MACROPHAGES AS A CELLULAR EXPRESSION OF INHERITED NATURAL RESISTANCE

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Natural resistance to Arbor B viruses in the Princeton (PRI) and the BRVR strains of mice has been attributed to the presence of a single dominant gene in the genotypes of these mice.[1, 2] Recent experimentation *in vivo* and *in vitro* strongly suggests that the phenotypic expression of the dominant gene for virus resistance lies in cells of the histiocytic-macrophage-polyblastic type of the genetically-resistant mice.[3] The present study presents further evidence that the gene conferring virus resistance is expressed as virus-resistant macrophages.

In breeding experiments among PRI mice, C3H/He mice, and their hybrids, the effects of inheritance of the dominant resistance gene have been studied, and at the same time a strain of mice virus-resistant and coisogenic with C3H/He mice has been created.[3] Mice of the eighth generation of backcrossing to C3H/He mice (BC-8) are theoretically coisogenic with the C3H/He mice,[4] and have been shown by skin transplantation, tumor transplantation, and red-blood-cell agglutination studies to be similar to C3H/He mice at all loci tested.[3] The single locus at which the coisogenic strains differ[5] should be the one determining resistance to Arbor B viruses, since the resistance character has been selected at each generation of backcross breeding. The effect of this single genotypic difference between the coisogenic strains of mice could thus be investigated. Mature BC-8 mice were either challenged intracerebrally with an Arbor B virus or sacrificed for the preparation of cultures of macrophages which were then infected with the virus. Results obtained both *in vivo* and *in vitro* indicated that resistance to Arbor B viruses was distributed among members of the BC-8 generation in a pattern of segregation suggesting a cross between a simple dominant heterozygote (BC-7 virus-resistant male mouse) and a recessive homozygote (C3H/He susceptible female mouse).

Materials and Methods.—Cultures of peritoneal macrophages were prepared from individual mice. Exudates were induced in these mice by intraperitoneal inoculation of 2 ml of 2% suspension of cornstarch in saline. Twenty-four hours later, each mouse was killed by cervical dislocation, and the peritoneal wall exposed. Medium consisting of twice-concentrated Eagle's solution in Earle's saline, 20% calf serum, and 10 units of heparin per ml was injected into each mouse intraperitoneally. The fluid was aspirated with syringe and needle, and the cell suspension harvested from each mouse was inoculated into a 60-mm Petri dish. Each Petri dish was inoculated with approximately 3 ml of cell suspension containing 2 to 3 million cells per ml. The cultures were incubated at 37°C in an atmosphere of 5% CO_2 in air. After 24 hr, the heparinized medium was removed and replaced with medium without the addition of heparin. Within 24 hr the cultures were ready for use. The average density of cells in each 60-mm culture dish was 2 to 3 million macrophages (Fig. 1).

Kidney cell cultures were prepared by the technique of Manaker *et al.*[6] Trypsinized cells were planted either into Petri dishes or 16-mm culture tubes. Cells were incubated at 37°C in an atmosphere of 5% CO_2 when necessary.

Arbor B viruses used in these experiments were the 17D strain of yellow fever and the Egypt 101 strain of West Nile virus. Ampules of 17D strain of yellow fever virus (obtained through courtesy of The National Drug Company, Philadelphia, Pennsylvania) contained desiccated suspensions of infected chick embryos. Adult mice of strains susceptible to Arbor B virus died

