

A New Look at Viruses in Type 1 Diabetes

Hee-Sook Jun and Ji-Won Yoon

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Abstract

Type 1 diabetes results from the destruction of pancreatic beta cells. Genetic factors are believed to be a major component for the development of type 1 diabetes, but the concordance rate for the development of diabetes in identical twins is only about 40%, suggesting that non-genetic factors play an important role in the expression of the disease. Viruses are one environmental factor that is implicated in the pathogenesis of type 1 diabetes. To date, 14 different viruses have been reported to be associated with the development of type 1 diabetes in humans and animal models. Viruses may be involved in the pathogenesis of type 1 diabetes in at least two distinct ways: by inducing beta cell-specific autoimmunity, with or without infection of the beta cells, (e.g. Kilham rat virus) and by cytolytic infection and destruction of the beta cells (e.g. encephalomyocarditis virus in mice). With respect to virus-mediated autoimmunity, retrovirus, reovirus, Kilham rat virus, bovine diarrhoea-mucosal disease virus, mumps virus, rubella virus, cytomegalovirus and Epstein-Barr virus are discussed. With respect to the destruction of beta cells by cytolytic infection, encephalomyocarditis virus, mengovirus and Cocksackie B viruses are discussed. In addition, a review of transgenic animal models for virus-induced autoimmune diabetes is included, particularly with regard to lymphocytic choriomeningitis virus, influenza viral proteins and the Epstein-Barr viral receptor. Finally, the prevention of autoimmune diabetes by infection of viruses such as lymphocytic choriomeningitis virus is discussed.

Key Words: beta cell-specific autoimmunity; environmental factor; type 1 diabetes; virus; virus-mediated autoimmunity

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Introduction

Type 1 diabetes (T1D), also known as insulin-dependent diabetes mellitus or juvenile onset diabetes results from the destruction of beta cells by a beta cell-specific autoimmune process [1-7]. Genetic factors are thought to be a major component for the development of T1D, as there is a strong association between susceptibility to T1D and specific alleles of MHC class II genes, particularly HLA-DR and HLA-DQ [8-12]. Although genetic susceptibility appears to be a prerequisite, studies on the risk of developing T1D using identical twins have shown that the concordance rate for the disease approaches only 40% [13], suggesting that environmental factors such as viruses, diet, and beta cell toxins may be involved in the initiation and/or progression of beta cell destruction leading to T1D [14,15].

Viruses have long been suspected to contribute to the onset of T1D. The earliest observations were that the onset of T1D sometimes followed acute infections [16] and occurred with greater frequency at certain times of year [17,18], which often indicates a viral cause. More recently, epidemiological studies have shown the presence of virus-specific IgM antibodies in recent-onset T1D patients [19-22]. The most convincing evidence comes from studies in which viruses isolated from the pancreata of patients that died from acute T1D caused diabetes in animals by the destruction of beta cells [23,24].

To date, over a half-dozen human viruses have been reported to be associated with human type 1 diabetes (Table 1). These include Cocksackie B virus [25-27], rubella virus [28,29], mumps virus [30,31], cytomegalovirus [32-34], Epstein-Barr virus [35,36], varicella zoster virus [37], retrovirus [38,39] and rotavirus [40]. About nine viruses have been reported to be associated with the development of type 1 diabetes in animals (Table 2). These include encephalomyocarditis (EMC) virus [41,42], mengo virus [43], reovirus [44] and retrovirus [45-47] in mice; Cocksackie B virus, particularly B4, in mice [48,49] and non-human primates [50]; foot-and-mouth virus in pigs and cattle [51]; rubella virus in hamsters and rabbits [52,53]; bovine viral diarrhoea-mucosal disease virus in cattle [54] and Kilham rat virus (KRV) in rats [55]. In addition to inducing diabetes, there is also some evidence that viruses such as lymphocytic choriomeningitis virus (LCMV) [56,57] and mouse hepatitis virus (MHV) [58] can protect against the development of autoimmune T1D in two spontaneously diabetic animals,

Table 1 Viruses associated with the development of type 1 diabetes mellitus (IDDM) in humans

Virus	Involvement of genetic factors	Remarks
RNA Viruses:		
Picornaviridae		
Coxsackie B virus	Not determined	Evidence from epidemiological studies, anecdotal reports and isolated viruses causing diabetes in infected animals
Retroviridae		
Retrovirus	Not determined	Association of beta cell-specific expression of retroviral gene with development of human autoimmune IDDM
Togaviridae		
Rubella virus	Not determined	Possible association with autoimmune IDDM, especially congenital rubella syndrome
Paramyxoviridae		
Mumps virus	Yes	Possible induction of islet-cell autoantibodies
Reoviridae		
Rotavirus	Not determined	Association with islet autoimmunity
DNA Viruses:		
Herpesviridae		
Cytomegalovirus	Not determined	Association with autoimmune IDDM
Epstein-Barr virus	Not determined	Possible induction of autoimmune IDDM

the BioBreeding (BB) rat and the nonobese diabetic (NOD) mouse.

Viruses may be involved in the pathogenesis of T1D in at least two distinct ways (Figure 1). First, viruses may trigger beta cell-specific autoimmunity leading to diabetes with or without direct infection of the beta cells. Second, viruses may directly infect and destroy insulin-producing pancreatic beta cells, resulting in clinical T1D. The various mechanisms by which viruses may act to induce diabetes and conversely the mechanisms involved in the prevention of T1D by viruses will be discussed in this review.

Virus-mediated Autoimmune Type 1 Diabetes

While it is well established that autoimmune T1D results from the T cell-mediated destruction of pancreatic beta cells, the event that triggers this autoimmune destruction is not known. Evidence from animal models suggests that viruses can trigger T1D in some cases. Viruses have also been implicated as possible triggers of other autoimmune disorders, including multiple sclerosis, autoimmune chronic active hepatitis, Sjögren's syndrome, juvenile rheumatoid arthritis and systemic lupus erythematosus [59,60].

Several mechanisms have been proposed for virus-mediated autoimmunity (Figure 1). First, a virus could alter the target tissue of the host such that the tissue becomes recognized as foreign by the host's immune system, thus triggering an autoimmune response. These alterations could include modification of surface antigens into immunogenic

forms, induction of new antigens, release of sequestered antigens during host cell lysis, incorporation of cellular antigens with viral envelope, or upregulation of MHC class I and/or class II molecules on the target tissue of the host. Second, the virus could alter the immune system of the host, resulting in autoimmune attack of beta cells. These alterations could include: a) polyclonal B cell activation leading to production of autoantibodies, b) release of lymphokines such as interferon (IFN)- α and tumor necrosis factor (TNF), which in turn recruit immunocytes to the hosts tissues, c) activation of immune cells that result in a break-down of immune tolerance or d) disruption of the Th1/Th2 immune balance. Third, antigenic epitopes on the virus could be similar to molecules on the host tissue (molecular mimicry), thus causing the generation of antigen-specific T effector cells and/or antibodies that recognize the host target cell, leading to the development of autoimmunity. Fourth, it has been proposed that antiviral antibodies arising as a result of viral infection could lead to the formation of an anti-idiotypic antibodies. These secondary antibodies could be autoreactive if the first antibody was made against the part of the virus that reacts with the host.

Retrovirus

Most mammalian species contain endogenous retroviruses as part of their DNA. Endogenous retroviruses consist of two identical molecules of single-stranded RNA of positive polarity that are linked together by hydrogen bonds at their 5' termini. The virion contains a reverse transcriptase en-

Table 2 Comparative clinical and genetic features of spontaneous autoimmune diabetes: characteristics of spontaneous autoimmune diabetes mellitus in humans, NOD mice, and three rat model systems^a

	Human	NOD mouse	BBDP/Wor	LEW.1AR1/ Ztm-iddm	KDP
Age at onset:	Adolescence	Up to 6 mo	7-14 wk	6-12 wk	8-16 wk
Ketosis:	Severe	Mild	Severe	Severe	Severe
Insulin deficiency:	Severe	Mild to severe	Severe	Severe	Severe
Associated autoimmune diseases:	Thyroiditis, celiac disease, vitiligo, PA, polyendocrine syndromes	Sialadenitis, thyroiditis	Thyroiditis	Thyroid, adrenal, salivary glands free of infiltration	Thyroid and kidney infiltrates present
Autoantibodies:	Insulin, GAD, ICA, ICSA, BSA, CPH, EC, IA-2, IAA	Insulin, GAD, ICA	ICA present; GAD and IAA are controversial	ICA and antibodies to GAD, IA-2 not found	Unknown
MHC genes:	HLA-DQ and DR	Unique I-A ^{g7} Absent I-E	RT1 ^{u/u/u}	RT1 ^{u/u/a}	RT1 ^{u/u/u}
Non-MHC genes:	CTLA-4. At least 2 loci, perhaps ≥ 16	$\beta 2$ microglobulin; possibly CTLA-4; ≥ 27 loci	>3 loci. Ian4L1 mutation causes lymphopenia; Iddm4	At least 1 recessive locus	Cblb
Gender effect:	M = F	F > M	M = F	M = F	M = F
Response to general immunosuppression:	Cyclosporin prolongs endogenous insulin production if given at onset	Cyclosporin, tacrolimus prevent diabetes	Cyclosporin, tacrolimus, thymectomy, ALS, radiation prevent diabetes	Unknown	Unknown
Response to environmental perturbation	Diet and viral infection implicated in pathogenesis, but no definitive proof	More than 150 interventions prevent disease	LCMV prevents disease; Certain diets and bacterial vaccines reduce diabetes frequency	Unknown	Unknown

^aALS, anti lymphocyte serum; BSA, bovine serum albumin; CPH, carboxypeptidase H; CTLA-4, cytotoxic T-lymphocyte antigen 4; DR, diabetes resistant; EC, endothelial cell surface; GAD, glutamic acid decarboxylase; HLA, human leukocyte antigen; IAA, insulin autoantibodies; ICA, islet cell autoantibodies; ICSA, islet cell surface antibodies; LCMV, lymphocytic choriomeningitis; MHC, major histocompatibility complex; PA, pernicious anemia.

zyme that produces a DNA copy of the RNA genome. This DNA becomes circularized and integrated into host chromosomal DNA as a provirus. Viral DNA can be transmitted vertically to the next generation via germ-line DNA.

