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Determination of Encephalomyocarditis Viral Diabetogenicity by a Putative Binding Site of the Viral Capsid Protein

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EMC, encephalomyocarditis; FITC, fluorescein isothiocyanate; MEM, minimal essential medium; PBS, phosphate-buffered saline; PFU, plaque-forming units; RIA, radioimmunoassay; TRITC, tetramethyl rhodamine isothiocyanate.

The molecular mechanism by which some, but not all, variants of encephalomyocarditis (EMC) virus selectively infect pancreatic β -cells in mice and induce IDDM has been an enigma for more than a decade. We report here that the binding site of the EMC viral capsid protein VP1 determines viral diabetogenicity. Recombinant chimeric EMC viruses containing threonine, serine, proline, aspartic acid, or valine at position 152 of the major capsid protein VP1 bind poorly to β -cells. In contrast, recombinant chimeric EMC viruses containing alanine or glycine at position 152 of the VP1 bind efficiently to and infect β -cells, resulting in the development of diabetes. Three-dimensional molecular modeling reveals that the van der Waals interactions are greater and the residues surrounding position 152 of the VP1 are more closely packed in recombinant chimeric viruses containing threonine, serine, proline, aspartic acid, or valine at position 152 than in recombinant chimeric viruses containing alanine or glycine at the same position. Our studies reveal that the surface areas surrounding alanine or glycine at position 152 of the VP1 are more accessible, thus increasing the availability of the binding sites for attachment to β -cell receptors and resulting in viral infection and the development of diabetes. *Diabetes* 47:576–582, 1998

Substitution of a single amino acid in a viral protein can have profound effects on viral pathogenesis (1–6). Because of the diabetogenic capabilities of encephalomyocarditis (EMC) virus (7–12), the molecular basis of EMC virus-induced diabetes in mice has received considerable attention. The D variant of this virus selectively destroys pancreatic β -cells, resulting in the onset of IDDM. In contrast, the B variant does not destroy pancreatic β -cells. When SJL/J mice are infected with EMC-D virus, over 90% of the infected animals develop

diabetes, whereas none of the mice infected with EMC-B virus develop diabetes (13). However, these two variants cannot be distinguished antigenically by a sensitive plaque neutralization assay or a competitive radioimmunoassay (RIA) (13,14). Examination of the complete nucleotide sequences of the genomes of the two variants revealed a total of 14 nucleotide differences between them (15). Further investigation, using 21 different nondiabetogenic and 15 different diabetogenic mutant viruses generated from stocks of EMC-B and EMC-D variants, revealed that all nondiabetogenic variants contained Thr at position 152 of the VP1, whereas all diabetogenic variants contained Ala at the same position (16). Recently, we demonstrated that a single point mutation at nucleotide position 3155 or 3156 (the first or second base in the codon for the amino acid at position 152 of the VP1, also the amino acid at position 776 of the viral polyprotein), which causes an amino acid change at position 152, results in a loss or gain of viral diabetogenicity (17). The present study was initiated to investigate the possibility that alterations in the binding of the recombinant chimeric EMC viruses to β -cells are responsible for the changes in viral diabetogenicity.

RESEARCH DESIGN AND METHODS

Animals. Six-week-old male SJL/J mice were purchased from Jackson Laboratory (Bar Harbor, ME). The animals were maintained under pathogen-free conditions at the Animal Resource Centre, University of Calgary (Calgary, Alberta, Canada).

Viruses and viral RNA. Diabetogenic EMC-D and nondiabetogenic EMC-B viruses were grown in L-929 cells (13), purified by CsCl and sucrose gradient ultra-centrifugation, and used in the preparation of viral RNA by phenol-chloroform extraction (14,18). The integrity and purity of the viral RNA was confirmed by denaturing gel electrophoresis and measurement of the A_{260}/A_{280} ratio (19).

Construction of mutated recombinant chimeric RNA and production of recombinant chimeric viruses. EMC-B and EMC-D cDNAs were synthesized, and full-length cDNA was constructed as described elsewhere (16,19). Site-specific mutagenesis was performed at nucleotide position 3155 (the first base of the codon for the 152nd amino acid of the VP1) with various mutagenic oligonucleotides, as follows: Ala¹⁵²-CCAACTGGAACGCCAGGCCAAGCCAACCACTC; Thr¹⁵²-CCAACTGGAACGCCAAACCAAGCCAACCACTC; Ser¹⁵²-CCAACTGGAACG CCATCCAAGCCAACCACTC; Pro¹⁵²-CCAACTGGAACGCCACCCAAGCCAACCACTC; Asp¹⁵²-CCAACTGGAACGCCAGACAAGCCAACCACTC; Val¹⁵²-CCAACTGGAACGCCATCAAGCCAACCACTC; and Gly¹⁵²-CCAACTGGAACGCCAG GCAAGCCAACCACTC (Fig. 1). We constructed the chimeric RNA by attaching the poly(C) region of the viral RNA to the 5'-truncated RNA transcript of the EMC viral cDNA (20). After transfection of the chimeric RNA into L-929 cells, progeny viruses were produced (Fig. 1). The nucleotide sequences of the mutated regions of the progeny viruses were determined by direct RNA sequencing.

