Perspectives on the Role of Viruses in Insulin-dependent Diabetes

JI-WON YOON, Ph.D., AND USHA R. RAY, Ph.D.

Insulin-dependent diabetes mellitus (IDDM) results from the destruction of pancreatic beta cells. Viruses have been suggested as one of the possible causes. The evidence for viruses comes largely from experiments in animals, but several studies in humans also point to viruses as a trigger of this disease in some cases. Encephalomyocarditis (EMC) virus, Mengovirus (2T), and Coxsackie B4 virus infect and destroy pancreatic beta cells when inoculated into mice. This results in hypoinsulinemia and hyperglycemia. The development of EMC virus–induced diabetes is dependent on the genetic background of the host and genetic makeup of the virus. Animals with diabetes for several months show some long-term complications, including glomerulosclerosis, ocular changes, and decreased bone formation and mineralization in addition to acute metabolic changes. EMC virus–induced diabetes can be prevented by a live-attenuated vaccine. The capacity of Coxsackie B4 virus to induce diabetes is also influenced by the genetic background of the host. However, Mengovirus-induced diabetes is not dependent on the genetic background of the host. In contrast to the EMC, Mengo, and Coxsackie B4 viruses, reovirus type 1 seems to be somehow associated with an autoimmune response producing a diabetes-like syndrome in suckling mice. This virus produces an autoimmune polyendocrinopathy that results in very mild and transient glucose intolerance. Several common human viruses including mumps, Coxsackie B3 and B4 viruses, and reovirus type 3 can infect human beta cells in culture and destroy them. A variant of Coxsackie B4 virus has been isolated from the pancreas of a child who died of acute-onset IDDM. Serologic studies revealed a rise in the titer of neutralizing antibody to the virus. This virus produced diabetes when inoculated into certain inbred strains of mice. Support for the idea that viruses can trigger some cases of diabetes in man has been strengthened lately by additional case reports and epidemiologic studies. However, it is evident that diabetes is not a common consequence of virus infection. In conclusion, based on the experiments in mice and studies in humans, it appears that at least an occasional case of IDDM may be triggered by a viral infection. DIABETES CARE 1985; 8 (SUPPL. 1):39–44.

Insulin-dependent diabetes mellitus (IDDM) results from the destruction of pancreatic beta cells. What causes beta cell destruction? Several possibilities (genetic factors, autoimmunity, and viral infections) have been extensively studied during the last several decades. The idea that viruses might cause some forms of IDDM comes from numerous case reports showing a temporal relationship between the onset of certain viral infections (e.g., rubella, mumps, and Coxsackie B group) and subsequent development of diabetes.[1–4] Recent case reports and epidemiologic studies support the idea by showing (1) viral specific antigens in the islets of Langerhans[5] and destruction of beta cells in the pancreas of a diabetic patient;[6] (2) the presence of viral antibodies with rising titers in paired sera from newly diagnosed diabetic patients;[6,7] (3) high frequency of Coxsackie B virus–IgM specific antibody in newly diagnosed diabetic children;[8] (4) beta cell damage in children who died of well-documented, overwhelming viral infections;[9] and (5) the isolation of Coxsackievirus B4 and B5 from two patients with acute-onset diabetes and the demonstration that these isolates could produce diabetes in mice.[6,7] The best experimental evidence indicating that viruses have an etiologic role in diabetes appears to be that of mice infected with encephalomyocarditis (EMC) virus, which induces a diabetes-like syndrome in a genetically

susceptible host by infecting and destroying pancreatic beta cells.[10,11] In this brief review, we would like to summarize some of our studies on virus-induced diabetes and some epidemiologic studies from other laboratories.

Animal Model for Virus-induced Diabetes

Encephalomyocarditis virus. The M-variant of EMC virus, a positive-stranded small RNA virus in the picornaviridae group, can infect murine pancreatic beta cells and destroy them. As a result of the beta cell destruction, mice developed hypoinsulinemia and subsequently hyperglycemia.[12-14] Viral specific antigens were found in the pancreatic beta cells at 2–3 days after infection.[13,15] Histopathologic examination of the pancreatic sections revealed moderate to severe changes including lymphocytic infiltration and coagulation necrosis of beta cells at 5–7 days after infection.[12,15]