The expression of endogenous retroviruses by beta cells is associated with insulinitis and T1D in NOD mice [45-47]. In NOD mice, the islet cells express various retroviral messenger RNAs (mRNAs) encoded by the *gag*, *pol* and *env* genes, and the beta cells in particular express the group-specific antigen p73 of the A-type retrovirus [61]. In addition, the presence of both A-type and C-type retroviral particles was found in pancreatic beta cells of NOD mice [46,47,62] and was considered to be associated with the development of autoimmune T1D in these animals.

It is not certain how retroviruses may be involved in the

pathogenesis of autoimmune T1D in NOD mice. The presentation of a retroviral antigen on the beta cells by antigen-presenting cells, such as macrophages and dendritic cells, may be the initial step in the autoimmune destruction of beta cells. An immune response to a specific antigen on a target cells involves the activation of CD4⁺ T cells by antigens presented on the surface of a macrophage or other antigen-presenting cells. Our experimental results support this possibility, as elimination of macrophages resulted in the prevention of beta cell-specific autoimmune processes in NOD mice [63-65]. Another possible mechanism whereby retroviruses could be involved in the initiation of autoimmune T1D in NOD mice is the alteration of the expression of cellular genes by the retroviral genomes in the beta cells, possibly resulting in a beta cell-specific altered antigen(s).

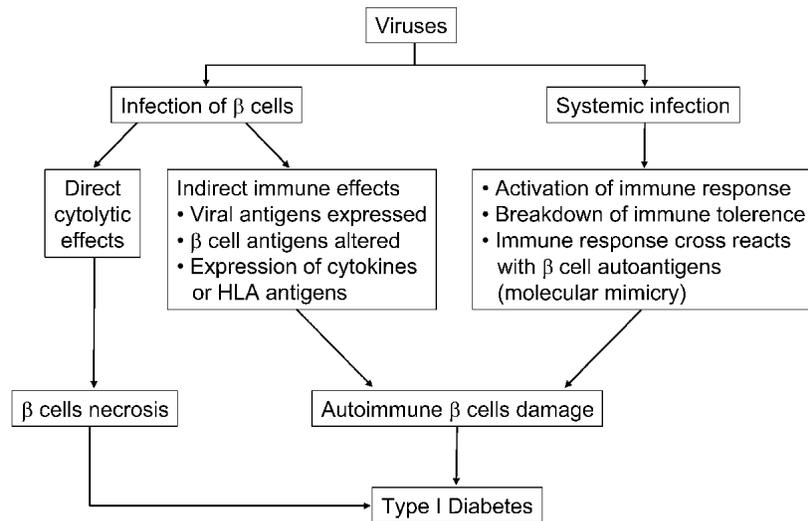


Figure 1 Possible mechanisms for virus-induced diabetes. Viruses can induce diabetes either by infection of beta cells or systemic infection. Viruses can kill beta cells by the replication within the cell, by altering the beta cell-expression of potential autoantigens or other immunogenic molecules or by the induction of a general immune response that is misdirected against beta cells.

An altered antigen might be recognized as foreign by immunocytes, leading to beta cell-specific autoimmunity. In addition, it is possible that cellular proteins taken up in the retroviral envelope may elicit an autoimmune response or that IFN- α -induced expression of HLA-II may trigger autoimmunity through CD4⁺ lymphocytes.

In addition to animal models such as the NOD mouse, endogenous retroviruses have also been implicated in human T1D. Anti-insulin autoantibodies from T1D patients and their non-diabetic, first-degree relatives have been found to crossreact with the retroviral p73 antigen in up to 75% of cases, whereas only 3% of non-diabetic unrelated controls had p73-binding antibodies [66]. These results indicate that anti-insulin autoantibody-positive sera contain antibodies that recognize both insulin and p73. On the basis of studies on beta cell-specific expression of retroviruses in NOD mice and the cross-reaction between human anti-insulin autoantibodies and retroviral group-specific antigen p73, it is suggested that endogenous retroviruses may be involved in the pathogenesis of autoimmune T1D in humans.

In other autoimmune diseases, nucleotide sequence homologies are being discovered between human retroviruses and self-antigens, in particular between ribonucleoproteins and the p30 C-type retroviral gag-gene product [67-69]. Moreover, our electron-microscope studies have demonstrated retrovirus-like particles in the cytoplasm of beta cells of T1D patients who died shortly after the onset of diabetes, but in none of the non-diabetic controls. All diabetic pancreata showed islet destruction with insulinitis. Staining with monoclonal antibodies revealed that most of the infiltrating immune cells were macrophages, CD8⁺ (cytotoxic) T lymphocytes and natural killer (NK) cells, with only a minority of CD4⁺ T-helper lymphocytes. None of the non-diabetic

pancreata showed any features of insulinitis. On the basis of these observations, we speculate that retroviral antigens released from beta cells during beta cell turnover might be processed by antigen-presenting cells such as macrophages, dendritic cells or B cells and presented to T-helper cells (CD4⁺) in association with HLA class II antigens. The activated CD4⁺ T cells secrete interleukin (IL)-2, which amplifies retroviral antigen-specific CD8⁺ cytotoxic T cells. These cells could recognize retroviral antigens expressed on beta cells in conjunction with MHC class I antigen, resulting in CD8⁺ cytotoxic T cell-mediated beta cell destruction.

A novel human endogenous retroviral gene, designated IDDMK_{1,22}, thought to belong to the mouse mammary tumor virus-related family of human endogenous retroviruses (HERV)-K, was reported to be expressed in the plasma of recent-onset type 1 diabetes patients but not in non-diabetic control subjects [38]. However, careful studies have shown that a sequence identical to that of IDDMK_{1,22} was not present in either the plasma or peripheral lymphocytes from either diabetic or control subjects [70]. Instead, a related human endogenous retrovirus with 90-93% sequence homology with IDDMK_{1,22} was present equally in both diabetic and nondiabetic subjects, indicating that this identified human endogenous retrovirus is unlikely to be associated with the development of autoimmune type 1 diabetes in humans [70,71].

Even though it appears that the endogenous retroviral gene homologous with IDDMK_{1,22} is not associated with type 1 diabetes, it does not necessarily exclude the involvement of other human retroviruses or endogenous retrovirus genes in the pathogenesis of autoimmune diabetes. An interesting report showed that the expression of the defective retroviral gene, the HERV-K18 provirus encoding superantigen, is induced by IFN- α and subsequently

stimulates V β 7 T cells [39], which was correlated with the onset of T1D. Whether the HERV-K18 provirus is truly involved in the development of autoimmune diabetes remains to be determined.

Reovirus

Reovirus is a double-stranded RNA virus that is believed to cause mild infections of the upper respiratory and gastrointestinal tract of humans. It has also been associated with T1D in animals; however its mode of action is not known. Mice infected with beta cell-passaged reovirus type 3 showed abnormal glucose tolerance tests within 10 days after infection, but glucose tolerance returned to normal after 3 weeks [44]. Specific viral antigens were present in some beta cells as well as in acinar cells of these animals, and viral particles were detected by electron microscopy in the cytoplasm of some beta cells (Figure 2), suggesting that the diabetic symptoms were caused by direct infection of the beta cells. Other evidence suggests that reovirus might cause transient diabetes through an immune reaction. Mice infected with beta cell-passaged reovirus type 1 developed transient diabetes, and their sera contained autoantibodies that reacted with cytoplasmic antigens from the islets of Langerhans, the anterior pituitary, and the gastric mucosa of uninfected mice [72]. Administration of immunosuppressive drugs to reovirus-infected SJL and NFS mice reduced or prevented the development of reovirus-induced diabetes and mortality [73], indicating that an autoimmune mechanism might be involved in the disease. Recent studies suggest that a Th1 response induced by the increased expression of IL-12 may be responsible for the development of diabetes in newborn DBA/1 mice infected with reovirus [74]. Human beta cells are also susceptible to reovirus 3 infection *in vitro* [75]; however there is little evidence for the involvement of reovirus in the pathogenesis of human T1D.

Kilham Rat Virus

Kilham rat virus (KRV) belongs to the parvovirus family. The parvovirus virion has a diameter of 18 to 26 nm and a relatively simple structure composed of three proteins and a linear, single-stranded DNA molecule. Parvoviruses replicate in the nucleus of dividing cells, hence the predilection of these viruses for bone marrow, lymphoid organs, gut and the developing embryo, and the genome becomes integrated with that of the infected cells. KRV was originally isolated from a rat sarcoma, and has been found to cause a fatal neonatal disease, physical deformities and mental retardation in newborn rats.