Glucose and insulin assay. Glucose levels were measured in blood by a glucose oxidase assay with *O*-dianisidine dihydrochloride as the indicator dye (13). Insulin was extracted from the frozen pancreas by the acid-ethanol extraction method (21). The concentration of immunoreactive insulin was determined with an insulin RIA kit (Pharmacia Biotech, Uppsala, Sweden). Data were expressed as microgram of insulin per gram of pancreas.

Histology. Histological examination of the pancreas of each mouse was performed as described previously (22). The pancreas was removed at 17 days after virus infection. Fifty percent of each pancreas was fixed in 10% buffered formalin phosphate. Paraffin-embedded sections were stained with hematoxylin and eosin. The islets (17–25 per mouse) were examined under a light microscope.

Double-labeled antibody staining. To identify EMC virus-infected β -cells, sections of each pancreas from EMC virus-infected mice, obtained 3 days after infection, were fixed on slides in acetone and stained with tetramethyl rhodamine isothiocyanate (TRITC)-labeled anti-insulin antibody and fluorescein isothiocyanate (FITC)-labeled anti-EMC antibody (23) overnight at 4°C. The slides were washed three times with phosphate-buffered saline (PBS), mounted with Elvanol, and examined for

immunofluorescence by fluorescent microscopy with rhodamine filters and then fluorescein filters (23).

Competitive virus binding assay. The pancreases from each of 50 5-week-old male SJL/J mice were injected with collagenase solution (Worthington Type IV) through the common bile duct. Each pancreas was then dispersed by aspiration with a Pasteur pipette. After ficoll gradient centrifugation, the islets were handpicked under a stereomicroscope and then dispersed into single cells (24). The number of pancreatic β -cells was adjusted to 2×10^6 cells/ml in Eagles's minimal essential medium (MEM) (Life Technologies, Gaithersburg, MD) with 2% fetal bovine serum.

Unlabeled purified recombinant chimeric EMC virus (10^4 plaque-forming units [PFU] per cell) was added to 500 μ l of the β -cells suspension (1×10^6 cells). The suspension was incubated at 4°C for 1 h with rotation at 100 rpm. Unbound unlabeled virus was removed by washing three times with PBS (centrifugation at 12,000 rpm). 35 S-labeled purified wild-type EMC-D virus (10^8 PFU/ 10^6 cells – 10^2 PFU/cell) was added to the suspension, and unbound virus was removed by washing with PBS as described above. The precipitate was then dissolved in 0.15 mol/l NaOH, mixed with an aqueous cocktail solution, and the radioactivity was measured. A similar competitive virus binding assay was performed using L-929 cells.

To determine the competitive binding of each chimeric EMC virus with a labeled wild-type EMC-D virus, we calculated the percentage of 35 S-labeled EMC-D virus binding after incubation of a saturated level of each unlabeled chimeric EMC virus with β -cells. Control binding was determined in the absence of unlabeled chimeric EMC virus. The specific binding of each recombinant chimeric virus to β -cells was calculated as a percentage of inhibition of 35 S-labeled EMC-D virus binding by incubation of each unlabeled recombinant chimeric EMC virus with the labeled virus. The formula used to calculate the specific binding of each recombinant chimeric virus is as follows: $(1 - [\% \text{ mean of labeled EMC-D virus binding after addition of unlabeled chimeric virus} / \% \text{ mean of control binding of labeled EMC-D virus}]) \times 100$.

Plaque assay. The viral titer was determined as previously described (9). Briefly, confluent monolayers of secondary mouse embryo cells were inoculated with various dilutions (0.2 ml) of each virus preparation (recombinant chimeric viruses and wild-type EMC viruses). After adsorption for 1 h at 37°C, the cultures were overlaid with 2% methylcellulose in MEM containing 5% fetal calf serum. Monolayers were stained 2 days later with neutral red. Plaques were counted within 24 h.

Molecular modeling. Based on the structure of the Mengo virus reported by Luo et al. (25), molecular models were constructed with the computer program Insight II, Version 2.3.5 (Biosym Technologies, San Diego, CA).

RESULTS AND DISCUSSION

We recently demonstrated that the amino acid at position 152 of the viral capsid protein VP1 of EMC virus determines viral diabetogenicity (17). Recombinant chimeric viruses containing Ala at position 152 of the VP1 caused diabetes in SJL/J mice, whereas viruses with Thr, Ser, Pro, Asp, or Val at the same position did not. In the present study, we tested a recombinant chimeric virus containing Gly at position 152 of the VP1. SJL/J mice infected with the Gly¹⁵² chimeric viruses developed diabetes comparable in severity to that found in mice infected with the Ala¹⁵² chimeric viruses (Fig. 2A). In addition, the pancreatic insulin levels of the Gly¹⁵² chimeric virus-infected animals were markedly reduced (Fig. 2B). In contrast, mice infected with Thr¹⁵², Ser¹⁵², Pro¹⁵², Asp¹⁵², or Val¹⁵² chimeric viruses did not develop diabetes, and their pancreatic insulin levels were within the normal range (Fig. 2A and B). The examination of the pancreases of mice infected with Ala¹⁵² or Gly¹⁵² chimeric viruses showed marked destruction of the pancreatic β -cells as compared with mice infected with the other chimeric viruses (data not shown). Moreover, immunohistochemistry, using antibodies against EMC virus and insulin, revealed EMC viral antigens in the β -cells of animals infected with the Ala¹⁵² or Gly¹⁵² chimeric viruses but not in animals infected with the other chimeric viruses, as reported previously (17).