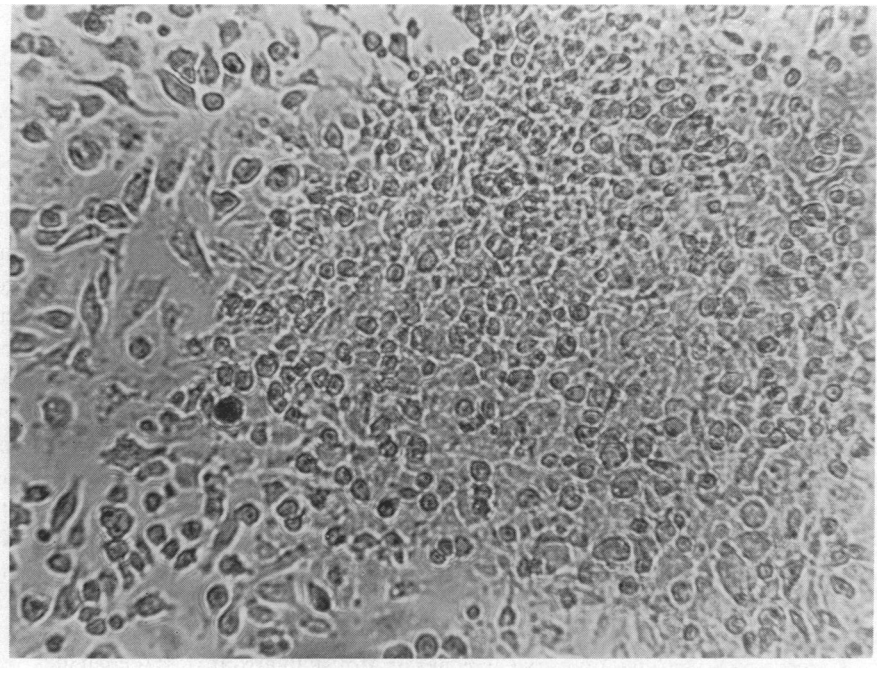

FIG. 1.

after intracerebral inoculation of 17D virus, while intracerebral injection of the same dilution of virus into virus-resistant mice did not cause death. Pools of E101 strain of West Nile virus were prepared according to the method of Clarke and Casals,[7] from suspensions of infected suckling mouse brains and stored in aliquots of 1 ml at −70°C. The virus content of each pool was determined by titrating the contents of several randomly-selected ampules on monolayers of chick embryo fibroblasts. The virus concentration was then expressed in plaque-forming units per ml (pfu/ml).

Cultures were infected with the Egypt 101 strain of West Nile virus by exposing the monolayers for 90 min to a 10% suspension of infected mouse brain from a standardized West Nile virus pool. The virus was then thoroughly washed from the monolayers and fresh medium added to the cultures. Virus content of the tissue culture fluid was determined by titration of fluids on monolayers of chick embryo fibroblasts. Titers of virus were expressed in plaque-forming units per ml (pfu/ml).

Results.—In creating the virus-resistant mouse strain coisogenic with C3H/He mice, resistant male PRI mice were bred to C3H/He female mice, and the hybrid progeny were challenged with 17D virus. Virus-resistant hybrid males were then backcrossed to C3H/He females, and the mice of the first backcross generation were challenged with 17D virus. Resistant males of the first and each subsequent backcross generation were bred to C3H/He females. In this way the PRI gene for Arbor B virus resistance was introduced into the C3H/He genotype and maintained by selection.[3] By the eighth backcross generation (BC-8), the genotype of the hybrid mice was theoretically 99.8 per cent of C3H/He chromation.[4] Challenge of BC-8 mice, as in all preceding generations of backcrosses between a dominant heterozygote and susceptible homozygote, resulted in a 1:1 distribution of resistance and susceptibility (Table 1). 104 out of 209 BC-8 mice survived infection by 17D virus, those mice apparently carrying the dominant gene conferring Arbor B virus resistance. These resistant BC-8 mice were taken as the progenitors of the virus-

TABLE 1

DISTRIBUTION OF ARBOR B VIRUS RESISTANCE AMONG MICE CHALLENGED WITH 17D VIRUS

BRVR	BC-5		BC-8		C3H/He
% Resistance	Ratio resistance	% Resistance	Ratio resistance	% Resistance	% Resistance
100	83/150	55	104/209	50	0

resistant strain of mice coisogenic with the C3H/He mice. Breeding of the coisogenic strains is presently in progress in this laboratory.

Challenge of C3H/He × BRVR hybrid mice with Arbor B viruses has indicated that the BRVR resistance factor behaves as a single dominant autosomal gene. There has been no evidence that the BRVR gene for resistance to Arbor B viruses and its mechanism of expression is different from the PRI gene for virus resistance.[3]

The cellular mechanism through which the gene conferring virus resistance is expressed in PRI or BRVR mice appears to be in macrophages of the reticuloendothelial system. When cultures of macrophages from spleens[3] or from peritoneal exudates of PRI or BRVR mice were infected with West Nile virus, the cells failed to support virus multiplication,[3] although in some instances small amounts of virus were detected in the culture fluid (Table 2). On the other hand, West Nile–infected macrophages from susceptible C3H/He mice always supported growth

TABLE 2

GROWTH OF WEST NILE VIRUS IN CULTURES OF MOUSE PERITONEAL MACROPHAGES

Day harvest of medium after virus infection	Virus in medium susceptible cells (pfu/ml)	Virus in medium resistant cells (pfu/ml)
1	6.5×10^2	0
2	6.0×10^3	30
6	2.5×10^3	0

of virus (Table 2). Although virus has been shown to multiply for extended periods of time in infected macrophages cultured from susceptible mice, no cytopathic effect of West Nile virus on the macrophages has been observed.