KRV has been shown to induce diabetes by provoking autoimmune responses against the beta cells, rather than by direct beta cell infection in diabetes-resistant BB (DR-BB) rats [55,76]. Diabetes-prone (DP)-BB rats, like NOD mice,

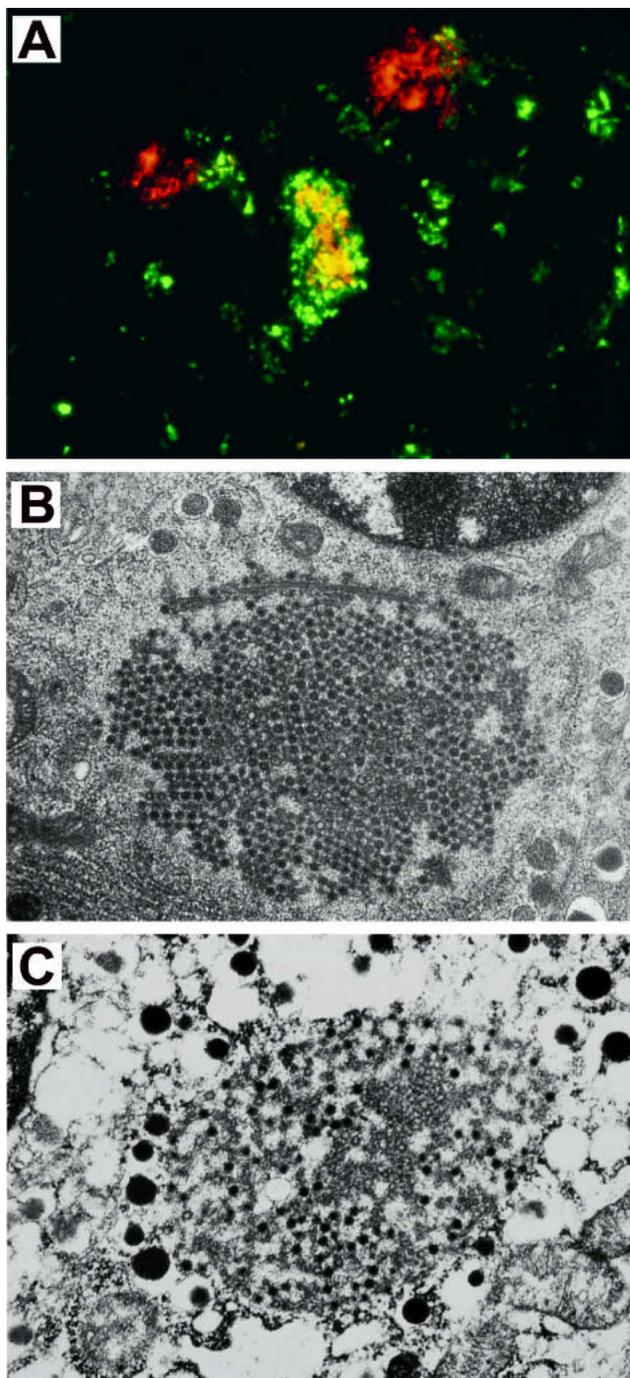


Figure 2 Detection of reoviral antigen and reovirus particles in pancreatic beta cells of reovirus-infected SJL/J mice. (A) Pancreatic sections from reovirus-infected SJL/J mice were stained with anti-insulin antibody (brownish orange) and anti-reovirus antibody (green); a double-stained (light orange) reovirus-infected islet is shown in the center. (B) Crystallized reovirus particles are present in the cytoplasm of intact pancreatic beta cells. (C) Reovirus particles are present in beta cells with severe destruction.

spontaneously develop a diabetic syndrome that resembles human T1D in many respects [77]. DP-BB rats are lymphopenic and 80-100% of the animals become diabetic at about 120 days of age. DR-BB rats are derived from DP-BB rats, but do not normally develop diabetes. When DR-BB rats were infected with KRV at 3 weeks of age, about 30% of these animals developed autoimmune diabetes within 2-4 weeks after infection and a further 30% showed insulinitis without diabetes [55].

Role of Macrophages in KRV-induced Diabetes in DR-BB Rats

The inactivation of macrophages with liposomal dichloromethylene diphosphonate (lip-Cl₂MDP), which selectively destroys macrophages by apoptosis, results in the near complete prevention of insulinitis and diabetes in KRV-infected DR-BB rats [78]. Measurement of the macrophage-derived cytokines IL-12, TNF- α and IL-1 β revealed a selective increase in expression after KRV infection in the splenic lymphocytes and pancreatic islet infiltrates. Measurement of CD4⁺ T cell-derived cytokines revealed that IL-2 and IFN- γ cytokine gene expression are closely correlated with an elevation in the level of IL-12 gene expression, but that IL-4 and IL-10 gene expression did not change. Concanavalin-A (ConA)-activated splenic lymphocytes isolated from macrophage-depleted DR-BB rats injected with KRV/poly I:C did not induce insulinitis and diabetes in young DP-BB rats, indicating that the depletion of macrophages resulted in the loss of the ability to transfer diabetes. These studies show that macrophages and macrophage-derived cytokines play a critical role in the cascade of events leading to the destruction of pancreatic beta cells, culminating in the development of autoimmune diabetes in KRV-infected DR-BB rats.

Role of T Cells in KRV-Induced Autoimmune Diabetes in DR-BB Rats

KRV infects lymphoid organs such as the spleen, thymus and lymph nodes. DR-BB rats harbor autoreactive T cells, which are naturally held silent by immunoregulatory control involving the RT6.1⁺ subset of T cells [79,80]. Experimental data suggests that KRV infection leads to the activation of silent autoreactive T cells in DR-BB rats [81]. These activated T cells are thought to be specific for beta cells. However, the precise mechanisms by which KRV induces autoimmune type 1 diabetes without the infection of beta cells is poorly understood. In addition, it was unclear how KRV-specific autoreactive T cells destroy pancreatic beta cells without direct infection of the cells by KRV. It was hypothesized that KRV antigen-specific T cells generated by KRV peptides might cross-react with pancreatic beta cells and attack them, resulting in the development of insulinitis and, subsequently, diabetes. To test this hypothesis, recombinant vaccinia viruses (rVVs) expressing various KRV proteins were used. rVV was chosen because the wild-type strain of vaccinia virus does not induce insulinitis or

diabetes in DR-BB rats and rVVs have been used as a vehicle for the expression of foreign proteins that successfully induced humoral and cell-mediated immune responses. Infection of DR-BB rats with rVVs expressing the KRV peptides VP1, VP2 (completely overlapped by VP3), non-structural protein 1 or 2 resulted in the generation of viral peptide-specific T cells and antibodies against the KRV peptides. However, none of the DR-BB rats developed insulinitis or diabetes [82]. This result indicates that molecular mimicry between KRV peptides and beta cell-specific autoantigens in DR-BB rats is unlikely to be a mechanism by which KRV induces beta cell-specific autoimmune diabetes.

Another possibility is that KRV infection of DR-BB rats might disturb the finely tuned immune balance and activate autoreactive T cells that are cytotoxic to beta cells, resulting in T cell-mediated autoimmune diabetes similar to that seen in DP-BB rats (Figure 3). To test this hypothesis, the CD4⁺ and CD8⁺ T cell populations were examined in the splenocytes of DR-BB rats after KRV infection. The percentage of CD8⁺ T cells increased considerably, whereas the percentage of CD4⁺ T cells decreased, although the absolute number of both CD4⁺ and CD8⁺ T cells was increased during KRV infection. In addition, CD8⁺ T cells preferentially proliferated as compared with CD4⁺ T cells in KRV-infected DR-BB rats [82]. Treatment of KRV-infected DR-BB rats with OX-8 monoclonal antibody significantly decreased the incidence of diabetes, indicating that CD8⁺ T cells are clearly involved in the destruction of beta cells. It has been reported that the treatment of DP-BB rats with anti-NK cell antibody failed to prevent diabetes, while OX-8 monoclonal antibody treatment successfully prevented diabetes [83]. Therefore, it is more likely that CD8⁺ T cells may play a major role in KRV-induced diabetes, although the possibility of the involvement of NK cells cannot be absolutely excluded, because OX-8 monoclonal antibody also depletes NK cells.