Because it is well known that the diabetogenic EMC virus selectively infects and destroys pancreatic β -cells (9–10,13), and that the initial step in viral infection is the attachment of the virus to the target cell, we compared the specific binding activity of diabetogenic recombinant chimeric viruses to that of nondiabetogenic recombinant chimeric viruses. We found that a significantly higher amount (87–90%) of diabetogenic chimeric viruses (containing Ala¹⁵² or Gly¹⁵²) attached to pancreatic β -cells when compared with the attachment of non-diabetogenic chimeric viruses (5–7%) ($P < 0.0001$) (Table 1). We also examined the amount of attachment of diabetogenic and nondiabetogenic chimeric viruses to a non- β -cell line (L-929 cells). We found that the amount of attachment of diabetogenic chimeric viruses to L-929 cells was significantly higher (86–91%) than that of nondiabetogenic chimeric viruses (41–48%) ($P < 0.001$) (Table 1). A competitive binding assay revealed that, when compared with the amount of attachment to β -cells (5–7%) (Table 1), nondiabetogenic chimeric viruses exhibited a significantly higher amount of attachment to L-929 cells (41–47%). This greater amount of attachment may be due to the greater number of viral receptors expressed on cultured L-cells.

We measured viral replication in pancreatic β -cells at different times (1–5 days) after infection. We found that the viral titer in the pancreatic islets of mice infected with diabetogenic chimeric viruses was significantly higher than that in the islets of mice infected with nondiabetogenic viruses (Fig. 3A). The titer of the diabetogenic chimeric viruses peaked at 2 days after infection, reaching close to 5×10^5 PFU/ml of pancreatic extract, and then decreased sharply from 3 through 5 days after infection (Fig. 3A). This decrease in infectious virus titer may be due to an increase in the titer of antibodies against the viruses, as reported previously (13). In contrast, the replication of the nondiabetogenic chimeric viruses in the pancreatic islets appears to be minimal. The peak viral titer, at 2 days after infection, was only just over 10^3 PFU/ml of pancreatic extract, and the titer decreased slightly from 3 through 5 days after infection (Fig. 3A). These results show an ~500-fold difference between the viral titers of diabetogenic and nondiabetogenic chimeric viruses in the pancreatic β -cells at 2 days after infection.

The detection of some infectious viruses in the pancreatic islet cell preparation from mice infected with nondiabetogenic chimeric EMC viruses may be due to viremia, since it is difficult to completely remove viruses from the circulating blood, or may be due to replication of the virus in pancreatic fibroblasts or ductal cells, which are contaminants during the preparation of pancreatic β -cells. However, we could not exclude the possibility of replication of nondiabetogenic viruses in the pancreatic β -cells if they do attach to the β -cells. To determine whether any pancreatic β -cells were, in fact, infected by nondiabetogenic chimeric EMC viruses, we double-stained pancreatic sections prepared from mice infected with the nondiabetogenic chimeric viruses with TRITC-labeled anti-insulin antibody and FITC-labeled anti-EMC viral antibody. None of the pancreatic β -cells were stained with anti-EMC viral antibody (data not shown). This result indicates that the pancreatic β -cells are not visibly infected by the nondiabetogenic chimeric EMC viruses, and that the detection of infectious viruses in the pancreatic islet cell preparation could be due to contamination during preparation of the islet cells or replication of the virus in contaminating non- β -cells, including fibroblasts and/or ductal cells. As a matter of fact, the replication of the viruses in the pancreatic β -cells appears to reflect the attachment of the viruses to the β -cells.

However, a different situation exists with respect to the attachment and replication of the viruses in cultured non- β -cells, such as L-929 cells. Although the diabetogenic chimeric EMC viruses bound more efficiently to the L-929 cells than did the nondiabetogenic chimeric EMC viruses, the nondiabetogenic chimeric viruses showed a significantly higher amount of attachment to L-929 cells than to β -cells (Table 1). In addition, there appeared to be no significant difference in the rate of viral replication between diabetogenic and nondiabetogenic chimeric viruses in L-929 cells, even though the viral titers of diabetogenic chimeric viruses

were significantly higher than those of nondiabetogenic chimeric viruses (Fig. 3B). This difference may be due to the differing susceptibilities of the β -cells and the non- β -cells to the viruses.

To elucidate the differences observed in the binding activity of diabetogenic and nondiabetogenic chimeric viruses to pancreatic β -cells, we examined the three-dimensional structure of the capsid protein VP1 of a Thr¹⁵² chimeric virus (representative of nondiabetogenic chimeric viruses), and we compared this structure to that of an Ala¹⁵² chimeric virus (representative of diabetogenic chimeric viruses). Because the amino acid sequence of the VP1 of Mengo virus, another cardiovirus, and that of the diabetogenic EMC-D virus are remarkably similar, sharing 96.4% homology (19,26), and because the three-dimensional structure of the VP1 of Mengo virus is known (25), we used the backbone coordinates of the Mengoviral VP1 to construct our models of the Thr¹⁵² and Ala¹⁵² chimeric EMC viruses. Luo et al. (25) showed that the 152nd amino acid of the capsid protein VP1 is located in the deep pit, which is the putative binding site of the Mengo virus to host cell receptors. Furthermore, the 152nd amino acid of the EMC-D virus, Ala, lies in a highly conserved strongly hydrophilic region of the VP1, containing three proximal prolines (Pro-ThrGly-Thr-Pro-Ala¹⁵²-Lys-Pro). This site is the most likely site for cardiovirus attachment to host cell receptors (19,27).

