secondary viremia was detectable in blood from resistant mice (*Goodman* and *Koprowski* 1962b; *Groschel* and *Koprowski* 1965).

Cell cultures derived from various tissues of resistant mice produce lower yields of flaviviruses than do comparable cultures of cells from congenic susceptible mice. This phenomenon was first observed by (*Webster* and *Johnson* (1941), who studied the replication of St. Louis encephalitis virus in brain cell cultures from resistant and susceptible animals. *Goodman* and *Koprowski* (1962a) reported a similar difference in yield with WNV. Resistant brain cell cultures infected with WNV yielded 100-fold less infectious virus than did susceptible cultures. Cultures of spleen cells, peritoneal exudate cells, and embryofibroblasts from resistant animals displayed a similar diminished ability to support flavivirus replication when infected with either 17D-YFV or WNV (*Goodman* and *Koprowski* 1962a; *Vanio* 1963a, b; *Hanson* and *Koprowski* 1969; *Darnell* and *Koprowski* (1974). The differential ability to replicate flaviviruses was maintained in established cell lines developed from SV40-transformed resistant and susceptible embryofibroblasts (*Darnell* and *Koprowski* 1974). WNV adsorption and penetration occur normally in resistant cells, since the same percentage of cells show virus-positive immunofluorescence in resistant and susceptible cultures by 6 to 8 h after infection (*Darnell* and *Koprowski* 1974). Recent studies indicate that the level of flavivirus RNA and protein synthesis is signif-

icantly lower in resistant cells as compared to susceptible ones, indicating that inhibition of flavivirus replication within resistant cells occurs at an early step in virus replication (*Brinton* 1981b). We have isolated a number of temperature-sensitive mutants of WNV from persistently infected cultures of resistant and susceptible cells and are currently using these as tools for gaining a further understanding of the steps involved in flavivirus replication, as well as the differences in the virus-host interaction in resistant and susceptible cells.

The genesis of defective interfering (DI) virus particles, one type of viral deletion mutant, has been found to occur in cells infected with virtually any animal virus (*Huang* and *Baltimore* 1976) and seems to be controlled by host cell factors. Tissue culture experiments indicate that flavivirus DI particles are produced more readily and/or interfere with standard infectious virus replication more efficiently in resistant cells than in susceptible one (*Darnell* and *Koprowski* 1974). Assessment of the ability of serially passaged culture fluids from infected resistant and susceptible cell cultures to interfere with the replication of infectious homologous standard virus revealed that detectable interference was only observed with samples from resistant cells (Table 2). Since the ratio of defective to infectious particles determines the extent of interference, the lack of an observable interference by samples from susceptible cultures apparently was due to the presence of an insufficient number of DI particles to cause a detectable interference in the test. Susceptible cultures probably do produce DI particles as indicated by the cycling titer of infectious virus observed during serial undiluted passage in susceptible cells (Fig. 2). An identical decline in infectious vesicular stomatitis virus (VSV) was observed after serial undiluted passage in either flavivirus-resistant or -susceptible cell cultures, indicating that similar numbers of defective VSV particles are synthesized by both cultures and that the extent of interference is similar (*Huang*, personal communication).

The reduced yield of flaviviruses observed in resistant cultures and animals was not accompanied by an earlier or enhanced production of interferon (*Vanio* et al. 1961). As

Table 2. Interference between serial undiluted passage WNV and brain-produced WNV[a]

Cell type	Moi of brain-produced WNV	48 h WNV yield (log_{10} PFU/ml)		
		[b]Brain-produced WNV	[b]Brain-produced WNV plus 3rd passage T-MEF-HE WNV	[b]Brain-produced WNV plus 3rd passage T-MEF-RV WNV
T-MEF-RV	1.82	2.7	-	1.9
	0.18	2.35	2.2	0.9
	0.018	1.0	1.75	Undetectable
T-MEF-HE	1.42	7.3	-	6.3
	0.14	6.6	6.5	5.75
	0.014	5.5	6.25	4.35

[a] WNV, West Nile virus. Reproduced with permission from *Darnell* and *Koprowski* 1974