In the rat, CD4⁺ T cells can be divided into Th1-like CD45RC⁻CD4⁺ T cells, which express IL-2 and IFN- γ and play an important role in cell-mediated immune responses, and Th2-like CD45RC⁻CD4⁺ T cells, which express IL-4 and IL-10 and play an important role in humoral immune responses. It has been suggested that the dominance of Th1 cells over Th2 cells is associated with the development of autoimmune T1D, whereas the dominance of Th2 cells over Th1 cells is associated with the prevention of T1D [84-86]. KRV infection in DR-BB rats increased the expression of Th1-type cytokines in the splenocytes and pancreatic infiltrates [78]; therefore, it is possible that the proportions of Th1 and Th2 cells are altered during KRV infection in DR-BB rats. Subsequent experiments showed that the number of Th2-like CD45RC⁻CD4⁺ T cells was significantly decreased and the number of Th1-like CD45RC⁺CD4⁺ T cells significantly increased in the splenocytes of KRV-infected DR-BB rats as compared with PBS-treated controls [82]. In addition, Th1-like CD45RC⁺ CD4⁺ and CD8⁺ T cells isolated from DR-BB rats after infection with KRV could induce diabetes in 88% of recipient DP-BB rats when both

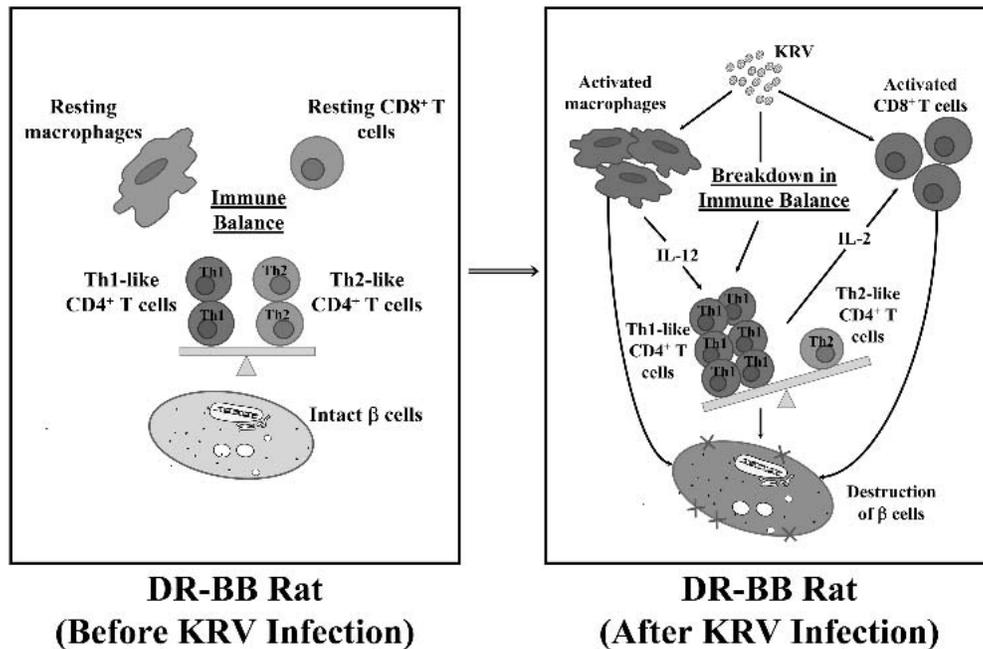


Figure 3 Schematic diagram of the possible mechanism for KRV-induced diabetes in DR-BB rats. In DR-BB rats before KRV infection (left panel), there is a solid immune balance between Th1-type CD4⁺ and Th2-type CD4⁺ T cells. Pre-existing macrophages and CD8⁺ T cells are in the resting state. However, in DR-BB rats after KRV infection (right panel), there is a preferential activation of Th1-type CD4⁺ T cells, which secrete IL-2. IL-2 secreted from the activated Th1-type CD4⁺ T cells activates CD8⁺ T cells. In addition, KRV activates macrophages which secrete IL-12, which also activates Th1-type CD4⁺ T cells. Thus, there is a substantial increase in Th1-type CD4⁺ T cells and, subsequently, an increase in CD8⁺ T cells. Through this process, the finely tuned immune balance breaks down, resulting in the upregulation of effector T cells (Th1-type CD4⁺ and CD8⁺ T cells), leading to the destruction of beta cells.

CD45RC⁺ CD4⁺ and CD8⁺ T cells were transferred [82]. This result indicates that CD45RC⁺ CD4⁺ and CD8⁺ T cells are major effector T cells that can induce autoimmune diabetes. The incidence of diabetes in DP-BB rats that received either CD45RC⁺ CD4⁺ or CD8⁺ T cells alone was, however, significantly lower as compared with that in rats that received a combination of CD45RC⁺ CD4⁺ and CD8⁺ T cells. These results indicate that Th1-like CD4⁺ and CD8⁺ T cells from KRV-infected rats work synergistically to destroy pancreatic beta cells, as proposed previously [78]. In contrast, none of the recipients of both CD45RC⁻ CD4⁺ and CD8⁺ T cells developed diabetes, indicating that CD45RC⁻ CD4⁺ T cells play a role as regulatory T cells. Therefore, infectious KRV, rather than KRV proteins expressed in rVVs, is absolutely required to disturb or breakdown the finely tuned immune balance, resulting in the upregulation of preexisting beta cell-specific autoreactive T cells that can destroy beta cells.

Bovine Viral Diarrhoea-Mucosal Disease Virus

Bovine viral diarrhoea-mucosal disease (BVD-MD) virus belongs to Pestivirus genus of the Flavivirus family and is widespread in livestock such as cattle. BVD-MD virus has been reported to be associated with T1D in cattle, however not all animals with BVD-MD infection develop diabetes

[54]. This may be attributable to the existence of different variants of the virus or to genetic differences among the hosts. In a more recent study, BVD-MD-infected cattle with T1D showed the presence of BVD-MD genes in the pancreas, however not in the islet cells. Many of these cattle also had islet cell autoantibodies. This suggests that T1D associated with BVD-MD is not a direct effect of BVD-MD on islet cells [87]. More research is needed to determine if BVD-MD truly induces autoimmune responses that result in T1D in genetically susceptible animals.

Mumps Virus

The mumps virus is an enveloped single-stranded virus belonging to the paramyxovirus family. Mumps virus was one of the first viruses implicated in the development of human T1D; several cases were reported in which mumps infection appeared to precede the onset of T1D [30,31]. It has been hypothesized that infection with mumps virus may induce autoimmunity, as some children appear to develop islet cell autoantibodies during parotiditis [31], however the mechanisms by which this might occur are unknown. *In vitro* studies have shown that human beta cells could be infected with mumps virus [88], that mumps infection of a human insulinoma cell line induced the release of IL-1 and IL-6 and upregulated the expression of HLA class I and II anti-

gens [89], and that pancreatic beta cells infected with mumps virus had increased expression of only HLA class I molecules [90]. In addition, mumps virus has been shown to be capable of replicating in the exocrine pancreas [91]. On the basis of these studies, it may be suggested that cytokines released by mumps virus-infected cells and increased expression of HLA molecules by infected beta cells may lead to an immune response against the beta cells or may increase pre-existing autoimmune processes directed against beta cells.

Several studies have explored the impact of mumps vaccinations on either increasing or decreasing the incidence of T1D. One investigation concluded that the elimination of natural mumps infections by vaccination may have been responsible for the decreased risk of developing T1D over the time period studied [92]. Other studies concluded that there is no association with childhood mumps vaccinations and the development of islet autoimmunity [93] or T1D [94]. Further studies are required to determine whether or not mumps virus is definitely involved in the development of autoimmune T1D.

Rubella Virus

Rubella virus is a non-segmented, single-stranded RNA enveloped virus that belongs to the togavirus family. It has been implicated in T1D, as patients with congenital rubella syndrome (CRS) had a higher incidence of T1D than the general population, with approximately 10-20% developing diabetes between the ages of 5-20 years [28,95-102]. Islet cell and anti-insulin antibodies were found in 50-80% of diabetic patients with CRS, whereas these antibodies were present in about 20% of non-diabetic patients with CRS [29], suggesting an underlying autoimmune disorder. Genetic susceptibility may also be involved, as patients with CRS and diabetes had a significantly increased frequency of HLA-DR3 and a significantly decreased frequency of HLA-DR2 [28]. While rubella virus appears to be involved in the development of T1D in patients with CRS, more research is required to discover if infection by rubella virus after birth plays any role in the induction of T1D.

Rubella virus appears to be able to directly infect beta cells, as shown by *in vivo* and *in vitro* studies. Neonatal golden Syrian hamsters infected with beta cell-passaged rubella virus developed hyperglycaemia and hypoinsulinaemia between 7 and 10 days of age, and their beta cells were positive for rubella virus antigen. An autoimmune process may be involved, as 40% of infected animals had cytoplasmic islet cell antibodies and 34.5% had insulinitis [53]. Human islets are also susceptible to direct rubella infection under culture conditions. Human fetal islets exposed to rubella virus contained rubella viral antigens in both beta and non-beta cells and had lowered levels of insulin production [103], although without any demonstrable cytopathology [104]. It is possible that the virus may insert, expose, or alter antigens in the plasma membrane of the infected host as it

buds through the cell membrane. Rubella viral antigens on beta cells or rubella virus-altered antigens on the surface of beta cells may be perceived as foreign by the host's immune system, leading to beta cell-specific autoimmunity.

Alternatively, *in vitro* studies suggest that rubella virus may induce autoimmune T1D by molecular mimicry. When a panel of monoclonal antibodies that recognize rubella virus capsid and envelope glycoproteins were tested for reactivity with islet cell antigens, one monoclonal antibody that recognized a domain within the rubella virus capsid protein was found to react with extracts from rat and human islets, as well as with extracts from a rat insulinoma line [105]. Further testing showed that the shared epitope was on a 52 kD protein. In addition, it was reported that T cells of diabetic patients recognize cross-reactive protein determinants from rubella virus and glutamic acid decarboxylase (GAD) 65 and 67 [106], which is considered to be an important β cell autoantigen in the pathogenesis of T1D [107]. This suggests that rubella virus exposure may lead to the generation of viral antigen-specific cytotoxic T cells that also recognize beta cell-specific antigen(s) in susceptible individuals.