The model of the nondiabetogenic Thr¹⁵² chimeric virus reveals that the side chain hydroxyl group of Thr¹⁵² forms hydrogen bonds with the side chain guanidino group of Arg⁸⁵. Two water molecules are found in the pit area; one water molecule forms hydrogen bonds with Arg⁸⁵ and Thr²²⁴. The replacement of Thr¹⁵² by Ala¹⁵² perturbs the hydrogen-bonded network that maintains the interaction between Thr¹⁵² and Arg⁸⁵. In this case, the hydrogen bonds formed between the guanidino group of Arg⁸⁵ and the hydroxyl group of Thr¹⁵² are missing (Fig. 4: A152). Hydrophobic interactions may also contribute to the conformation of the VP1. The residues surrounding Thr¹⁵² of the capsid protein VP1 of the nondiabetogenic Thr¹⁵² are closer together (Fig. 4: T152) than those of the diabetogenic Ala¹⁵² chimeric EMC virus. Similarly, compact structures are predicted for viruses substituted with Ser, Pro, Asp, or Val at position 152 of the VP1. Additionally, the ionic interaction between Asp¹⁵² and Arg⁸⁵ of the Asp¹⁵² chimeric virus favorably contributes to the compact structure of the proposed receptor binding site. Chimeric viruses containing Gly¹⁵² exhibit a to those containing Ala¹⁵² (Fig. 4: G152). The van der Waals forces may be stronger and accessible surface areas smaller in the capsid protein VP1 containing Thr¹⁵², Ser¹⁵², Pro¹⁵², Asp¹⁵², or Val¹⁵² (Fig. 5: T, S, P, D, V). The loosely packed residues surrounding Ala¹⁵² and Gly¹⁵² may permit recognition of the β -cell receptors by diabetogenic EMC viruses, because the binding site on the virus is more "open." Because Ala¹⁵² is common to all diabetogenic viruses, it has been speculated that the flexibility Arg⁸⁵ provides may play an important role in the recognition of β -cell receptors on diabetogenic EMC viruses. On the basis of our results, we conclude that the interaction of the amino acid at position 152 with neighboring amino acids, such as Arg⁸⁵, may influence the configuration of the binding site of the EMC viral major capsid protein VP1, determining whether the virus can attach to and infect β -cells.

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TABLE 1 The specific competitive binding of various diabetogenic and nondiabetogenic recombinant chimeric EMC viruses to β -cells and L-929 cells was determined, as described in METHODS. The data represent the mean of three individual determinations. * $P < 0.0001$ when compared with the specific competitive binding of Thr¹⁵²-, Ser¹⁵²-, Pro¹⁵²-, Asp¹⁵²-, or Val¹⁵²-containing chimeric virus. † $P < 0.001$ when compared with the specific competitive binding of Thr¹⁵²-, Ser¹⁵²-, Pro¹⁵²-, Asp¹⁵²-, or Val¹⁵²-containing chimeric virus.

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FIG. 1 Schematic representation of the procedure for the production of recombinant chimeric EMC viruses containing a mutated amino acid at position 152 of the capsid protein VP1 (at amino acid position 776 of the polyprotein). The G base at nucleotide position 3155, which is the first nucleotide in the codon for Ala¹⁵² (diabetogenic virus) was changed to an A base by site-specific mutagenesis with a mutagenic oligonucleotide (CCAACTGGAACGCCAACCAAGCCAACCACTC) using a Muta-Gene kit (Bio-Rad, Richmond, CA). Similarly, the A base at nucleotide position 3155 in the codon for Thr¹⁵² (nondiabetogenic virus) was changed to a G base with a mutagenic oligonucleotide (CCAGACTGGAACGCCAGCCAAGCCAACCACTC) using pB104, the EMC-B cDNA clone containing nucleotide bases 1120–3530. Mutants containing the desired nucleotides were screened by sequencing with the M13 universal primer (United States Biochemicals, Cleveland, OH). To create a full-length mutated cDNA clone containing the poly(C) region, the *Sph* I to *Sst* I fragment of the pD104 or pB104 plasmid containing the mutated nucleotide was substituted into the corresponding site of the pEDfH or pEBfH vector, respectively. The vector was renamed pEDfH(Thr¹⁵²) or pEBfH(Ala¹⁵²). The RNA transcript containing A³¹⁵⁵ or G³¹⁵⁵ (nucleotide bases 477–7830) was synthesized with T7 RNA polymerase after linearization of pEDfH(Thr¹⁵²) or pEBfH(Ala¹⁵²) with *Sal*I. The viral RNA fragment containing the poly(C) region (nucleotide bases 1–476) was isolated by RNase-H cutting at nucleotide bases 476/477 (28). Chimeric RNA containing A³¹⁵⁵ or G³¹⁵⁵ was constructed by attaching the transcribed RNA (nucleotide bases 477–7830) to the viral RNA fragment (nucleotide bases 1–476) with RNA ligase and a brace primer. The chimeric RNA was introduced into L-929 cells by calcium-phosphate transfection, and mutated progeny virus was produced. Male SJL/J mice (6 weeks old) were injected intraperitoneally with the progeny virus (1×10⁵ PFU). Any mouse with a glucose level >243 mg/dl (5 SD above the mean blood glucose level of PBS-injected mice) was classified as diabetic. Other substitutions for amino acid 152 were similarly produced using the appropriate mutagenic oligonucleotides.