[b] Titers of different virus preparations used for infection: brain-produced WNV = $10^{8.9}$ PFU/ml; 3rd passage T-MEF-HE WNV = $10^{4.5}$ PFU/ml; 3rd passage T-MEF-RV WNV = $10^{2.5}$ PFU/ml; T-MEF-RV = SV40 transformed resistant C3M/RV embryofibroblasts; T-MEF-HE = SV40 transformed susceptible C3M/HE embryofibroblasts

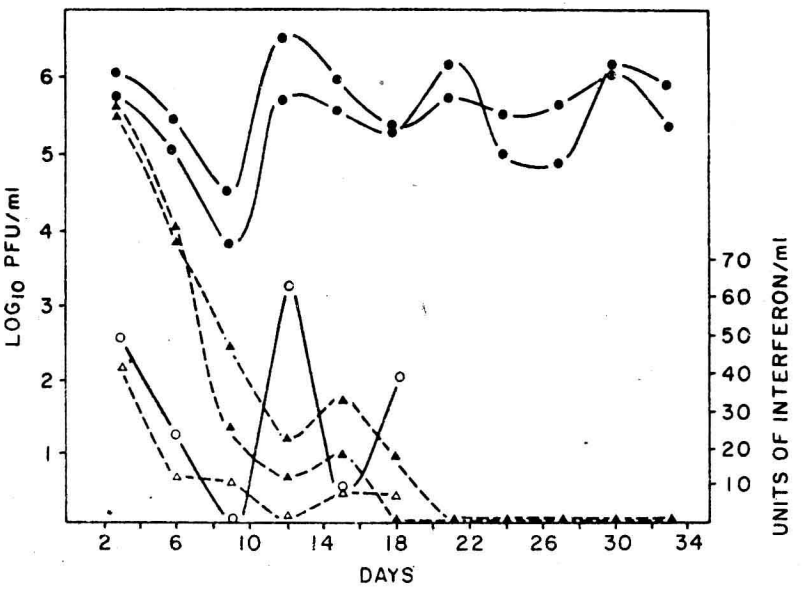

Fig. 2. Serial undiluted passage of West Nile virus (WNV) in cultures of SV40-transformed resistant and susceptible cells. Cultures were infected with WNV (10 PFU/cell) and culture media was transferred to fresh cultures every 3 days. Titers of WNV in transferred culture fluids from susceptible (●-●) and resistant (○-○) cultures and interferon from susceptible (▲-▲) and resistant (△-△) cultures are shown. (Reproduced with permission from *Darnell* and *Koprowski* 1974)

shown in Fig. 1, when 10- to 12-week-old C3H/HE (susceptible) or C3H/RV (resistant) mice were injected intracerebrally with 10^5 plaque-forming units (PFU) of WNV, a dose sufficient to kill all the mice, the maximum levels of interferon measured in brain tissues were similar. Virus titers were characteristically lower in the resistant brains and rose later as compared to those in susceptible brains. Interferon production by WNV-infected resistant and susceptible cell cultures is low, but interferon levels were consistently higher in fluids obtained from susceptible cell cultures (*Darnell* and *Koprowski* 1974) (Fig. 2).

Vesicular stomatitis virus and Sindbis replicate equally well in flavirus-resistant or -susceptible cells. The replication of these viruses was suppressed to an equal degree by treatment with crude interferon of both resistant and susceptible cultures, but interferon treatment caused an apparently greater suppression of WNV replication in resistant cultures than in susceptible ones (*Hanson* and *Koprowski* 1969; *Darnell* and *Koprowski* 1974) (Fig. 3). However, it must be remembered that the yield from non-interferon-treated resistant cells was already lower than that from susceptible cells. The apparent enhanced interferon mediated suppression of WNV production in resistant cells may be merely a synergism between interferon-induced inhibition and inhibition induced by the flavivirus resistance gene product.

In order to attempt to further clarify the role of interferon in the expression of flavivirus genetic resistance, the effect of pretreatment of animals and cell cultures with anti-interferon globulin was investigated. This study was done in collaboration with Drs.