Cytomegalovirus

Cytomegalovirus (CMV) belongs to the herpesvirus family and is a double-stranded DNA enveloped virus. Like rubella virus, human cytomegalovirus (CMV) can be congenital, although the disease may not appear until later in life. CMV infections can also be transmitted perinatally, or postnatally through close contact or breast milk, as the immaturity of infant immune systems favours the establishment of persistent viral infections.

CMV has been implicated in T1D by a number of clinical studies. Case reports describe a child with congenital CMV infection [32] and a woman with CMV infection [33] who both developed T1D, the latter after extensive pancreatitis. In a study of children with fatal viral infections, viral cytopathology of the pancreas and characteristic inclusion bodies in the beta cells were found in 20/45 cases of CMV infection [27], indicating that CMV can infect pancreatic beta cells. In a study following 73 infants with congenital CMV infection, one developed T1D, compared with 38/19,483 non-infected control subjects, which the investigators believed indicated no statistical correlation between CMV infection and the development of T1D [108].

Correlations between CMV infection and T1D also have been found by studies using molecular biological methods. A study using both dot and *in situ* hybridization techniques showed that 20% of T1D patients had cytomegalovirus genomic DNA in their lymphocytes, compared to only 2% of normal controls. Furthermore, 80% of patients who had both anti-CMV antibodies and the CMV genome also had islet cell autoantibodies [34]. Another study found that non-diabetic siblings of T1D patients had a significant association between high titres of anti-CMV antibodies and islet

cell autoantibodies, but no correlation between anti-CMV antibodies with HLA-DR antigens [109]. These results suggest that chronic CMV infection may be associated with islet cell autoantibody production, but that other factors may be needed for the development of clinical T1D.

It is possible that molecular mimicry may be involved in some cases of CMV-induced diabetes. In this situation, immune responses against similar epitopes shared by antigenic determinants of CMV and islet cell-specific proteins may lead to islet cell-specific autoimmunity. Evidence for this is the finding that human CMV can induce an islet cell antibody that reacts with a 38 kD autoantigen expressed in human pancreatic islets [110]. As well, a study showed that a CD4⁺ T cell clone reactive to GAD65 isolated from a prediabetic Stiffman syndrome patient crossreacted with a peptide of human CMV major DNA binding protein, suggesting that human CMV may be involved in the induction of autoimmunity by molecular mimicry of the beta cell autoantigen, GAD65 [111].

The evidence on the association of CMV with T1D remains circumstantial. Further studies are needed to determine whether CMV is actually involved in the development of T1D in man and/or animals.

Epstein-Barr Virus

Epstein-Barr virus (EBV) is a double-stranded DNA enveloped virus that belongs to the herpesvirus family. EBV has been implicated in the aetiology of autoimmune diseases [112,113]. A temporal link between EBV infection and the onset of T1D has been reported in a rare number of cases, including one where the child also had concurrent adenovirus and Coxsackie B viral infections [114].

There is some evidence that EBV may be potentially capable of triggering autoimmune T1D by molecular mimicry. An 11 amino acid sequence of the EBV protein, BOLF1, was found to be homologous to residues in the Asp-57 region of the HLA-DQw8 beta chain peptide, although sera from the diabetic patients tested in this study did not bind to DQw8 beta [115]. It was also found that a pentapeptide sequence in the Asp-57 region of the HLA-DQβ chain is successively repeated six times in the EBV-BERF4-encoded epitope [9,113]. Two patients who produced antibodies against this epitope during acute EBV infection soon developed T1D, while five individuals also acutely infected but not producing antibodies against this epitope did not develop T1D [113]. Further investigation is needed to find the relationship between EBV and T1D.

Studies on the Pathogenesis of Autoimmune T1D Using Mice Transgenic for Viral Antigen

Transgenic animal models have been developed to study virus-induced autoimmune diabetes. This strategy has pri-

marily employed mice transgenic for lymphocytic choriomeningitis virus (LCMV) [116-119] and influenza viral proteins [120] and the Epstein-Barr viral receptor [121].

One of the best studied models is the expression of LCMV proteins under the rat insulin promoter (RIP) in pancreatic beta cells [116-119]. The transgenic mice did not develop diabetes; however, more than 90% of these mice developed diabetes after infection with LCMV. Two different RIP-LCMV transgenic models have been established. In one, the viral transgene is expressed only in pancreatic beta cells and diabetes develops rapidly within 2 weeks of LCMV challenge. In the other, the viral transgene is expressed in both the pancreas and thymus and diabetes develops slowly within 2 months after LCMV challenge in H-2^d mice or within 3-6 months after LCMV challenge in H-2^b mice. It was shown that CD4⁺ T cells are not required for the development of diabetes in transgenic (H-2^d) mice with fast onset, but are necessary for the development of diabetes in the transgenic (H-2^b) mice with slow onset. The number of LCMV-specific cytotoxic T lymphocytes correlate with the onset of diabetes [118,119]. The challenge of the transgenic mice with vaccinia virus expressing the LCMV transgene did not induce diabetes because of the low number of LCMV-specific cytotoxic T lymphocytes that were induced [122]. However, if self reactive cytotoxic T lymphocytes are proliferated locally by the expression of the costimulatory molecule B7.1 or the cytokine TNF-α in the beta cells, then vaccinia virus-LCMV is able to induce diabetes [122,123].

The role of cytokines in the development of T1D has been studied using double-transgenic mice expressing both LCMV protein and a cytokine in pancreatic beta cells or cytokine knockout mice expressing LCMV protein in the beta cells [119]. IFN-γ expression in the beta cells of RIP-LCMV mice resulted in the spontaneous development of diabetes without LCMV infection. When IL-2 was expressed in the beta cells of RIP-LCMV mice, spontaneous T1D did not occur, although the onset of T1D was significantly enhanced and accelerated in the slow onset model. The expression of TGF-β or IL-10 did not affect the incidence of diabetes in RIP-LCMV transgenic mice. Transgenic mice that express both IL-12 and the LCMV antigen in the beta cells did not spontaneously develop diabetes, but the onset of T1D was accelerated when the mice were infected with LCMV [124]. The expression of TNF-α in beta cells during the early phase enhanced the incidence of diabetes, whereas TNF-α expression in the late phase abrogated the autoimmune process, suggesting the dual role of TNF-α in the development of autoimmune diabetes [125]. In addition, abrogation of signal transducers and activators of transcription (STAT)4, which is involved in the IL-12 signaling pathway, significantly reduced the development of CD4⁺ T cell-dependent diabetes in the RIP-LCMV transgenic model [126].

Recent evidence suggests that antigen-presenting cells also play an important role in the development of T1D in RIP-LCMV transgenic mice. Activated macrophages infil-

trate the pancreatic islets soon after infection with LCMV in these mice. Activation of LCMV-specific cytotoxic T lymphocytes by dendritic cells expressing the immunodominant epitope gp33 of LCMV activate and expand beta cell-specific cytotoxic T lymphocytes, contributing to the destruction of pancreatic beta cells [127]. Immunotherapeutic approaches have been tried using the RIP-LCMV transgenic model. Oral administration of porcine insulin prevented T1D, but one (Ala B30 to Thr) or two (Phe B25 to Asn: Ala B30 to Thr) amino acid substitutions completely abrogated the protective effect of insulin [119]. It was recently reported that vaccination of RIP-LCMV transgenic mice with plasmid DNA encoding the insulin B chain prevented T1D by induction of insulin-reactive regulatory CD4⁺ T cells [128].

Transgenic mice expressing the influenza viral protein hemagglutinin (HA) in the beta cells (RIP-HA) developed a very low incidence of hyperglycemia, and infection of these mice with the influenza virus did not significantly increase the incidence of diabetes or insulinitis [120], which is a different result from the LCMV-transgenic mouse model. This might be due to the difference in precursor frequencies of cytotoxic T lymphocytes, as mice are susceptible to LCMV, but not to influenza virus [119]. Double transgenic mice expressing HA in beta cells and with a T cell receptor specific to MHC class II-presented HA peptide showed only mild insulinitis, but no diabetes [129]. However, double transgenic mice expressing HA and MHC class I-restricted HA-specific TCR developed diabetes immediately after birth with a massive infiltration of CD8⁺ cytotoxic T lymphocytes in the pancreas [130]. Perforin/granzyme and fas/fas ligand pathways are involved in the destruction of beta cells by these CD8⁺ cytotoxic T lymphocytes [131].

In addition to LCMV- or influenza viral protein-transgenic mouse models, a transgenic model in which the human Epstein-Barr virus receptor CR2 is expressed in the beta cells of mice has been established [121]. Transgenic mice which have genetic backgrounds H-2b (C57BL/6) or H-2k (CBA) did not develop diabetes. After CR2-expressing islets from these mice were transplanted under the kidney capsules of genetically matched recipients, examination of the transplanted islets revealed peri-insulinitis. In addition, antibodies against CR2 appeared to precede the onset of the peri-insulinitis. On the basis of these studies, two distinct phases have been suggested for the induction of diabetes: one involving peri-islet infiltration and antibody response, and the other involving some second signal for islet destruction.