FIG. 2 Effects of various amino acids at position 152 of the capsid protein VP1 of the EMC virus on the induction of diabetes in SJL/J mice. Six-week-old male SJL/J mice were infected by intraperitoneal injection with various chimeric viruses (1×10⁵ PFU/mouse). PBS-injected and wild-type EMC-D virus-infected mice were used as negative and positive controls, respectively. *A*: Nonfasting (NF) blood glucose levels were measured at 7 and 14 days after infection. Glucose tolerance tests (GTT) were conducted at 10 and 17 days after infection. A glucose index (GI) was calculated for each mouse as follows:

$$GI = \frac{(4 \times \text{NF day 14}) + (3 \times \text{NF day 7}) + (2 \times \text{GTT day 17}) + (1 \times \text{GTT day 10})}{10}$$

Any mouse with a GI >243 mg/dl (5 SD above the mean for nondiabetic control PBS-injected mice) was classified as diabetic. *B*: Concentration of pancreatic immunoreactive insulin was determined 17 days after infection as described in METHODS. Data were expressed as micrograms of insulin per gram of pancreas. **P* < 0.0001 when compared with Thr¹⁵²-, Ser¹⁵²-, Pro¹⁵²-, Asp¹⁵²-, or Val¹⁵²-

containing recombinant chimeric viruses. Each group was composed of 17–32 mice. Male mice were used because of the higher incidence of diabetes they exhibit when compared with female mice.

FIG. 3 A: Replication of recombinant chimeric EMC viruses in pancreatic islets. Six-week-old SJL/J mice were infected intraperitoneally with 1×10^6 PFU of each recombinant chimeric virus (three mice/group). At 1, 2, 3, 4, and 5 days after infection, the pancreatic islets were isolated and virus was extracted from the isolated islets. The infectious virus was assayed. Data represent the mean of three individual determinations (PFU/ml of islet extract). ●, Ala¹⁵²-; ○, Thr¹⁵²-; ▲, Ser¹⁵²-; △, Pro¹⁵²-; ■, Asp¹⁵²-; □, Val¹⁵²-; and ◇, Gly¹⁵²-containing recombinant chimeric virus. B: Replication of recombinant chimeric EMC viruses in L-929 cells. At zero time, $\sim 1 \times 10^6$ L-cells were inoculated with 1×10^7 PFU of each recombinant chimeric virus. The viral titer was determined at 2, 8, 10, 20, 30, 40, and 50 h after infection and expressed as PFU/plate. ●, Ala¹⁵²-; ○, Thr¹⁵²-; ▲, Ser¹⁵²-; △, Pro¹⁵²-; ■, Asp¹⁵²-; □, Val¹⁵²-; and ◇, Gly¹⁵²-containing recombinant chimeric virus.

FIG. 4 Models of the capsid protein VP1 of Thr¹⁵² (T152) (nondiabetogenic), Ala¹⁵² (A152), and Gly¹⁵² (G152) (diabetogenic) chimeric EMC viruses, based on the structure of Mengo virus VP1 reported by Luo et al. (25). Here is a view of the atoms in the region surrounding the 152nd amino acid. Only the regions involved in the proposed receptor-binding site are shown for the purpose of clarity. The Ala and Gly mutations at the 152nd position of VP1 result in the loss of two hydrogen bonds with Arg⁸⁵. Nitrogen atoms are depicted in blue, oxygen in red, and protein carbon in green. The image was generated with the Insight II program, version 2.3.5 (Biosym Technologies).

FIG. 5 A van der Waals surface comparison of Thr¹⁵²-containing virus and six mutants. Mutants of the corresponding residues to Thr¹⁵² in the VP1 were modeled by a direct replacement approach without further geometric optimization. The side chains of the mutants were selected from the rotamer library and further adjusted in such a way that the hydrogen bonding modes of the new models mimic those of Thr¹⁵² in Mengo virus. The van der Waals surfaces are shown in yellow, with the exception of the 152nd residue shown in purple. The images were generated with the Insight II program (Biosym Technologies).