However, the frequency of diabetes induced by the M-variant of EMC virus (EMC-M) varied with different virus pools and passage history of the virus.[16] Plaque purification of EMC-M resulted in isolation of two stable variants.[17] One is highly diabetogenic (designated EMC-D) and the other is nondiabetogenic (designated EMC-B). When the D-variant was inoculated into SJL/J male mice, diabetes developed in >90% of the animals. In contrast, none of the mice inoculated with the B-variant developed diabetes.[17]

Light microscopy showed that EMC-D, but not EMC-B, induced severe beta cell damage. Fluorescent microscopy using fluorescein-labeled anti-EMC antibody revealed that approximately 10 times more beta cells became infected in mice inoculated with EMC-D as compared with mice inoculated with the same dose of EMC-B. Tissue culture experiments showed that the B-variant induced considerably more interferon than the D-variant.[17]

Despite these differences, the D- and B-variants could not be distinguished antigenically by a sensitive plaque neutralization assay.[17] Antibody made against the D-variant neutralized both the D- and B-variants. Conversely, antibody made against EMC-B neutralized the B- and the D-variants equally well. These studies illustrate the potential difficulty in identifying diabetogenic viruses in nature.

In contrast to biologic differences, the size morphology and capsid polypeptides on polyacrylamide gels of these two variants could not be distinguished.[18] Molecular hybridization studies with radiolabeled DNA complementary to D- and B-variant RNAs failed to distinguish the D- and B-variants.[19] However, analysis of the RNA of both variants by fingerprinting after digestion with RNAse T1 did reveal a difference in their genomes. The nondiabetogenic B-variant was missing a unique oligonucleotide of 20–25 bases long, which was present in the diabetogenic D-variant.[19]

The separation of the M-variant of EMC virus into the D- and B-variants has made it possible to study some of the long-term complications of diabetes.[20,21] The D-variant by itself produces far more severe and prolonged diabetes than the original M-variant of EMC virus. Diabetic mice 4–6 mo after infection with EMC-D show some of the similar types of long-term complications as seen in humans with diabetes mellitus. Light microscopy revealed both diffuse and nodular types of glomerulosclerosis in sections of kidney from those animals. Electron microscopy showed a two- to fourfold increase in the thickness of the glomerular basement membrane. In addition to the glomerular changes, the diabetic animals showed some of the similar types of early ocular changes found in retinal vessels (e.g., a decrease in pericytes) of patients with diabetes mellitus.[20] Furthermore, decreased bone formation and mineralization, which were found in diabetic patients, were also seen in diabetic mice at 30–180 days after infection with EMC-D.[21] These endochondral bone changes are not due to the virus-induced tissue damage, but to the persistent metabolic alterations. Thus, the animal model is valid in the sense that virus can produce both the early metabolic changes and at least some of the long-term complications of IDDM.

EMC virus–induced diabetes can be prevented by a live-attenuated vaccine.[22] Mice were immunized with the nondiabetogenic EMC-B and challenged 30, 43, or 90 days later with the diabetogenic EMC-D. Diabetes did not develop in any of the immunized mice, but it did develop in approximately 80% of the unimmunized controls.[22] In addition to prevention of diabetes by live-attenuated vaccine, EMC virus–induced diabetes can also be prevented by the repeated administration of interferon or an interferon inducer (e.g., poly I:C).[23]

Mengovirus 2T. Mengovirus, another member of the cardiovirus genus of the picornaviridae group, produces a rapidly fatal encephalitis in mice.[24] Earlier studies with a neurovirulent Mengovirus pool failed to demonstrate beta cell damage or hyperglycemia in mice.[24] Plaque purification of the virus resulted in the isolation of a clone (Mengo-2T) that caused diabetes in addition to encephalitis.[25] Microscopic examination of the pancreas from Mengo-2T–infected mice revealed necrosis in the islets of Langerhans and infiltration of inflammatory cells. By immunofluorescence, viral antigens were found in the islets of Langerhans at 2–3 days after infection with Mengovirus 2T. Radioimmunoassays demonstrated a substantial decrease in pancreatic immunoreactive insulin.