Direct Cytolytic Infection and Destruction of Pancreatic Beta Cells by Viruses and Development of T1D

As well as triggering beta cell-specific autoimmunity, viruses may cause diabetes by directly infecting and destroying beta cells. Studies on animal models have shown that three picornaviruses are associated with this mode of devel-

opment of T1D: encephalomyocarditis (EMC) virus, menogovirus, and the Coxsackie viruses. Picornaviruses have a small non-enveloped icosahedral particle and a single stranded, positive sense, RNA genome. The capsid is comprised of four polypeptides, of which the major capsid protein VP1 is responsible for viral attachment to cells. When susceptible cells are infected with picornaviruses, the cells are destroyed due to the inhibition of DNA, RNA and protein synthesis of the infected cells.

Encephalomyocarditis Virus

There is unequivocal evidence that diabetogenic variants of EMC virus can induce diabetes in animals [41,42], although there is little evidence that this occurs in humans. Infection of genetically susceptible mice with the M variant of EMC virus resulted in the selective destruction of pancreatic beta cells [132] (Figure 4). Further research found that beta cell destruction in EMC virus-infected mice is dependent on the genetic makeup of the virus and the genetic background of the host [133-140].

Two different animal models have been established with respect to pathogenic mechanisms for EMC virus-induced diabetes. The first model involves animals infected with a high dose (10⁵ PFU/mouse) of EMC virus, in which replication of EMC virus within the beta cells plays a major role, whereas recruitment of macrophages plays a minor role in beta cell destruction. In contrast, the second model involves animals infected with a low dose (< 10² PFU/mouse) of EMC virus, in which activated macrophages that are recruited to the beta cells play a major role, whereas replication of the virus within the beta cells plays minor role in beta cell destruction (Figure 5). In the following subsections, the genetic makeup of the EMC virus, genetic susceptibility of the host and the pathogenic mechanisms of EMC virus-induced diabetes will be discussed.

Genetic Makeup of the Virus: Identification of the Diabetogenic EMC Viral Gene

The highly diabetogenic EMC (EMC-D) and nondiabetogenic EMC (EMC-B) viruses were isolated from EMC-M virus by plaque purification [42]. EMC-D virus produces diabetes in over 90% of infected animals, and EMC-B virus does not produce diabetes in any of the animals that it infects [42] (Figure 6). These two variants are antigenically similar and could not be distinguished by either neutralization assay or competitive radioimmunoassay [42]. Examination of the complete nucleotide sequences of the genomes of both variants showed that they were different in only 14 nucleotide positions [136,137]. Further investigation using several mutant viruses generated from stocks of both EMC-D and EMC-B variants revealed that only one amino acid, alanine (776th amino acid on the polyprotein), is critical for the diabetogenicity of the EMC virus [138] (Figure 6). A "G" base at nucleotide position 3155 ([GCC] Ala-776) is common to all diabetogenic variants, while an "A" base

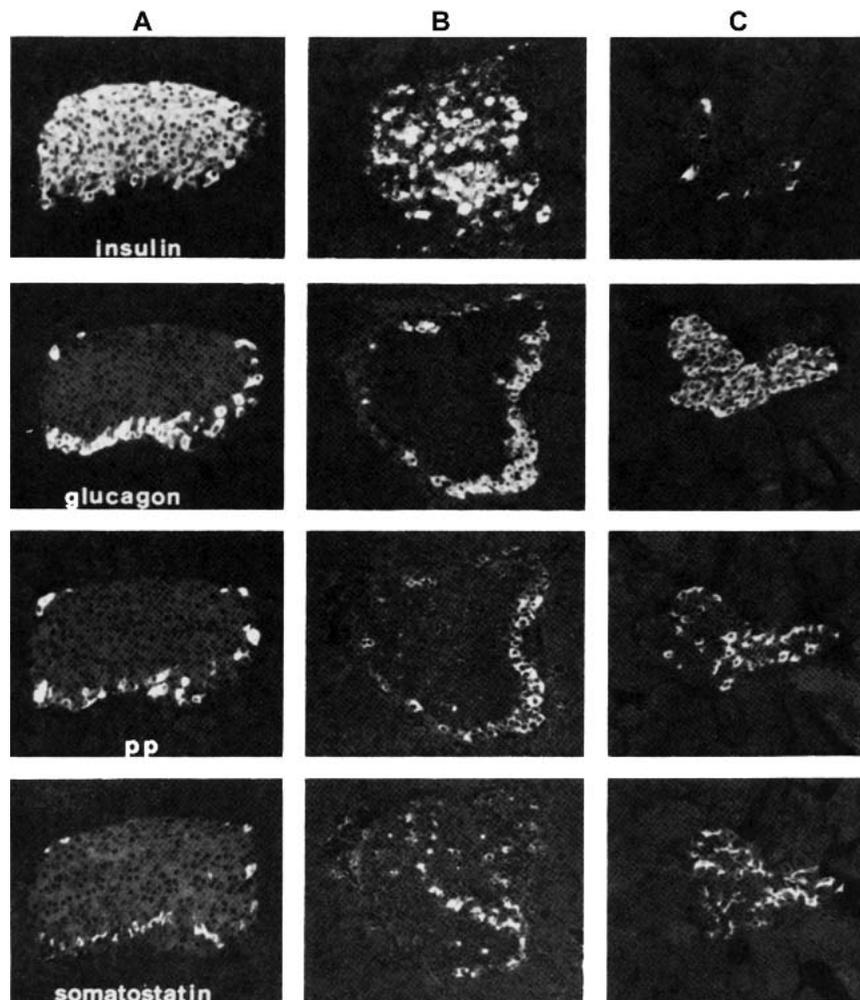


Figure 4 Beta cell-specific destruction in pancreatic islets of EMC virus-infected SWR/J mice. (A) Uninfected control; (B) 3 days after infection; (C) 21 days after infection. Serial section of pancreas from uninfected (A) and infected (B and C) mice were stained with fluorescein-labeled anti-insulin, anti-glucagon, anti-pancreatic polypeptide (pp) and anti-somatostatin antibodies. Insulin-producing cells are localized in the central area and other cells (alpha, pp and delta cells) are localized in the periphery. Beta cells showed necrosis at 3 days, whereas other cells did not change, with the exception of relocalization of delta cells. 21 days after infection, most of the beta cells were destroyed, while other cells were relocalized in the islet due to the absence of beta cells. (H 165).

at the same position ($[\overline{\text{ACC}}]$ Thr-776) is common to all non-diabetogenic variants. The single point mutation of “G” to “A” at position 3155 (Ala-776 to Thr-776) results in the conversion of the diabetogenic variant to a non-diabetogenic variant of EMC virus. Substitution of other amino acids, including serine, proline, aspartic acid or valine, for Ala-776 in the EMC-D viral genome also resulted in a loss of diabetogenicity [139,140]. Therefore, a single amino acid at position 776 of the polyprotein determines viral diabetogenicity.

The 776th amino acid lies in the highly conserved, strongly hydrophilic patch of the VP1. This site contains three proximal prolines (Pro-Thr-Gly-Thr-Pro- X^{776} -Lys-Pro) and is involved in attachment of the virus to beta cells. Changing the amino acid in position 776 may shift the hydrophilicity of the region such that the initial interactions

between the virus and beta cells are affected. It was found that a change from Thr-776 (non-diabetogenic) to Ala-776 (diabetogenic) reduced the hydrophilicity of the region by 37%, which may increase the efficiency of viral attachment to pancreatic beta cells [141]. This hypothesis is supported by a previous finding [142] that six times more EMC-D virus than EMC-B virus attached to primary beta cells in mice.

The attachment of the recombinant chimeric EMC viruses to pancreatic β cells was determined in further studies. The recombinant chimeric EMC viruses containing Thr, Ser, Pro, Asp or Val at position 152 of the major capsid protein VP1 (amino acid position 776 of the polyprotein) bound poorly to beta cells. In contrast, recombinant chimeric EMC viruses containing Ala at position 152 of the VP1 bound efficiently to and infected beta cells, resulting in the development of diabetes. Three-dimensional molecular

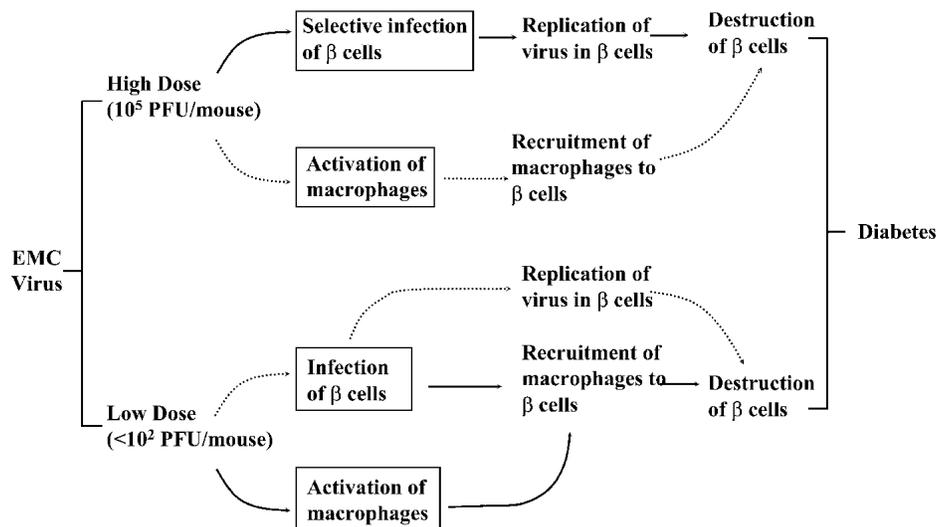


Figure 5 Two animal models for EMC virus-induced diabetes. Infection of a genetically susceptible mouse with a high dose (10^5 PFU/mouse) of EMC-D virus results in the development of diabetes within 3 days. EMC-D virus selectively infects pancreatic beta cells and replication of the EMC-D virus in the beta cells plays a major role (solid lines), whereas activated macrophages play a minor role (dashed lines) in the destruction of beta cells, leading to the development of diabetes. In contrast, in mice infected with a low dose ($<10^2$ PFU/mouse) of EMC-D virus, activated macrophages play a major role (solid lines), whereas replication of the EMC-D virus in the beta cells plays a minor role (dashed lines) in the destruction of beta cells, leading to the development of diabetes.

modeling revealed 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 Thr, Ser, Pro, Asp or Val at position 152 than in recombinant chimeric viruses containing Ala at the same position (Figure 6). The surface area surrounding Ala at position 152 of the VP1 is more accessible, thus increasing the availability of the binding sites for attachment to beta cell receptors and resulting in viral infection and the development of diabetes [143].