Effects of various amino acids at position 152 of the capsid protein VP1 of the EMC virus on the specific competitive binding of the virus to pancreatic β -cells and L-929 cells

| Unlabeled virus | Specific binding (%) | |
|-----------------------------------|----------------------|-------------|
| | β -cells | L-929 cells |
| EMC-D | 90.1 | 90.7 |
| Ala ¹⁵² chimeric virus | 87.8* | 88.9† |
| Gly ¹⁵² chimeric virus | 86.9* | 86.1† |
| Thr ¹⁵² chimeric virus | 6.4 | 44.1 |
| Ser ¹⁵² chimeric virus | 7.2 | 47.7 |
| Pro ¹⁵² chimeric virus | 5.3 | 41.4 |
| Asp ¹⁵² chimeric virus | 7.3 | 44.7 |
| Val ¹⁵² chimeric virus | 6.7 | 43.5 |

TABLE 1

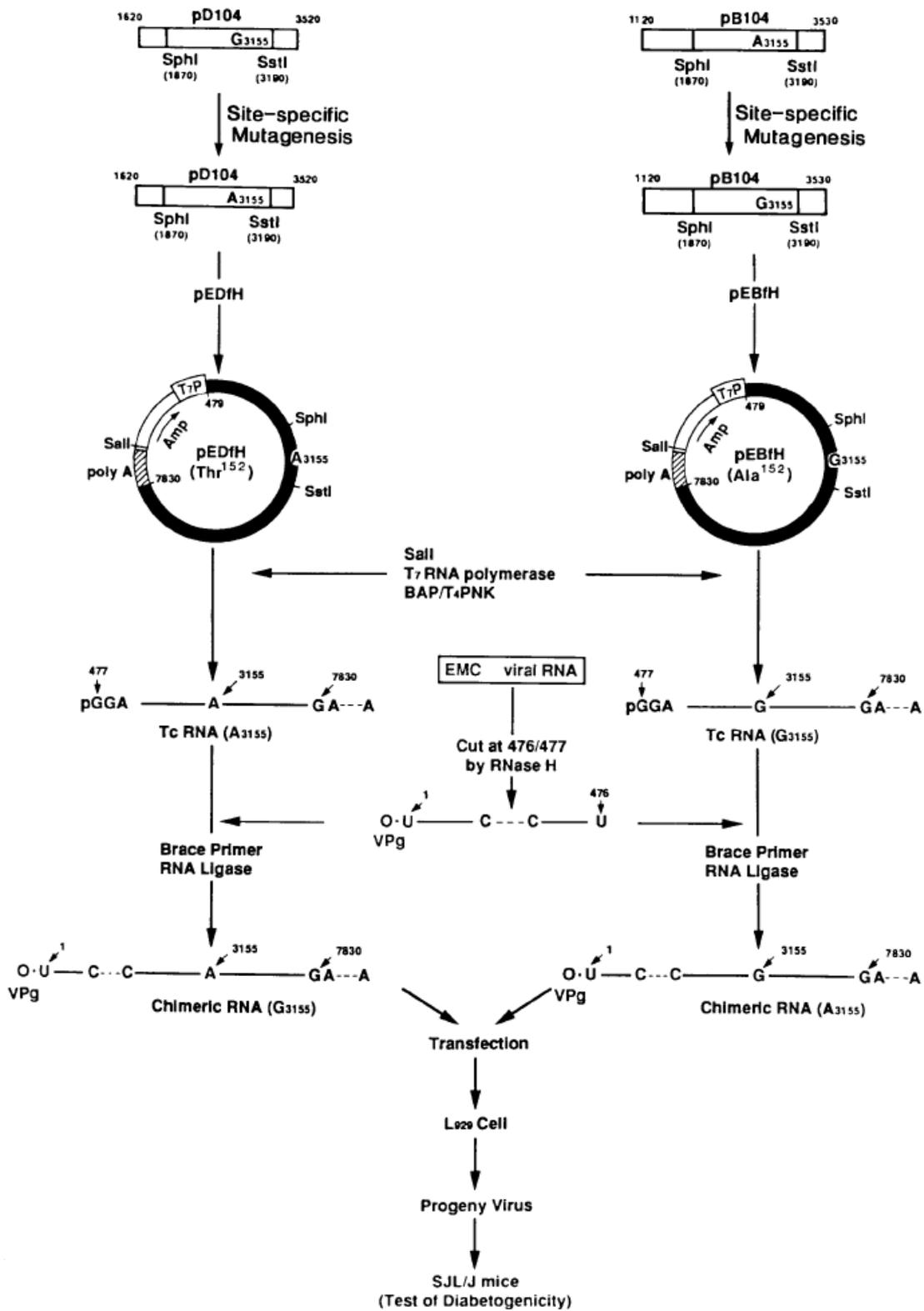


FIG. 1

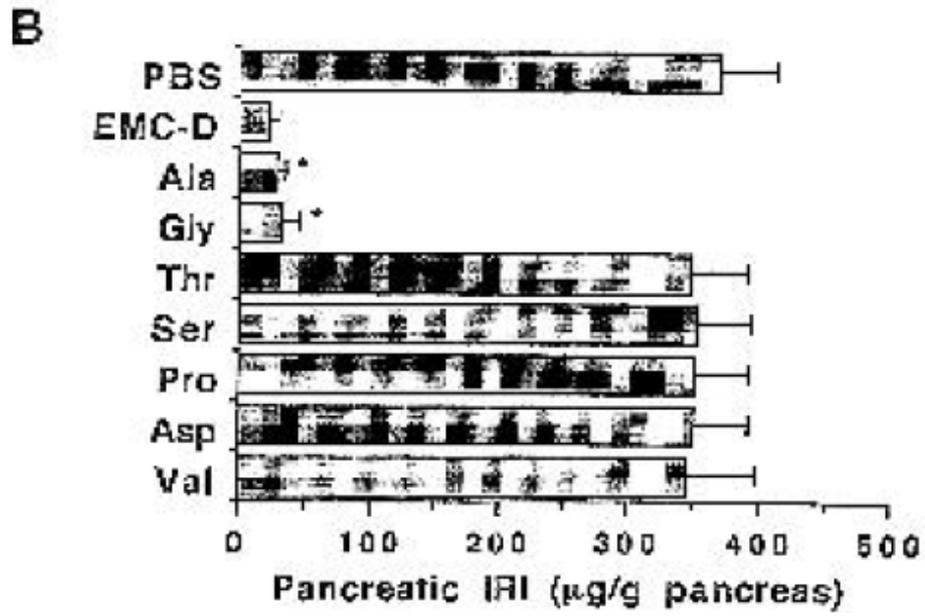
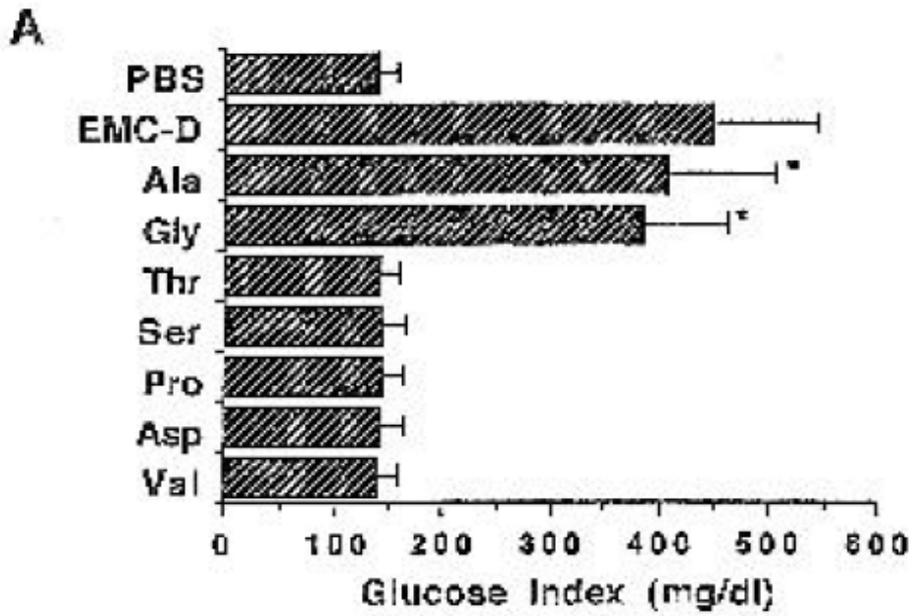


