# VIRUS-INDUCED DIABETES MELLITUS VI. Genetically Determined Host Differences in the Replication of Encephalomyocarditis Virus in Pancreatic Beta Cells

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Infection of mice with the M variant of encephalomyocarditis  $(EMC)^1$  virus results in beta cell damage and a clinical picture characterized by hyperglycemia, glycosuria, polydipsia, polyphagia, and hypoinsulinemia (1-5). The severity of the diabetes-like syndrome was found to correlate closely with the degree of viral-induced beta cell damage (6).

Recent experiments with inbred strains of mice revealed that the development of EMC virus-induced diabetes is genetically determined. Only certain strains of mice (e.g., SWR/J, SJL, CD-1, Swiss NIH, DBA/1, and DBA/2) develop hyperglycemia and/or abnormal glucose tolerance tests after infection with EMC virus. Other strains (e.g., C57BL/6J, AKR, and C3H/He) do not develop overt diabetes or abnormal glucose tolerance tests (7). Matings between susceptible and resistant strains of mice showed that in the  $F_1$  generation the development of EMC virus-induced diabetes is inherited as a recessive trait (8). Analysis of the  $F_2$  data suggests that more than one gene is involved. The genetically determined host factors responsible for differences in susceptibility to EMC virus-induced diabetes, however, are not known. The present investigation was initiated to study the ability of EMC virus to replicate in vivo and in vitro in beta cells isolated from inbred strains of mice susceptible (SWR/J) and resistant (C57BL/6J) to the development of diabetes.

#### Materials and Methods

*Mice.* SWR/J, C57BL/6J, DBA/J, CBA, and AKR mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Swiss NIH inbred mice were obtained from the breeding colony at the National Institutes of Health. Mice were maintained on Purina NIH mouse ration containing 5.0% fat and 23.5% protein. Except where noted, 4-5-wk-old male mice were used in all experiments.

Virus. The preparation and source of M variant of EMC virus was described elsewhere (1, 4). Virus pools were prepared from CAF<sub>1</sub> secondary mouse embryo cells or from the hearts of C57BL/ 6J mice. Except where noted, mice were inoculated intraperitoneally with  $5.0 \times 10^3$  plaqueforming units (PFU) of virus.

Plaque Assay. The virus titer was determined by inoculating 0.2 ml of appropriate dilutions of

<sup>&</sup>lt;sup>1</sup> Abbreviations used in this paper: EMC, enecephalomyocarditis; FITC, fluorescein isothiocyanate; MEF, mouse embryo fibroblast; MEM, minimal essential medium; PBS, phosphate-buffered saline; PFU, plaque-forming units.

samples into confluent monolayers of CAF, secondary mouse embryo cells in 60-mm plastic Petri dishes. After adsorption for 1 h at 37°C, cultures were overlayered with 2% methylcellulose medium (Eagle's minimal essential medium [MEM] containing 5% calf serum). Monolayers were stained 2 days later with 1:20,000 dilution of neutral red. Plaques were counted within 24 h.

To determine the amount of virus within infected cells (e.g., pancreatic beta cells), the samples were resuspended in Eagle's MEM, frozen and thawed three times, homogenized, and appropriate dilutions assayed as described.

Pancreatic Beta Cells. Pancreatic beta cells were isolated from mice by modifications of methods described elsewhere (9, 10). Briefly, aseptically removed pancreas were minced and washed in calcium-magnesium-free phosphate-buffered saline (PBS), pH 7.4. Floating debris was decanted, and the tissues were minced and washed an additional four to five times. Small pieces of pancreatic tissues were then transferred to an Erlenmeyer flask containing prewarmed collagenase solution (3 mg/ml of PBS) and incubated at 37°C for 15-20 min with vigorous shaking. The mixture was transferred to a conical tube, resuspended in PBS, centrifuged at 500 rpm for 2 min, and the sediment was washed. This procedure was repeated three times. The resuspended material (containing islets, acinar cells, fibroblasts, and other cell types) was transferred to a 35mm Petri dish, and the islets were collected with a Pasteur pipette under a steromicroscope (9). The islets (150-200 islets/mouse) were disrupted by pipetting or by stirring very gently in a prewarmed collagenase solution (7 mg/ml of PBS) for 5 to 6 min at 37°C. The suspension was filtered through sterile gauze to eliminate large aggregates and to break up small clumps of cells. The filtered cells were carefully layered on a 13-36% preformed Ficoll density gradient and centrifuged at 1,200 rpm for 10 min. Cells collected from the heavy visible band near the top of the gradient were washed to eliminate residual Ficoll and used directly in all experiments, except where indicated.