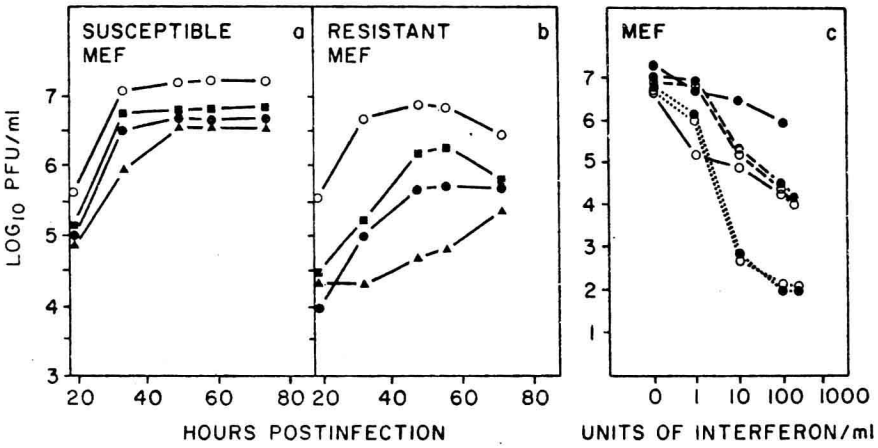

Fig. 3a–c. Inhibition of West Nile virus (WNV), Sindbis virus and vesicular stomatitis virus (VSV) production by crude interfeon in resistant and susceptible mouse embryofibroblasts (MEF). a Effect of interferon on WNV replication in susceptible cells. b Effect of interferon on WNV replication in resistant cells. Units of interferon used per ml of medium: (O–O) = 0 U/ml, (■–■) = 10 U/ml, (●–●) = 100 U/ml, and (Δ–Δ) = 1000 U/ml. c Comparison of interferon-mediated suppression of Sindbis, VSV, and WNV replication in susceptible (●–●) and resistant (▲–▲) cells. –, WNV; --, Sindbis virus, and -- VSV. (Reproduced with permission from *Darnell* and *Koprowski* 1974)

Arnheiter, Haller, and *Lindenmann*, employing sheep antimouse interferon (type I) antisera (AIF) obtained from Dr. *Gresser*. In the case of genetically determined resistance to orthomyxoviruses, injection of small amounts of AIF rendered resistant mice fully susceptible to lethal influenza virus infections (*Haller* et al. 1979). AIF-treated resistant animals showed a corresponding increase in virus production, so that virus titers similar to those in susceptible mice were observed. Normal sheep serum globulin (NSG) or AIF (neutralizing titer = 1.2×10^{-6} against 8 units of mouse interferon) was diluted 1:3 with PBS, and 0.1 ml was injected into mice intravenously immediately before intracerebral injection of 17D-YFV (10^3 PFU/0.03 ml/animal). Two animals were killed at the indicated times after infection and their brains removed and frozen for subsequent titration (Table 3). Four animals from each group were observed throughout the course of the

Table 3. Virus titer in brain (PFU/brain)[a]

Mouse strain	Treatment	Days after infection (log_{10})			
		3	6	9	12
C3H/HE	NSG[b]	2[b]	5	5	-
	AIF	2	5	6	-
C3H/RV	NSG	-	1	1	2
	AIF	-	1	1	2

[a] NSG, normal serum globulin; AIF, anti-mouse interferon serum. These were diluted 1:3 with PBS and 0.1 ml was injected intravenously immediately before virus was inoculated

[b] Titers represent average titer of virus in two brains homogenized and titrated separately

Table 4. Effect of AIF on mortality after YFV infection of C3H/HE and C3H/RV mice[a]

Mouse strain	Treatment	Mortality	
		Proportion	Mean day of death
C3H/HE	NSG[b]	4/4	12
	AIF[b]	4/4	13.5
C3H/RV	NSG	0/4	-
	AIF	0/4	-

[a] Mice were given an intracerebral injection of 0.03 ml undiluted 17D-YFV (yellow fever virus) vaccine (titer = 10^4 PFU/ml). NSG, normal serum globulin; AIF, anti-mouse interferon serum
[b] NSG and AIF were diluted 1:3 with PBS and 0.1 ml was injected intravenously immediately before virus was inoculated

infection (Table 4). In contrast to what was observed with myxovirus-resistant mice, AIF did not cause flavivirus-resistant C3H/RV mice to succumb to flavivirus infection (Table 4). Moreover, an increased production of flavivirus in the brains of resistant mice treated with AIF was also not observed (Table 3). AIF-treated resistant mice remained resistant when WNV was administered by the intraperitoneal route. In contrast, AIF treatment of susceptible C3H/HE mice led to a lethal infection after injection of a ten-fold sublethal intraperitoneal dose of WNV (Table 5).