FIG. 2

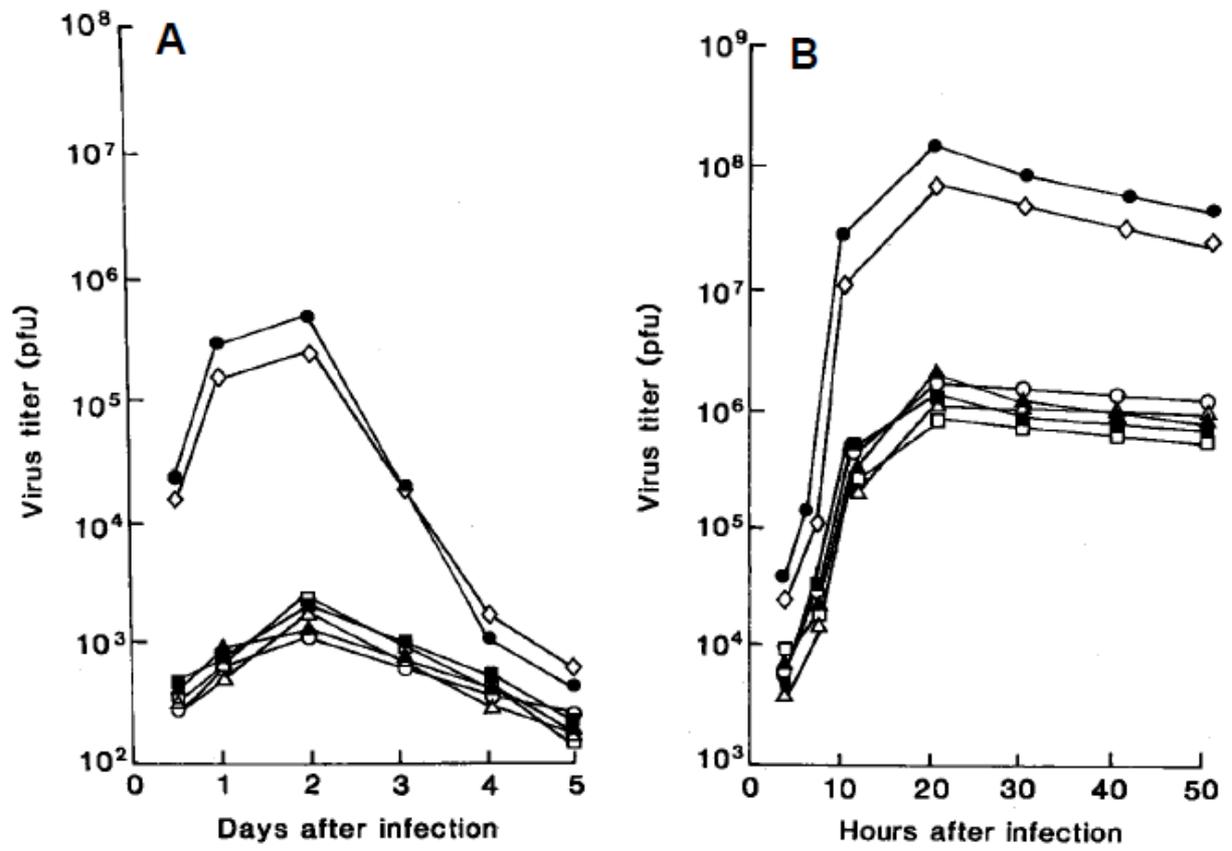


FIG. 3

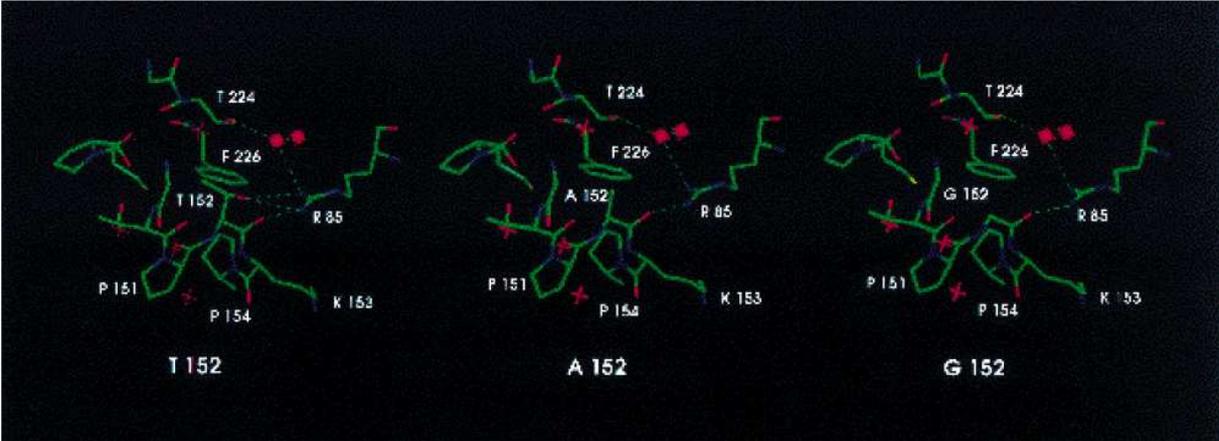


FIG. 4

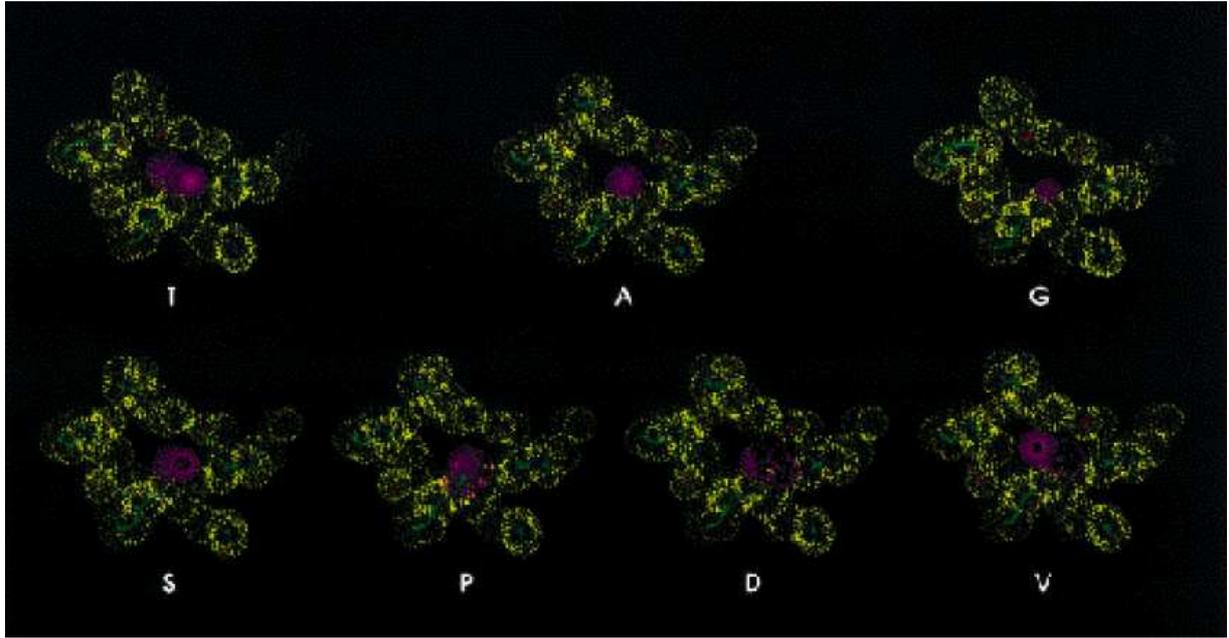


FIG. 5