To prepare monolayers, cells from 3-wk-old mice were processed as described above, except they were not placed on a Ficoll gradient. Instead, the cells that had been filtered through gauze were suspended in prewarmed culture medium (medium 199 containing 7% fetal calf serum and 150 mg/ml of p-glucose) and adjusted to  $5 \times 10^4$  viable cells per ml. Plastic dishes (35 mm) then were seeded with 2.5 ml of cell suspension and incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. 12-15 h later, the nonadherent beta cells were decanted, thereby eliminating the large numbers of fibroblastoid cells which had adhered to the dish. The beta cells then were seeded in 35mm plastic dishes. At the end of 48 h, the culture medium was replaced with chemically defined growth medium (MPNL 65/C plus 7% heat-inactivated calf serum) and refed at 2-3-day intervals (10). At the end of 2 wk, nearly complete monolayers had formed in 20-30% of the plates.

Primary Mouse Embryo Fibroblast (MEF). Primary MEF cells were prepared from 16-18-dayold SWR/J or C57BL/6J embryos by trypsinization. Cells were grown in Eagle's MEM supplemented with 10% calf serum, 100 U of penicillin, and 100  $\mu$ g of streptomycin per ml. After monolayers had formed, the growth medium was replaced with Eagle's MEM containing 2% calf serum.

Primary Mouse Kidney Cells. Kidneys were removed from 4-5-wk-old uninfected SWR/J or C57BL/6J mice, minced, and then trypsinized. Approximately  $2.0 \times 10^5$  cells were seeded in 35-mm Petri dishes and fed as described above.

Infectious Center Assay. Beta cells were isolated from SWR/J or C57BL/6J mice which had been infected 48 h earlier. Appropriate twofold dilutions of beta cells (ranging from 5 to 500 cells/ plate) in Eagle's MEM containing 5% calf serum were inoculated onto confluent monolayers of secondary mouse embryo cells and overlayed with methylcellulose medium. Monolayers were stained 4 days later with neutral red, and plaques were counted.

Fluorescein Isothiocyanate (FITC) Labeled Anti-EMC Antibody. For the preparation of anti-EMC virus antibody, mice were immunized intraperitoneally every 2 wk with increasing doses of EMC virus beginning with  $3.0 \times 10^3$  PFU and ending with  $1.0 \times 10^4$  PFU. Sera were obtained after five or more injections, passed through a Sephadex G-200 column, and the IgG fraction collected. The neutralization titer of this material was greater than 1:640. The gamma globulin fraction then was labeled with FITC (11). Unconjugated FITC was removed by dialysis against 0.01 M PBS (pH 7.5) and by gel filtration through Sephadex G-25. The labeled gamma globulin was subsequently adsorbed with acetone-treated mouse liver powder to eliminate nonspecific fluorescence.

The isolated beta cells from EMC virus-infected mice were smeared on clean glass slides and fixed with acetone for 10-20 min at room temperature. The slides were flooded with labeled

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antibody and incubated in a moist chamber at room temperature for 2 h or 37°C for 60 min. The slides were then washed three times for 10 min with PBS, mounted with a few drops of buffered glycerol, and observed with a Zeiss microscope (Carl Zeiss, Inc., New York), using a UG-1 exciter filter system. Beta cells from the uninfected mice served as controls. The specificity of the labeled gamma globulin was determined by inhibition tests using unlabeled anti-EMC antibody (6, 12). To determine the percentage of cells containing viral antigen, approximately 400 cells were counted.