Genetic Susceptibility of the Host: Genetic Control of EMC Virus-Induced Diabetes

Diabetes only develops in some strains of mice after EMC-D viral infection; SJL/J, SWR/J, DBA/1J, and DBA/2J strains are susceptible to EMC-D-induced diabetes, whereas C57BL/6J, CBAI/J, and AKR/J mouse strains are resistant [42]. Results from genetic and *in vitro* viral attachment studies showed that susceptibility to EMC-D virus-induced diabetes is determined by a single autosomal recessive gene that is inherited in a Mendelian mode [144]. The gene may operate by modulating the expression of viral receptors on beta cells in genetically susceptible mice [134,135,144-147].

Pathogenic Mechanisms for EMC Virus-Induced Diabetes

The infection of SJL/J mice with a high dose of EMC virus (10^5 PFU/mouse) results in the development of diabetes

within 3 days of infection by acute destruction of pancreatic beta cells [42]. The development of diabetes in these infected mice is mainly due to the replication of the EMC virus within the beta cells, rather than to the involvement of humoral and/or cell-mediated immune responses. However, one study suggested that T lymphocytes might be involved in the pathogenesis of EMC-D virus-induced diabetes [148]. In contrast, later studies showed that depletion of lymphocytes failed to alter the incidence of the disease [149]. Also, EMC-D virus-infected athymic nude mice showed a diabetogenic response that was nearly identical to that of their heterozygous littermates [149], and treatment of EMC virus-infected mice with cyclosporin A did not prevent the disease, but rather enhanced both its incidence and severity [150]. Thus, the contribution of T cell-mediated immune responses to the development of diabetes appears to be negligible in mice infected with a high dose of EMC-D virus. Although T cells do not appear to be involved, it is possible that macrophages might contribute to the destruction of pancreatic beta cells in mice infected with a high dose of EMC virus. Macrophages predominate in the pancreatic islets during the early stages of EMC-D viral infection [151], and it is possible that activated macrophages might migrate to EMC-D virus-infected beta cells as scavengers and secrete cytotoxic cytokines such as IL-1, TNF- α and IFN- γ and nitric oxide. In this way, the active replication of EMC-D virus within the beta cells and the production of cytokines and oxygen free radicals from activated macrophages could act synergistically to destroy beta cells, leading to the development of diabetes.

Natural viral infections in animals and man generally

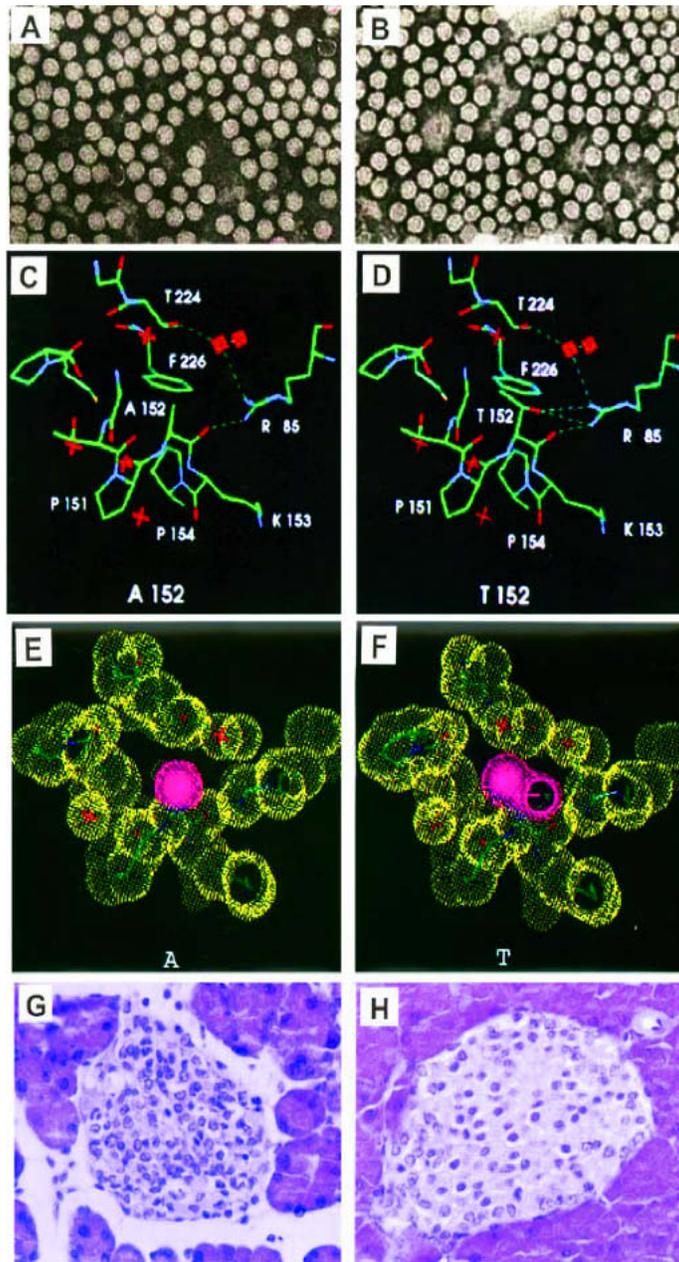


Figure 6 Molecular differences between the diabeticogenic EMC-D viruses and the non-diabeticogenic EMC-B viruses. There are no differences in morphology between (A) EMC-D viruses and (B) EMC-B viruses. Models of the capsid protein VP1 of (C) Ala¹⁵² (A152) from the diabeticogenic EMC-D virus and (D) Thr¹⁵² (T152) from the non-diabeticogenic EMC-B virus, based on the structure of the Mengo virus VP1 showing a view of the atoms in the region surrounding the 152nd amino acid. Ala of the 152nd position of VP1 results 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 Inc., San Diego CA). A van der Waals surface comparison is shown for (E) Ala¹⁵² and (F) Thr¹⁵². The van der Waals surfaces are shown in yellow, with the exception of the 152nd residue, which is shown in purple. The van der Waals forces may be stronger and the accessible surface area smaller in the capsid protein VP1 of the EMC viruses containing Thr¹⁵². The loosely packed residues surrounding Ala¹⁵² may permit recognition by beta cell receptors in viruses containing Ala¹⁵². The images were generated with the Insight II program (Biosym Technologies Inc.). Representative islets are shown from (G) a EMC-D virus-infected mouse with severe insulinitis and (H) a EMC-B virus-infected mouse with intact islets.

involve exposure to relatively low numbers of virus; exposure to the high viral titres used in the experiments described above would be unlikely in nature. Thus, another animal model was established to study the immune mecha-

nisms involved in the destruction of beta cells, in which mice were infected with a low dose (100 PFU/mouse) of EMC-D virus [152]. In mice infected with a low dose of EMC-D virus, macrophages play a central role in the de-

struction of pancreatic beta cells, as activation of macrophages prior to viral infection results in a statistically significant increase in the incidence of diabetes, and inactivation of macrophages prior to viral infection almost completely prevents EMC-D virus-induced diabetes [152]. Additional studies showed that the selective EMC-D viral infection of pancreatic beta cells results in an initial recruitment of macrophages into the islets, followed by infiltration by other immunocytes including T cells, NK cells and B cells [151]. Further study revealed that EMC-D virus infects macrophages and activates them, but does not replicate within the macrophages [153]. The expression of IL-1 β , TNF- α and inducible nitric oxide synthase (iNOS) was selectively detected in the pancreatic islets of mice infected with a low dose of EMC-D virus. In addition, treatment of EMC-D virus-infected mice with antibody against IL-1 β or TNF- α or with the iNOS inhibitor, aminoguanidine, exhibited a significant decrease in the incidence of diabetes [154]. These results suggest that macrophage-derived soluble mediators play a critical role in the destruction of pancreatic beta cells, resulting in the development of diabetes in mice infected with a low dose of EMC-D virus.