Although EMC-D and Mengovirus 2T are antigenically indistinguishable by hyperimmune sera, these viruses have different host ranges for the induction of diabetes. EMC-D caused diabetes only in certain inbred strains of mice, whereas Mengovirus-2T caused diabetes in strains of mice susceptible and resistant to EMC virus–induced diabetes. The ability of Mengovirus-2T to induce diabetes in EMC-resistant mice was found to be due to the greater capacity of Mengovirus-2T as compared with EMC-D virus to replicate in and destroy the islets of the resistant animals. It was previously reported that these two viruses showed an estimated 20% difference in nucleotide sequences by cDNA-RNA hybridization. Furthermore, receptor binding experiments suggest that they may use different receptors on the surface of murine neuroblastoma cells.[26] Whether Mengovirus-2T and EMC-D viruses are distinct viruses that bind to different receptors on the surface of beta cells remains to be determined.

Coxsackievirus B4. Previous studies showed that Coxsack-

ievirus B4 did not produce diabetes when inoculated into mice. However, by repeatedly passaging Coxsackievirus B4 in murine-enriched pancreatic beta cell cultures (approximately 70% beta cells), it has been possible to enhance the diabetogenic capacity of this virus.[27,28] However, it is very difficult to get a pure beta tropic virus since the cells in primary cultures are a mixture of several different cell types.[27,28] Coxsackievirus B4 that had been passaged 14 times in cultures enriched for pancreatic beta cells prepared from SJL/J mice can infect and destroy the pancreatic beta cells in certain strains of mice. The destruction of beta cells resulted in a decrease in insulin content of the pancreas. This in turn led to hypoinsulinemia and the subsequent development of hyperglycemia. The reduction of immunoreactive insulin correlated inversely with the elevation of glucose in blood. Thus, the destruction of beta cells by the Coxsackievirus B4 appears to be responsible for the IDDM. As in the case of the M-variant of EMC virus, the degree of beta cell damage is, in all probability, responsible for the observed differences in the metabolic response of individual animals. In the majority of animals, hyperglycemia is transient. This may very well be due to the fact that a sufficient number of beta cells are left intact after the infection so that proliferation and/or hyperatrophy of these cells result in metabolic compensation. During the acute phase of the infection, viral antigens were found in the islets of Langerhans.

The capacity of Coxsackievirus B4 to induce diabetes is influenced by the genetic background of the host. As in the case of EMC virus, only certain inbred strains of mice were found to develop diabetes when exposed to Coxsackievirus B4, and male mice developed more severe diabetes than female mice. Moreover, the strains of mice known to be susceptible to EMC-induced diabetes were also found to be susceptible to Coxsackievirus B4–induced diabetes. Similarly, the strains of mice that were resistant to EMC-induced diabetes did not develop diabetes when exposed to Coxsackievirus B4. The only exception thus far appears to be DBA/1J and DBA/2J mice, which developed diabetes when infected with EMC virus but were resistant to the disease when exposed to Coxsackievirus B4.

Reoviruses. In contrast to the EMC, Mengo, and Coxsackie viruses, reovirus type 1 seems to be somehow associated with an autoimmune response producing a diabetes-like syndrome in suckling mice. Mice infected with reovirus type 1, which was passaged in enriched pancreatic beta cell cultures (about 70% beta cells), developed transient mild diabetes and a runting syndrome.[29] The runting syndrome consisted of retarded growth, oily hair, alopecia, and steatorrhea. Inflammatory cells and viral antigens were found in the islets of Langerhans (alpha, beta, and delta cells) as well as in the anterior pituitary (growth hormone–producing cells). Examination of sera from infected mice revealed autoantibodies that reacted with cytoplasmic antigens in the islets of Langerhans (anti-insulin antibody), anterior pituitary, and gastric mucosa of uninfected mice. To determine if these autoantibodies had a role in the pathogenesis of reovirus-induced diabetes, infected SJL and NFS mice were treated with different immunosuppressive drugs. The administration of antilymphocyte serum, antithymocyte serum, or cyclophosphamide reduced or prevented the development of reovirus-induced diabetes. In addition, virus-infected immunosuppressed mice gained weight at almost the same rate as uninfected controls, and mortality was greatly decreased. Thus, Onodera et al. concluded that autoimmunity does play a role in the pathogenesis of reovirus-induced diabetes.[30]

Precisely how reovirus infection triggers the development of autoantibodies is still unclear, but viruses have often been suspected as a cause of autoimmune disease. In contrast to reovirus type 1, reovirus type 3 generally does not induce autoantibodies to growth hormone in mice and does not infect the anterior pituitary. The critical difference between reovirus type 1 and type 3 seems to reside at the level of the sigma-1 polypeptide responsible for virus tropism. Therefore, it is speculated that a single viral protein appears to control pituitary infection and autoantibody production.