FITC-Labeled Anti-Insulin Antibody. Gamma globulin that was prepared by immunizing guinea pigs with glutaraldehyde polymerized porcine insulin and purified by Sephadex G-200 chromatography was purchased from Inolex Corp., Glenwood, Ill. This material was labeled with FITC as described above.

Beta cells isolated from uninfected mice were smeared on clean glass slides, fixed with buffered 6% glutaraldehyde and 10% formalin for 6 h, and then washed thoroughly in distilled water (13). The fixed cells were flooded with FITC-labeled anti-insulin antibody and then incubated in a moist chamber for 2 h at room temperature or overnight at 4°C. The slides were washed, mounted, and observed with a Zeiss microscope as described above. Mouse embryo fibroblasts prepared from CAF<sub>1</sub> mice served as controls.

Histochemical Staining. To identify insulin-containing beta cell granules, freshly isolated pancreatic cells or monolayer cultures were fixed with Bouin's solution and stained with aldehyde thionin (14).

*Extraction of Insulin and Measurement by Radioimmunoassay.* Insulin was extracted from the pancreas of infected or uninfected mice and from freshly isolated beta cells by methods described elsewhere (15). The concentration of insulin from pancreas was measured by radioimmunoassay techniques (16) using mouse insulin as a standard.

Glucose Assay. Blood was obtained from the retro-orbital venous plexus of infected or uninfected mice. Glucose levels were measured enzymatically by the glucose oxidase method with odianisidine dihydrochloride as the reactive dye (17).

#### Results

Isolation and Identification of Pancreatic Beta Cells. Pancreatic beta cells, before and after Ficoll gradient purification, were smeared on a clean glass slide and examined with an inverted phase contrast Zeiss microscope. Before purification, the cell population appeared heterogeneous, containing both large and small cells and many degranulated cells (Fig. 1A). In contrast, cells obtained from the Ficoll gradient appeared more homogeneous and were largely granulated (Fig. 1 B).

To determine what proportion of these cells were insulin-containing beta cells, a few drops of the cell suspension were placed on a glass slide and stained with either FITC-labeled anti-insulin antibody or aldehyde-thionin. Specific immunofluorescence was detected in the cytoplasm of the cells (Fig. 2 a). Staining with aldehyde-thionin revealed that the majority of the cells contained the characteristic dark granules of beta cells (not shown). By examining close to 800 cells, it was found that approximately 90% of the cells stained positively with either FITC-labeled anti-insulin antibody or aldehyde-thionin. Extraction of the purified cells and analysis by radioimmunoassay yielded 2,200 ng of insulin per  $10^5$  cells.

Beta Cells Infected in Vivo. Ficoll gradient-purified beta cells isolated from susceptible SWR/J or resistant C57BL/6J mice which had been infected in vivo 72 h earlier were placed on clean glass slides and examined for morphological changes using an inverted phase contrast microscope. Beta cells from mice that had been inoculated with a 10% normal mouse heart suspension served as controls. Fig. 3A shows that beta cells from infected C57BL/6J mice were heavily granulated, while beta cells from infected SWR/J mice were largely

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FIG. 1. Pancreatic islet cells from uninfected SWR/J mice before (A) and after (B) centrifugation through a Ficoll gradient. Greater homogeneity of cell population is observed in the material obtained from the Ficoll gradient ( $\times$  750).

degranulated (Fig. 3B). Beta cells from uninfected controls (SWR/J or C57BL/ 6J) were heavily granulated (not shown).

To see whether there was any difference in the ability of the virus to replicate in beta cells from susceptible SWR/J, as compared to resistant C57BL/6J mice, Ficoll gradient-purified beta cells were obtained at different times after infection and assayed for virus. The data in Fig. 4 show that at 40 h after infection a 10-fold difference existed in the amount of infectious virus recovered from beta cells of SWR/J as compared to C57BL/6J mice. At 120 h after infection, the virus titer in SWR/J beta cells had dropped by over 90%, while there was no significant change in the virus titer of C57BL/6J beta cells.