Although it is not yet possible to describe the precise mechanism of action of the flavivirus resistance gene, some general statements can be made. Since a decreased yield of flaviviruses is observed for resistant cell cultures, the action of the gene is most likely at the cellular level. This assumption is further supported by the fact that lower titers of virus are also observed in resistant animals even after immunosuppression. Both interferon and DI particles interfere with virus replication at the intracellular level. Therefore, it would not be surprising to find that interferon and DI particle interference appear to be enhanced in resistant cells against flaviviruses simply because their effect is superimposed on viral replication processes already limited by the product of the resistance gene. Data indicate that the resistance gene product does not affect the attachment or penetration of flaviviruses but acts at an early replication step, resulting in a reduction in the level of virus-specific RNA synthesis.

Table 5. Effect of AIF on mortality after WNV infection of C3H/HE and C3H/RV mice[a]

Mouse strain	Treatment	Proportion	Mean day of death
C3H/HE	NSG[b]	0/3	-
	AIF[b]	3/4	14
C3H/RV	NSG	0/4	-
	AIF	0/4	-

[a] 10^7 PFU/0.1 ml was given to each mouse by the intraperitoneal route. LD_{50} for C3H/HE by the intraperitoneal route = $10^{7.5}$ PFU. WNV, West Nile virus; NSG, normal serum globulin; AIF, anti-mouse interferon serum
[b] NSG and AIF were diluted 1:3 with PBS and 0.1 ml was injected intravenously immediately before virus was inoculated

3 Genetically Controlled Resistance to Lactate-Dehydrogenase-Elevating Virus-Induced Paralysis

In 1970, *Murphy* et al. observed a fatal paralytic disease in C58 mice characterized by destruction of motor neurons in the brain stem and spinal cord. The virus causing this disease has recently been identified as a strain of mouse lactate-dehydrogenase-elevating virus (LDV) (*Martinez* et al. 1980). LDV normally produces a persistent infection in mice which is not characterized by any type of overt pathology. LDV is a small lipid-enveloped virus which contains a single-stranded infectious RNA genome and is considered to be in the togavirus family (*Brinton-Darnell* and *Plagemann* 1975; *Brinton.* 1981a). LDV infects only mice and replicates to high titers. LDV-infected animals maintain a lifelong viremia with virus present in the bloodstream within infectious immune complexes, but no clinical signs of an immune complex disease are manifested. A permanent increase in the plasma levels of certain enzymes, including lactate dehydrogenase (LDH) and isocitrate dehydrogenase, is also characteristic of LDV infection and is considered to result from an LDV-induced impairment of enzyme clearance by reticuloendothelial cells (*Riley* et al. 1960). The virus normally replicates in macrophages and macrophage-like cells in vivo and multiplies most efficiently in vitro in primary peritoneal exudate cultures (*Brinton-Darnell* et al. 1975). LDV infection induces subtle alterations in the functioning of the immune system such as a transient suppression of T-cell function during the first 2 weeks after infection (*Howard* et al. 1969; *Michaelides* and *Schlesinger* 1974) and an adjuvant effect on the production of antibodies to unrelated antigens (*Notkins* et al. 1966; *Mergenhagen* et al. 1967).

Several lines of evidence reported by *Martinez* et al. (1980) indicated that a strain of LDV is the etiologic agent of the C58 paralytic disease, which has been variously identified in the literature as age-dependent polioencephalitis (ADPE) (*Murphy* et al. 1970) and immune polioencephalitis (*Lawton* and *Murphy* 1973; *Sager* et al. 1973). Serum from animals with paralysis induces an increase in serum enzyme levels after injection into mice characteristic of an LDV infection (*Martinez* et al. 1980), and the infectivity titer of such sera as assayed by enzyme elevation endpoint or by paralysis induction endpoint was found to be identical. Physiochemical and morphological analysis also revealed the similarity of the C58 agent to LDV (*Martinez* et al. 1980). Further, even though previously isolated strains of LDV had not been known to cause an overt disease, they were found to possess a low level of paralytogenic activity for immunosuppressed C58 mice (Table 6).