Lymphocytic chorinomeningitis virus. Recently, Oldstone and colleagues reported that lymphocytic chorinomeningitis (LCM) virus persistently infects murine pancreatic islet cells.[31] In their studies, viral nucleoprotein was detected predominantly in the pancreatic beta cells by double-labeled immunofluorescent antibody technique. Electron microscopy confirmed these findings by showing virions budding from the beta cells. Persistent infection was associated with the chemical evidence of diabetes including hyperglycemia and abnormal glucose tolerance. However, the virus-infected islet cells showed normal anatomy and cytomorphology. Neither beta cell destruction nor lymphocytic infiltration was routinely observed. The end result is a chemical and morphologic picture similar to that observed in early stages of adult-onset diabetes mellitus.

One of the possible mechanisms by which the virus might cause diabetes is through the establishment of an infection that might shut off the "luxury functions" of insulin-producing beta cells.[32] The other possibility is that the persistent infection may also result in a gradual reduction in the number of functioning beta cells since the regenerative capacity of beta cells is thought to be poor.[32] The precise mechanism by which LCM-virus induces diabetes remains to be investigated. Nevertheless, this new finding is very interesting and important for studies on diabetes in humans and animals.

ASSOCIATION OF VIRAL INFECTIONS IN HUMANS WITH IDDM

It has been thought for many years that viruses might cause some forms of IDDM by infecting and destroying pancreatic beta cells. Do viruses play any definite role in human IDDM? Two different approaches were taken to answer this question. The first was to determine whether human pancreatic beta cells, in culture, were susceptible to viral infection. Several common human viruses were tested in cultures enriched in beta cells. To prove unequivocally that the infected cells are insulin-containing beta cells, a double-labeled immunofluorescent antibody technique was used throughout these experiments.[32] By this method, it was

clearly shown that several human viruses, including mumps,[33] Coxsackie B3,[34] and Coxsackie B4,[6] could infect human beta cells and destroy them. Furthermore, the capacity of a virus (reovirus type 3) to infect human beta cells can be enhanced by repeated passage in human beta cell cultures.[35] Thus, at least under in vitro conditions, human beta cells are not inherently resistant to viral infection. However, this does not prove that these viruses actually do infect and destroy human pancreatic beta cells and cause diabetes in vivo.

The second approach was to try to isolate virus from the pancreas of children dying from acute-onset IDDM. Several years ago, such samples were obtained.[6] A 10-yr-old boy was admitted to the hospital with diabetic ketoacidosis within 3 days after the onset of a flu-like illness. Despite intensive therapy, the child's condition deteriorated and he died 7 days later. At autopsy, lymphocytic infiltration of the islets of Langerhans and necrosis of beta cells were observed.[6] The picture was very similar to that seen in the islets of Langerhans from mice that developed diabetes after infection with virus. A variant of Coxsackie B4 virus was successfully isolated from a small piece of the child's pancreas. Serologic studies on the patient's serum revealed a rise in the titer of neutralizing antibody to this isolate from <4 on the second hospital day to 32 on the day of death. This observation supported the idea that the virus actually came from the child. When several inbred strains of mice were inoculated with the human isolate, SJL/J mice developed diabetes, while the C57BL/6J, CBA/J, and Balb/c mice did not. Microscopic examination of the pancreas from diabetic mice showed extensive infiltration of inflammatory cells, destruction of beta cells, and viral antigens in the islets of Langerhans. Support for the idea that viruses can trigger some cases of diabetes in humans has been strengthened by two additional case reports. The first case was that of a 16-mo-old child who came down with a Coxsackie B5 virus infection and a few days later developed diabetes.[7] This virus, isolated from the feces of the child, produced abnormal glucose tolerance tests when inoculated into mice. The second case was that of a 5-yr-old girl who had myocarditis and diabetes 2–3 wk after open heart surgery.[5] At necropsy, her islets showed a lymphocytic infiltrate and beta cell necrosis. By immunofluorescence, Coxsackie B4 viral antigens were found in the islets and high levels of Coxsackie B4 antibody were found in the serum. In other studies, pancreatic sections from four of seven neonates who died of Coxsackie B virus infections showed insulitis and beta cell damage.[9] The insulitis and beta cell damage observed at autopsy do not prove that these children would have developed diabetes if they had survived after viral infection, but these studies provide further in vivo support for the idea that under certain circumstances some viruses are capable of infecting beta cells.