FIG. 2. (a) Pancreatic beta cells from uninfected SWR/J mice stained with FITC-labeled anti-insulin antibody ( $\times$  850). (b) Pancreatic beta cells from SWR/J mice 2 days after infection stained with FITC-labeled anti-EMC antibody ( $\times$  850).

To determine how many cells in the pancreas of SWR/J, as compared to C57BL/6J, were productively infected with EMC virus, Ficoll gradient-purified beta cells were isolated from mice 48 h after infection. The cells were counted, diluted, and assayed for infectious centers on confluent monolayers of secondary mouse embryo cells (Table I). Approximately 90% of the cells from SWR/J mice produced infectious centers, while only 11% of cells from C57BL/6J mice yielded virus.

In parallel experiments, pancreatic beta cells isolated from infected SWR/J and C57BL/6J were stained with FITC-labeled anti-EMC antibody to see what percentage of the cells contained viral-specific antigens (Fig. 2b). Specific immunofluorescence was detected in the cytoplasm of approximately 84% of the SWR/J cells as compared to only 7% of the C57BL/6J cells (Table I).

The capacity of the virus to replicate in beta cells from other inbred strains of mice was studied in two strains (Swiss NIH and DBA/J) that develop diabetes and two strains (CBA/J and AKR) that do not develop diabetes after infection with EMC virus (7). Ficoll gradient-purified beta cells were obtained 72 h after infection, and the amount of infectious virus was determined. The data in Table II, show that strains that developed diabetes contained more virus in their beta cells ( $6-8 \times 10^5$  PFU) than the strains that did not develop diabetes ( $6-9 \times 10^4$  PFU). Moreover, microscopic examination revealed that beta cells from susceptible strains were degranulated, while the majority of beta cells from resistant strains appeared to be intact.



FIG. 3. Beta cells isolated from susceptible and resistant strains of mice 72 h after infection with EMC virus. Beta cells from resistant C57BL/6J mice contain insulin granules (A), while beta cells from susceptible SWR/J mice are largely degranulated (B) ( $\times$  800).

Comparison of Insulin Content in Pancreas of Susceptible and Resistant Mice Infected In Vivo. To see whether virus-induced beta cell degranulation truly represented a decrease in insulin, the insulin content in the pancreas of SWR/J and C57BL/6J was measured at different times after infection. The data in Table III show that EMC virus resulted in a marked decrease in the insulin content of the pancreas of SWR/J mice. In contrast, infection of C57BL/6J mice resulted in only a transient or slight reduction in insulin content.



FIG. 4. Viral replication in beta cells of mice susceptible and resistant to EMC virusinduced diabetes. 5-wk-old SWR/J ( $\bigcirc - \bigcirc$ ) and C57BL/6J ( $\bigcirc - - \bigcirc$ ) mice were inoculated intraperitoneally with 5 × 10<sup>3</sup> PFU of EMC virus. At the times indicated, pancreas were removed from 10 mice, the beta cells were isolated, and virus titer determined. Data are expressed in PFU per pancreas.

TABL Percent Beta Cells Infec	TABLE I   Percent Beta Cells Infected with EMC Virus*				
A	Cells infected				
Assay	SWR/J	C57BL/6J			
	%				
Infectious center	90	11			
Immunofluorescence	84	7			

\* Ficoll gradient purified beta cells were isolated from mice 48 h after infection.

TABLE II
Viral Replication and Degranulation in Beta Cells from Strains of Mice Susceptible and
Resistant to the Diabetogenic Effect of EMC Virus*

Strain of mouse	Sex	Susceptible to EMC-induced diabetes	Degranulation of beta cells	Virus titer	
				Log <sub>10</sub> , PFU	
SWR/J	М	Yes	Yes	Yes 7.1 × 10 <sup>5</sup>	
Swiss NIH	М	Yes	Yes 8.0 ×		
DBA/J	М	Yes	Yes	<b>6.4</b> × 10 <sup>5</sup>	
C57BL/6J	М	No	No	$9.1 \times 10^4$	
CBA/J	М	No	No	$7.8 \times 10^4$	
	F	No	No	$6.8 \times 10^4$	
AKR	М	No	No	$8.1 \times 10^{4}$	
	F	No	No	$6.3  imes 10^4$	