The more paralytogenic LDV strain isolated from C58 mice may have been fortuitously selected by prolonged inadvertent passage in C58 mice as a contaminant of transplanted line Ib leukemia cell suspensions. Line Ib leukemia originated spontaneously in a 1-year-old C58 mouse in 1929 (*MacDowell* and *Richter* 1932) and has since been maintained by successive passages in C58 mice (*Murphy* et al. 1958). It is not known when the leukemic cell suspensions became contaminated with LDV (presumably from transfer of the leukemia cell suspension to a C58 mouse already persistently infected with LDV), but paralysis was not observed in C58 mice until the late 1960s (*Murphy* et al. 1970).

Although the LDV strains replicate in all inbred strains of mice tested and cause a nonsymptomatic persistent infection, LDV-induced paralysis has been observed in only two strains, AKR and C58 (*Duffey* et al. 1976b; *Martinez* et al. 1979). Further, AKR and C58 mice must first be immunosuppressed to become susceptible (*Duffey* et al. 1976b; *Murphy* et al. 1970). The genetic and immunologic factors involved in the induction of

Table 6. Test for paralytogenicity of LDV isolates in C58 mice

Virus injected[a]	Incidence of paralysis in			
	6-month-old mice		12-month-old mice	
	Proportion	Mean day ± SD	Proportion	Mean day ± SD
LDV-1	0/10		9/11	16.9 ± 5.1
LDV-2	1/10	18	6/10	15.2 ± 5.2
LDV-3	0/10		8/9	17.1 ± 5.2
LDV-4	0/18		6/19	19.0 ± 5.0
C58 Agent	10/10	12.1 ± 2.3	10/10	10.0 ± 0.5

[a] Mice were given cyclophosphamide 1 day before challenge with 10^7 ID_{50} of the indicated virus (as determined by enzyme elevation assay). LDV, lactate-dehydrogenase-elevating virus. Used with permission from *Martinez* et al. 1980

paralysis by LDV have been only partially characterized. The resistance of young adult C58 mice (up to 6 months) to induction of LDV-induced paralysis appears to be mediated by a thymus-dependent immune response which can be abrogated by neonatal thymectomy (*Duffey* et al. 1976a), immunosuppressive agents, or X-ray irradiation (*Duffey* et al. 1976b). Beginning at about 6 months, C58 mice spontaneously lose the function of a subpopulation of T cells and are then susceptible to LDV-induced paralysis without external immunosuppressive treatment (*Murphy* et al. 1970; *Murphy* 1979).

Virus infectivity titers in the plasma and tissues have been found to be maintained at higher levels after infection of immunosuppressed C58 mice with the C58 strain of LDV (*Nawrocki* and *Murphy* 1978; *Brinton* 1980a) (Fig. 4). Also, the injection of LDV immune complexes into immunosuppressed C58 mice does not lead to induction of paralysis, suggesting that antiviral antibody may be involved in the resistance to LDV-induced paralysis. Although an immunosuppressed state is required during the initial infection with LDV in C58 mice for the induction of paralysis to occur, an additional host genetic factor is involved; mouse strains which are completely resistant to paralysis induction apparent-

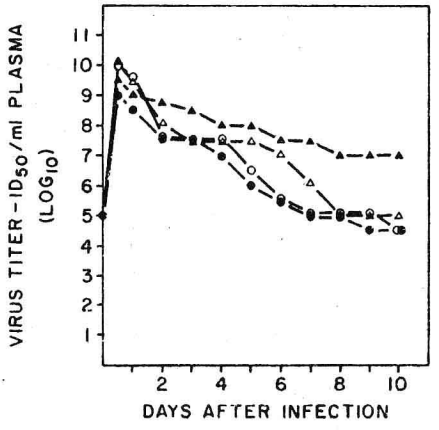

Fig. 4. Infectivity levels in plasma after infection of mice with the C58 strain of LDV. Δ-Δ, 3-month-old C58 mice; ▲-▲, 3-month-old C58 mice given 150 mg/kg cyclophosphamide 24 h prior to LDV inoculation; ○-○, 3-month-old Swiss mice; ●-●, 3-month-old Swiss mice given 150 mg/kg cyclophosphamide 24 h prior to LDV inoculation