Early reports showed that IDDM subjects with a recent onset of disease (within 3 mo) had a higher neutralizing antibody titer to Coxsackie B4 virus than either normal subjects or IDDM subjects with a duration >3 mo.[36] Other epidemiologic studies have not confirmed this finding.[37–40]

Among other numerous case reports regarding Coxsackievirus infections and IDDM, a couple of recent reports will be discussed. King et al.[8] detected Coxsackie B virus–specific IgM responses in 11 of 28 (39%) children 3–14 yr old with newly diagnosed IDDM, while only 5% of the age-matched control group showed Coxsackie B virus–specific IgM response. Five of 11 diabetic patients had only a homotypic IgM response to Coxsackie B4 virus, whereas one patient developed IgM response directed only against Coxsackie B5 virus. Sera from the remaining five children showed, in addition to the Coxsackie B4–specific IgM, heterotypic/cross-reactive IgM responses to Coxsackie B2 and/or Coxsackie B5. These findings allowed the authors to state that Coxsackie B4 is the virus most commonly associated with acute IDDM.[8]

In addition, Orchard et al.[41] also reported an interesting case in which a 13-yr-old boy, not sharing either HLA haplotype with his diabetic sister, had virtually normal glucose tolerance 80 days before symptomatic presentation. However, they showed serologic evidence of infection by Coxsackie B4 virus at the time of diagnosis of IDDM. They stated that this case might have developed IDDM in association with an acute Coxsackie B4 viral infection but with a less clear genetic and autoimmune background.[41]

So far, we have discussed the possible association between acute virus infection and IDDM. However, current knowledge indicates that a rather long pathologic process might precede the onset of IDDM in man. Therefore, if viruses are involved in the pathogenesis of IDDM, it seems important to consider the possible association of diabetes with relatively slow virus infections also. Numerous case reports have dealt with congenital rubella virus, mumps, and disseminated CMV infections.[4] Among several reports, the most convincing evidence that a persistent virus infection may cause IDDM comes from studies of patients with the congenital rubella syndrome.[42–44] Rubella virus has been isolated from the pancreas in a few cases of congenital infection.[42,43] Insulitis with severe beta cell depletion was also reported in the case of an infant with congenital rubella syndrome dying of acute-onset diabetes.[44] Epidemiologic studies have shown that the prevalence of IDDM among congenitally infected children is significantly high in both the United States and Australia (about 20%).[45,46] In contrast, in reports from England, IDDM does not appear to follow congenital rubella.[47]

In addition to rubella virus, the hypothesis that a preceding infection with mumps virus may also trigger at least some cases of IDDM has been suggested by recent reports that some children may develop islet cell antibody during parotitis.[48] Gamble showed that mumps infection apparently precedes the development of diabetes in some proportion of newly diagnosed diabetic children.[49] Other epidemiologic studies do not support the hypothesis that mumps infection is strongly related to the onset of IDDM.[50]

Regarding cytomegalovirus, characteristic inclusion bodies have been found in the beta cells of infants and children who died with disseminated cytomegalovirus infection.[2,9] Insulitis and CMV-like particles have been observed in the pancreas of a particular rodent (*Octodon degu*), which manifests spon-

taneous diabetes.[51] This observation supports the theory that CMV may also be associated with some forms of IDDM.

As previously indicated, IDDM most likely is not a single disease, but several diseases with different etiologies.[6] Therefore, if viruses can cause diabetes, they may be only one of the multiple causes and more than one virus or virus group may be involved. So far, most of the efforts have been directed toward viruses that produce an acute, lytic infection. This has been a too restrictive approach. Therefore, new approaches accompanying the present method should be made to look for viruses that produce cumulative insults, for viruses that produce slow or persistent infections, and for viruses that trigger autoimmune responses in man.[52]

From the Laboratory of Oral Medicine, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland.

Address reprint requests to Ji-Won Yoon, Ph.D., Division of Virology, Department of Microbiology and Infectious Diseases, Health Science Center, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1, Canada.

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