When cultures prepared from the kidneys of virus-resistant and virus-susceptible mice were infected with West Nile virus, the kidney cells from both mouse strains supported growth of virus at similar levels (Table 3). The same results were

TABLE 3

GROWTH OF WEST NILE VIRUS IN INFECTED MOUSE KIDNEY CELLS

Day medium harvest after virus infection	Virus in medium susceptible cells (pfu/ml)	Virus in medium resistant cells (pfu/ml)
1	4.0×10^2	8.0×10^2
2	2.5×10^4	1.5×10^4
3	4.0×10^4	5.0×10^4
6	4.0×10^3	2.5×10^3

obtained using infected lung cells of the two mouse strains.[3] Therefore, a difference in ability to support virus multiplication appeared to exist only between macrophages cultured from the virus-resistant and susceptible strains of mice. These observations suggested that the cellular expression of the gene conferring resistance to Arbor B viruses was in the population of macrophages of the resistant mice.

As described above, the mice of the BC-8 generation were created by backcross breeding of selected virus-resistant hybrids to susceptible C3H/He mice. These BC-8 mice were considered coisogenic with the susceptible mice, the single gene

differentiating the two coisogenic strains being the gene conferring virus resistance. In each backcross generation including BC-8, the presence of the virus-resistance gene was determined by virus challenge of mice. At each generation level the results of *in vivo* challenge indicated that nearly 50% of the hybrid individuals were resistant.[3] Thus, according to results of the intracerebral challenge, the 50 per cent of the BC-8 mice surviving 17D virus infection were the virus-resistant animals coisogenic with C3H/He mice (Table 1).

It was postulated that if the selected single dominant gene conferring Arbor B virus resistance were expressed at the cellular level in macrophages, then the macrophages from BC-8 mice which inherit this gene would fail to support virus growth when infected *in vitro*, as the macrophages from parental resistant mouse strains. Macrophages from the other 50 per cent of BC-8 mice should support virus multiplication at levels similar to macrophages from susceptible mice. To investigate this hypothesis, macrophages were cultured from individual BC-8 mice, and the virus yield from these infected cultures was compared with the level of virus in fluids from macrophage cultures of virus-resistant and virus-susceptible mouse origin.

In a representative experiment, peritoneal macrophages were cultured from 12 normal BC-8 mice, and the cells exposed to West Nile virus. Culture media were changed daily until the third day after infection, when fluids were harvested for determination of virus content. Of the 12 BC-8 mice tested, macrophages from 7 mice produced little or no virus, while macrophages from the remaining 5 mice supported virus multiplication at levels similar to those from the susceptible mouse controls (Table 4). Trace amounts of West Nile virus were detected in culture

TABLE 4

COMPARISON OF WEST NILE VIRUS IN MEDIA OF INFECTED PERITONEAL MACROPHAGE CULTURES FROM INDIVIDUAL BC-8 MICE

Mouse culture number		Virus in medium (pfu/ml)	Ratio resistance
BC-8	1	8.5×10^2	
	2	6.5×10^2	
	3	0	
	4	35	
	5	0	
	6	1.2×10^2	
	7	1.8×10^2	7/12
	8	0	
	9	0	
	10	60	
	11	3.2×10^2	
	12	0	
C3H/He		6.7×10^2	0/3
BRVR		0	3/3

fluid from macrophages of 2 BC-8 mice. However, these cultures were scored as resistant, since small amounts of virus were sometimes detected in macrophages cultured from virus-resistant BRVR mice (Table 2). Similar distribution of resistance and susceptibility to West Nile virus was observed in a second group of 12 BC-8 mice. Once again peritoneal macrophages from 5 BC-8 mice yielded amounts of virus similar to infected macrophages from susceptible C3H/He mice on the fourth day after infection, while 7 out of 12 BC-8 mouse macrophage cultures failed to support virus multiplication at significant levels. In summary, of 24 BC-8

mice tested, peritoneal macrophages from 14 BC-8 mice appeared to be as resistant to virus infection as the cells from virus-resistant BRVR or PRI mice, while cells from the remaining 10 BC-8 mice supported virus growth as well as macrophages from virus-susceptible C3H/He mice. Thus, 58 per cent of the BC-8 cultures were virus-resistant—

Total number BC-8 cultures tested	24
Ratio virus-resistant macrophage cultures	14/24+
Per cent resistance of BC-8 mouse macrophages	58

a distribution of resistance approximating the 1:1 distribution of resistance and susceptibility in the backcross generations, as predicted and observed by *in vivo* challenge of these mice (Table 1). It appears that in animals where the gene for resistance to Arbor B viruses is inherited, the macrophages obtained from these animals do not support virus multiplication.