Table 7. Susceptibility of inbred mice to paralytic disease

F_1 hybrids	H-2 type	FV-1 type	Incidence of paralysis age (months)		
			6	9	12
C58/wm X C3H/HeJ	k/k	n/n	2/8	7/12	
C58/wm X DBA/2J	k/d	n/n	2/8	5/8	
C58/wm X B10	k/b	n/b	0/26		0/10
C58/wm X B10.BR	k/k	n/b	0/20		0/14
C58/wm X BALB/c	k/d	n/b	0/11		0/19

Used with permission from *Pease* and *Murphy* 1980

ly are not dependent on immunologic restriction of LDV, since the resistance of these mice is unaffected by immunosuppression (*Duffey* et al. 1976b; *Martinez* 1979). Preliminary analysis of the inheritance of host genes involved in controlling susceptibility to LDV-induced paralysis indicated that susceptibility might be controlled by two genes, one of which is linked to the $H\text{-}2^k$ loci (*Martinez* et al. 1979). However, recent breeding studies have revealed that susceptibility may not be linked to the $H\text{-}2^k$ loci. A correlation with the $FV\text{-}1^n$ allele has been suggested (*Pease* and *Murphy* 1980). From the F_1 data so far obtained, several genes appear to be involved in the inheritance of susceptibility to LDV-induced paralysis (Table 7). However, further breeding studies are necessary to delineate the number and type of host genes controlling susceptibility.

Electron microscopic examination of spinal cords from C58-LDV-infected immunosuppressed C58 mice revealed the presence of LDV-like virions within neurons. Previously, LDV was only known to replicate in macrophage-like cells. Virions were observed budding through neuron cytoplasmic membranes, but no cytopathic effect was obvious in virus-containing neurons. Virions have not yet been observed replicating in neurons within spinal cords of mice which are resistant to induction of paralysis after infection with the C58 strain of LDV. Virus infectivity was measured in spinal fluids at various times after infection. The C58 strain of LDV was readily detectable in the spinal fluid by 3 days after infection in both normal and immunosuppressed resistant and susceptible mice (*Brinton* 1980b) (Table 8). This LDV can apparently readily gain access to the central nervous system even during an infection which does not lead to

Table 8. Titer of C58-LDV in CSF[a]

Mouse strain	Treatment	Days after infection		
		3	6	9
Swiss (r)	- - -	6.5[b]	6.0	6.0
Swiss (r)	Cyclo	7.0	6.1	5.5
C58 (s)	- - -	7.65	7.0	7.0
C58 (s)	Cyclo	7.58	7.5	6.0

[a] LDV, lactate-dehydrogenase-elevating virus; CSF, cerebrospinal fluid
[b] Log_{10} ID_{50}/ml

the induction of paralysis. Although these preliminary studies provide clues to the mechanism by which LDV can induce paralysis in a genetically susceptible immunosuppressed mouse, the exact sequence of events which produces lesions is not yet known. Further studies are necessary to determine the contribution to susceptibility of various T-cell subpopulations and the ability of neurons to replicate virus. It is not yet known whether neurons are damaged directly by virus replication or by attack by immune system components.

4 Summary

Resistance to flavivirus-induced disease is inherited in mice as a single autosomal dominant allele which shows no linkage to the H-2 loci. Resistant animals and cell cultures prepared from resistant animals produce two- to three-fold lower levels of flaviviruses than do comparable congenic susceptible mice or cell cultures. The product of the flavivirus resistance gene appears to act at the cellular level and, therefore, represents a virus-specific first-line defense mechanism. The resistance gene product does not affect the attachment or penetration of flaviviruses, but acts at an early step in virus replication, causing a reduction in the level of virus-specific RNA synthesis.

The genetic and immunologic factors involved in LDV-induced paralysis in mice have only partially been characterized. Although suppression of the function of a subpopulation of thymus-dependent cells does appear to be required prior to the induction of paralysis by LDV, an additional host genetically controlled factor is also necessary. Mouse strains which are resistant to LDV paralysis remain resistant after treatment with immunosuppressive agents. Further studies are needed to delineate the number and type of host genes which control susceptibility to LDV-induced paralysis. This system provides a unique opportunity to study the host factors involved in the production of a paralytic disease by a normally nonpathogenic virus.

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