\* Beta cells isolated from 10 mice, 72 h after infection, were pooled, the virus titer determined, and data expressed as PFU (log<sub>10</sub>) per 10 mice. Cells placed on glass slides were evaluated microscopically for degranulation.

u	un EMC virus		
Days after infection	IRI		
	SWR/J	C57BL/6J	
	μg/g po	uncreas	
0‡	$595 \pm 125$	$405 \pm 24$	
1	$350 \pm 50$	$455 \pm 121$	
2	$145 \pm 24$	$426 \pm 121$	
3	$76 \pm 27$	$132 \pm 20$	
4	$118 \pm 24$	$312 \pm 28$	
5	$169 \pm 42$	$255 \pm 28$	
6	$175 \pm 29$	$355~\pm~16$	
7	$34 \pm 8$	$302 \pm 24$	
11	$142 \pm 40$	NT	
15	$112 \pm 31$	NT	

TABLE III
Immunoreactive Insulin (IRI) in the Pancreas of Mice Infected
with EMC Virus*

\* At each of the times indicated, pancreas from three SWR/J or three C57BL/6J mice were weighed and the insulin content determined. Data are expressed as micrograms of insulin per gram of pancreas (wet weight).

‡ Uninfected mice.

§ Mean ± SD.

Not tested.

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Freshly Isolated Beta Cells Infected In Vitro. To see if there was a difference in viral replication and cell viability when beta cells from susceptible and resistant mice were infected in vitro, a suspension of freshly isolated Ficoll gradient-purified beta cells from SWR or C57BL/6J mice were infected in vitro with EMC virus at a multiplicity of infection of 100. After a 60-min adsorption period a 37°C, the cells were washed three times and resuspended in maintenance medium (Eagle's MEM containing 3% heat-inactivated fetal calf serum and 70 mg/ml of p-glucose). Approximately  $1.0 \times 10^5$  cells were plated on 35-mm Petri dishes, and at different times thereafter, the amount of virus in the beta cells was determined. Data from a representative experiment (Fig. 5) show that approximately 95% of the SWR/J and C57BL/6J beta cells were viable during the first 15 h of the experiment. At 52 h after infection, about 90% of the C57BL/6J beta cells were still viable. Similarly, over 90% of the beta cells from uninfected C57BL/6J and SWR/J mice retained their viability (data not shown). In contrast, only 50% of the infected SWR/J beta cells remained viable. Although there was relatively little difference in virus titer between the infected groups, beginning at about 24 h the titer of the virus in the SWR cultures began to decline, while the titer in the C57BL/6J cultures showed a slight rise. This trend occurred in three separate experiments.

Monolayers of Beta Cells Infected In Vitro. To see if differences in viral replication were more pronounced when cultured rather then freshly isolated beta cells were used, monolayers of beta cells (Fig. 6) were prepared as described in Materials and Methods. The cells in the culture were predominantly epithelioid in appearance. At 3 days, they contained heavy cytoplasmic granules in the perinuclear region which generally stained strongly positive with aldehyde-thionin. The intensity of staining, however, decreased with the length of time that the cells were in culture. A small percentage of fibroblasts were sometimes seen; these cells were morphologically distinct from the epithelioid cells and did not stain with aldehyde-thionin. The epithelioid cells, but not the fibroblasts, stained with FITC-labeled anti-insulin antibody. On the basis of staining with FITC-labeled anti-insulin antibody and aldehyde-thionin, it was estimated that 85–95% of the cells in the monolayers were beta cells.