Discussion.—The genetic basis of resistance to Arbor B viruses in mice of the PRI and BRVR strains has been established by extensive crossbreeding experiments and is apparently due to the presence of a gene in the genotype of these mice which is dominant, autosomal, and unifactorial.[3] This inherited resistance appears to be reflected in macrophages of the reticulo-endothelial system of PRI and BRVR mice. When cultures of peritoneal macrophages from resistant and susceptible mice are infected with Arbor B virus, the macrophages from the resistant mice fail to support virus multiplication, while the susceptible mouse macrophages support virus growth. In a previous study on the interaction of mouse hepatitis virus (MHV) and macrophages from liver explants, Bang and Warwick[8] suggested that the inherited susceptibility of PRI mice to the virus might be reflected in the complement of macrophages susceptible to infection and lysis by MHV. In contrast, liver macrophages from other strains of mice resistant to MHV apparently did not lyse or support virus growth.

When the single dominant gene conferring Arbor B virus resistance is selectively grafted into the genotype of susceptible C3H/He mice,[3] the gene confers resistance to all mice in which it is present. The present study investigates the effects of inheritance of this single dominant gene in mice of coisogenic strains which differ genotypically from one another at the single locus determining resistance or susceptibility to Arbor B viruses. Susceptibility and resistance were found to segregate in mice of the eighth backcross generation (BC-8) in a 1:1 ratio, when the mice were exposed to Arbor B viruses. Thus, approximately 50 per cent of the BC-8 mice inherited the gene for virus resistance from the parental PRI mice. The 1:1 segregation of resistance and susceptibility to Arbor B viruses, as observed after intracerebral challenge of BC-8 mice, has likewise been shown after virus infection *in vitro* of macrophages cultured from BC-8 mice. When the dominant gene for Arbor B virus resistance is present in the BC-8 genotype, macrophages from these mice will not support virus multiplication. The presence of the recessive allele for virus susceptibility is expressed in these mice in macrophages which, like macrophages from parental C3H mice, will support virus growth. Evidence that macrophages actually do represent the phenotypic expression of an inherited factor has been presented in *in vitro* studies on the distribution of resistance and susceptibility to Arbor B viruses in macrophages cultured from BC-8 mice.

In the BC-8 mice which comprise the virus resistant strain coisogenic with C3H mice, the single gene differentiating the two strains appears to be the gene conferring virus resistance, and its presence is apparently expressed in macrophages which cannot support multiplication of West Nile and probably all other Arbor B viruses.

Summary.—Tissue cultures of peritoneal macrophages prepared from individual mice of the eighth generation of backcrossing between virus-resistant hybrids and virus-susceptible C3H mice were exposed to West Nile virus. Half of the cultures failed to support virus multiplication, while the remaining cultures yielded infectious virus. This distribution of resistance and susceptibility in macrophage cultures reflected on the cellular level genes segregating for virus resistance and susceptibility on the whole animal level.

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† Including 4 cultures probably resistant.

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*A NEW HYPOTHESIS ON THE NATURE AND SEQUENCE OF MEIOTIC EVENTS IN THE FEMALE OF DROSOPHILA MELANOGASTER**

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A knowledge of the relation between synapsis, exchange, and disjunction is basic to an understanding of the meiotic process. That exchange in the female of *Drosophila melanogaster* is not a prerequisite for regular disjunction has been demonstrated by Sturtevant and Beadle[1] and by Cooper.[2] It is equally clear that when more than two chromosomal elements are mutually involved in disjunction, as happens with heterologues in the case of translocation heterozygotes[3] or with heteromorphs in the case of secondary nondisjunction,[1] the frequencies of exchange and regular disjunction are positively correlated. The role of a heterologue or of a heteromorph in these situations has been variously interpreted.

Bridges[4] postulated that competitive X,X,Y pairing, initiated prior to exchange (since secondary exceptions are almost invariably noncrossovers), is responsible