The monolayer cultures containing approximately  $5 \times 10^5$  cells were infected with EMC virus at a virus to cell ratio of 10. After a 60-min adsorption at 37°C, the cells were washed three times and placed on chemically defined maintenance medium (MPNL 65/C plus 1% heat-inactivated calf serum) (10). At different times thereafter, cells were harvested and the amount of infectious virus determined. Three separate experiments were performed. Data from a representative experiment (Fig. 7) show that more virus was produced by SWR/ J beta cells than C57BL/6J beta cells. Up to a 50-fold difference in virus titer was observed 18-24 h after infection. At about 30 h, the virus titer in the SWR/J cultures began to decline. In contrast, the virus titer in the C57BL/6J cultures did not change from 40 to 60 h after infection.

Examination of the SWR/J monolayers revealed marked cytopathology at 3 days after infection with almost complete destruction of the monolayers at 7 days. In contrast, the C57BL/6J cultures showed only minimal signs of cytopathology at 3 days after infection with slight progression at 7 days.

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FIG. 5. Replication of EMC virus in freshly isolated beta cells from SWR/J and C57BL/6J mice. At zero time, approximately  $1.0 \times 10^5$  cells were inoculated with  $1.0 \times 10^7$  PFU of EMC virus. At the times indicated, the amount of infectious virus and the percent viable cells per plate were determined. Virus titer (PFU, log 10); SWR/J (---), C57BL/6J (---). Viable cells (%); SWR/J (----), C57BL/6J ( $\Delta---\Delta$ ).

Viral Replication in Mouse Embryo and Kidney Cell Cultures. To see whether host differences in the capacity of the virus to replicate in pancreatic beta cells existed in other cell types from the same host, primary embryo and kidney cells from SWR/J and C57BL/6J were infected with EMC virus at a virus to cell ratio of 10. After a 60-min adsorption at 37°C, the monolayers were washed three times with Eagle's MEM. At different times thereafter, cells were harvested and the virus titer determined.

The data in Fig. 8 show that virus replicated equally well in cultures prepared from SWR/J and C57BL/6J mice. Maximum titers were reached at 16–20 h after infection. Marked cytopathology was observed 1–2 days after infection, followed by complete degeneration of the cells on days 3 and 4. This was considerably earlier than the appearance of cytopathologic changes in beta cell cultures from SWR/J mice that are susceptible to the diabetogenic effect of EMC virus.

# Discussion

The capacity of virus to replicate in beta cells has been difficult to study because the pancreas contains cells of several different types and because in



FIG. 6. Confluent monlayer of uninfected SWR/J pancreatic beta cells 2 wk after seeding ( $\times$  900).

vitro tissue culture methods have not been employed. By use of the purification methods described here, it has been possible to obtain preparations containing up to 90% beta cells and to prepare monolayers of beta cells. Moreover, these purification procedures greatly reduce the amount of viremic blood contaminating pancreatic tissue, making it possible to more accurately quantitate viral replication in beta cells isolated from in vivo-infected animals. By use of these procedures, we showed that in the pancreas of strains of mice susceptible to the diabetogenic effect of EMC virus, the virus replicated to a greater extent than in the pancreas of resistant strains. Moreover, examination of beta cells from susceptible and resistant strains revealed marked degranulation and a decrease in the insulin content of the beta cells from susceptible strains. To determine whether the lower viral titers recovered from beta cells of resistant strains represented less viral replication per cell or fewer cells infected, immunofluorescent and infectious center assays were used. These studies showed that the



FIG. 7. Replication of EMC virus in 2-wk-old monolayers of pancreatic beta cells from SWR/J (---) and C57BL/6J (---) mice. At zero time, approximately  $5.0 \times 10^{\circ}$  cells were inoculated with  $5.0 \times 10^{\circ}$  PFU of EMC virus. Data are expressed as PFU per plate.

number of beta cells infected with EMC virus in SWR/J mice was approximately 10 times greater than in C57BL/6J mice, thereby supporting the contention that the higher viral yield was due to more cells infected.

Further evidence that there was less viral replication in beta cells from mice resistant to the diabetogenic effect of EMC virus came from in vitro infection of beta cell cultures. Approximately 50 times more virus was recovered from monolayers of beta cells prepared from susceptible SWR/J mice as compared to resistant C57BL/6J mice. Moreover, SWR/J cultures were more readily destroyed by the infection than C57BL/6J cultures. Although less dramatic differences in viral titers were observed when freshly isolated beta cells were infected in vitro, the SWR/J cultures were still more rapidly destroyed than the C57BL/ 6J cultures. Loss of viral receptors during cell preparation or the altered physiologic state of freshly isolated beta cells, as compared to monolayers that had been in culture for 2 wk, could account for the difference.



FIG. 8. Replication of EMC virus in monolayer cultures of primary mouse kidney cells (A) and primary mouse embryo cells (B) from SWR/J (---) and C57BL/6J ( $\Delta - -\Delta$ ) mice. At zero time, approximately  $1.0 \times 10^6$  cells were inoculated with  $1.0 \times 10^7$  PFU of EMC virus. Data are expressed as PFU per plate.

Despite the failure of C57BL/6J mice to develop diabetes (7, 8), the demonstration here (Table I) that some of the Ficoll gradient-purified cells from these mice support viral replication suggests that resistance may not be complete. Microscopically, the C57BL/6J cells that stained with FITC-labeled anti-EMC virus antibody were largely epithelioid and looked like typical beta cells. The possibility exists, however, that there are subpopulations of beta cells in C57BL/6J mice with different degrees of susceptibility to EMC virus. Alternatively, the infected cells might be "contaminants" not removed during the initial purification procedure (e.g., fibroblasts, alpha cells, ductal cells).

The nature of the genetically determined factor(s) controlling the develop-

ment of viral-induced diabetes is not known. The fact that differences in viral replication can be demonstrated in vitro argues against differences in the host's immune response to the virus as an explanation for susceptibility versus resistance. Support for this argument also comes from studies that failed to show any differences in the time of appearance or amount of neutralizing antibody made against EMC virus in susceptible as compared to resistant mice (7, and unpublished data).

In contrast to the differences in viral replication observed in the pancreas, EMC virus replicated equally well in kidney and embryo cell monolayers from SWR/J and C57BL/6J mice. This suggests that the genetically determined factors controlling the development of viral-induced diabetes act at the level of the beta cell. A variety of factors could influence viral titer in beta cells: for example, genetically determined factors might control the number of viral receptors on the surface of beta cells; the ability of virus to replicate in beta cells; the rate of repair of damaged beta cells; or the responsiveness of beta cells to protection by interferon. These and other possibilities are now under investigation.

Our studies on EMC virus replication in kidney and embryo cells, as compared to beta cells of inbred strains of mice, suggest that genetically determined factors may affect the susceptibility of one cell type to a particular viral infection, without necessarily affecting the susceptibility of other cell types in the same host. In this connection, it should be emphasized that EMC virus is pantropic; it replicates in many organs, and death is usually due to myocarditis or encephalitis (18, 19). Lethality varies considerably among EMC-infected inbred strains of mice, but there does not appear to be any direct relationship between lethality and beta cell damage (4, 8, 19). The possibility that susceptibility of different organ systems to EMC infection may be under the control of different genes merits exploration. In addition, our studies in mice suggest that if a virus is capable of attacking beta cells in man, genetic differences in the susceptibility of these cells may determine whether or not the exposed individual will develop clinical diabetes.

## Summary

Beta cells were isolated from strains of mice that were susceptible and resistant to encephalomyocarditis (EMC) viral-induced diabetes mellitus. Beta cells from susceptible mice that were infected in vivo with EMC virus showed higher viral titers, more severe degranulation, and lower concentrations of immunoreactive insulin than beta cells from resistant mice. Immunofluorescence and infectious center assays revealed that pancreas from susceptible mice contained at least 10 times more infected cells than pancreas from resistant mice. Beta cell cultures prepared from susceptible mice and infected in vitro also showed higher viral titers and more severe cytopathologic changes than beta cell cultures from resistant mice. In contrast to beta cell cultures, virus replicated equally well in primary embryo and kidney cell cultures from susceptible and resistant strains of mice. It is concluded that the development of EMC virus-induced diabetes is related to genetically determined host differences in the capacity of the virus to infect beta cells.

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