Further investigations regarding the mechanisms that activate macrophages found that tyrosine kinase signaling pathways are involved in the EMC-D virus-induced activation of macrophages. Mitogen-activated protein (MAP) kinases such as extracellular signal-regulated kinases (ERK) 1/2, p38 MAPK and c-Jun-terminal activation kinase (JNK), were clearly activated in macrophages 5 hours after EMC-D viral infection. Treatment of mice infected with a low dose of EMC-D virus with a tyrosine kinase inhibitor, AG126, decreased the incidence of diabetes. The expression of IL-1 β , TNF- α and iNOS in the pancreata of AG126-treated, EMC-D virus-infected DBA/2 mice was clearly suppressed compared with vehicle-treated control mice [153]. It was recently found that a Src family kinase, hematopoietic cell kinase (hck), showed a dramatic increase in autophosphorylation and phosphorylation of Sam 68 (a substrate for Src kinase) in EMC-D virus-infected mice, whereas the autophosphorylating activity of lyn and fgr were barely detected and phosphorylation of Sam 68 was not increased. The protein level of hck had a peak at 48 hr after the infection of EMC-D virus [155]. These results suggest that hck is involved in the activation of macrophages in mice infected with EMC-D virus. Treatment of EMC-D virus-infected mice with a Src kinase inhibitor, PP2, resulted in the inhibition of hck activity, a decrease in the production of TNF- α and iNOS in the macrophages and the subsequent prevention of diabetes [155]. Further studies are needed to understand the molecular role of hck in the pathogenesis of EMC-D virus-induced diabetes using hck knockout mice.

Long-Term Complications of EMC Virus-Induced Diabetes

Susceptible strains of mice infected with EMC-D virus show extreme hyperglycaemia and develop long-term com-

plications, such as glomerulosclerosis, changes in the cornea and retinal vessels, and decreases in bone formation and mineralization, similar to those seen in humans with T1D [156,157]. Electron microscopic examination of kidneys from EMC-D virus-induced diabetic mice which had been diabetic for 6 months revealed a two- to four-fold increase in thickness of the glomerular basement membrane. Thickening of Bowman's capsule and the renal mesangial matrix has also been observed in EMC-M virus-infected DBA/2 mice by 2 months after infection, with the changes more clearly observable 5 months after infection [158].

Mengovirus

Mengovirus is a member of the cardiovirus family of picornaviruses, like EMC virus, and produces a fatal encephalitis in mice. While Mengovirus is antigenically similar to EMC virus, it is more neuropathic and lethal and has different tissue tropisms [159]. Plaque-purification of Mengovirus resulted in the isolation of a clone, Mengovirus-2T, which caused diabetes in strains of mice resistant to EMC-D infection [43]. Pancreata from Mengovirus 2T virus-infected mice revealed marked beta cell necrosis, severe inflammatory infiltration of the islets, and decreased insulin content, without evidence of autoimmune responses [43]. Like EMC-D virus, it appears that Mengovirus-2T acts by directly infecting beta cells. Mengovirus-2T virus may bind to a different beta cell receptor than EMC-D virus, thus accounting for the difference in strain susceptibility between the two viruses.

Coxsackie Viruses

There is considerable information that T1D may be associated with enterovirus infection, including infection with Coxsackie viruses and echo viruses. Epidemiological studies show a strong association between the development of T1D and enterovirus infection [25,160-162], and antibodies against enterovirus [163-169] or T cell responses to enterovirus [170-173] have been detected in new-onset diabetic patients. In addition, enteroviral RNA was detected with high frequency in the serum or lymphocytes of T1D patients [174-178]. Some case studies reported that T1D developed after enteroviral infection [179-181]; however the role of enteroviruses in T1D is still poorly understood [182].

Coxsackie B Viruses and Human T1D

There have been numerous epidemiological studies linking recent-onset T1D with Coxsackie B viral infections in humans [25]. Several studies have reported that patients with recent-onset T1D had significantly higher titres of antibody to Coxsackie virus, especially the B4 serotype, than did non-diabetic controls [20-22,160,163-168]. In addition, it

was reported that T cell responses to Coxsackie B4 non-structural protein of the virus increased in new-onset T1D patients [173]. While these studies support Coxsackie B viral involvement in the development of human T1D, results from epidemiological studies have been inconsistent. Some studies have found no evidence of a correlation between the onset of T1D and Coxsackie B viral infections [183-186], while other studies have found higher levels of anti-Coxsackie B virus-specific antibodies in non-diabetic control subjects than in recent-onset T1D patients [187,188]. The interpretation of such studies are complicated by the fact that there many variants of Coxsackie B4 virus [189], and only a minority are likely to cause diabetes. In one study, four Coxsackie B4 variants were tested and only one proved to cause diabetes in mice, while the remaining three variants did not [190]. In our own work, we are unable to distinguish between diabetogenic and non-diabetogenic variants using routine neutralizing antibody or ELISA testing, since the variants are cross-reactive. Therefore, prior exposure to a non-diabetogenic variant of Coxsackie B4 virus can render a person immune to a subsequent exposure to a rarer, diabetogenic variant, even if that person is genetically susceptible to virus-induced diabetes. Epidemiological studies in which this person is a subject will not find a correlation between Coxsackie B4 viral infection and the incidence of diabetes unless outbreaks of diabetogenic virus had occurred prior to outbreaks of non-diabetogenic virus. An immunization effect has been shown in animal models. Mice infected with non-diabetogenic EMC-B virus did not develop diabetes when subsequently infected with diabetogenic EMC-D virus [191]. Thus, the correlation between Coxsackie B viral infection and the development of diabetes seen in some epidemiological studies and the lack of correlation found in other studies may depend on the genetic makeup of the virus and differences in immune responses among individuals. In addition to these epidemiological studies, there have also been many anecdotal reports describing the development of T1D in patients with recent or concurrent Coxsackie B viral infections [176,177,192-199].

There are several direct supporting evidence on the association of Coxsackie B viral infection with the onset of T1D. A girl who died of myocarditis and diabetes shortly after open heart surgery had lymphocytic infiltration of the islets and beta cell necrosis [26]. Coxsackie B4 antigens were detected in the islets by immunofluorescence and high levels of antibody against Coxsackie B4 virus were present in the child's serum. In addition, a boy who died of diabetes showed lymphocytic infiltration of the islets and beta cell necrosis at autopsy (Figure 7). A variant of Coxsackie B4 virus was isolated from the pancreas of the child, which induced diabetes in SJL/J male mice, but not in CBA/J, C57BL/6J, and Balb/c mice [24]. A young girl developed diabetic symptoms for a 10-day period shortly after Coxsackie B5 viral infection, went into remission for 2 months, and then developed definite T1D [23]. The virus isolated from the girl's feces caused glucose intolerance in DBA/2,

SJL/J, and Swiss male mice, but not in BALB/c or C3H mice. Islet cell antibodies were found in the child a week before the onset of diabetes, and immunogenetic analysis revealed that the child had markers indicating a high risk for the development of T1D.

In vitro studies have shown that Coxsackie B3 virus and Coxsackie B4 virus can infect human beta cells [24,200,201]. These studies have shown that the insulin content of infected beta cells decreased rapidly, beginning at 24 h after infection, and that the decrease in insulin roughly paralleled the increase in viral titre. This indicates that Coxsackie B4 viral infection can impair human islet cell metabolism *in vitro*.

Coxsackie B Viruses and Animal T1D

Mice

Not all variants of Coxsackie B4 virus cause overt diabetes in susceptible animals. In early studies, mice inoculated with Coxsackie B4 virus did not develop diabetes [202]; however repeated passaging of Coxsackie B4 in murine-enriched pancreatic beta cell cultures resulted in the virus acquiring more diabetogenic capacity [48]. In the latter study, Coxsackie B4 virus was passaged 14 times in cultures enriched for pancreatic beta cells prepared from SJL/J mice. Mice infected with this virus showed lymphocytic infiltration of the islets (Figure 8) and beta cell destruction, which lead to hypoinsulinaemia and hyperglycaemia. An inverse correlation was observed between the reduction in immunoreactive insulin and the elevation in blood glucose levels [48,203]. As seen with the M variant of EMC virus, the varying degrees of beta cell damage resulting from Coxsackie B4 viral infection are most likely responsible for the observed difference in metabolic response of individual animals. In the majority of animals, hyperglycaemia is transient. It is possible that sufficient beta cells remain intact after some Coxsackie B4 viral infections so that proliferation and/or hypertrophy of these cells results in metabolic compensation. During the acute phase of Coxsackie B4 viral infection, viral antigens have been found in the islets of Langerhans (Figure 8).

Genetic susceptibility of the host plays an important role in Coxsackie B-induced diabetes in animals, as is the case with EMC virus. Some strains of mice, such as SJL/J and SWR/J mice, are susceptible to both EMC virus-induced diabetes and Coxsackie B4 virus-induced diabetes. Similarly, mouse strains resistant to EMC virus-induced diabetes are also resistant to Coxsackie B4 virus-induced diabetes. Exceptions are DBA/2J and DBA/1 mice, as they are susceptible to EMC, but not Coxsackie B4, virus-induced diabetes. Notwithstanding these strain differences, the capacity of Coxsackie B4 virus to induce diabetes appears to be definitely influenced by the genetic background of the host. Genetic studies showed that the 'db' diabetic mutation on chromosome 4 exerted the most effect on suscep-

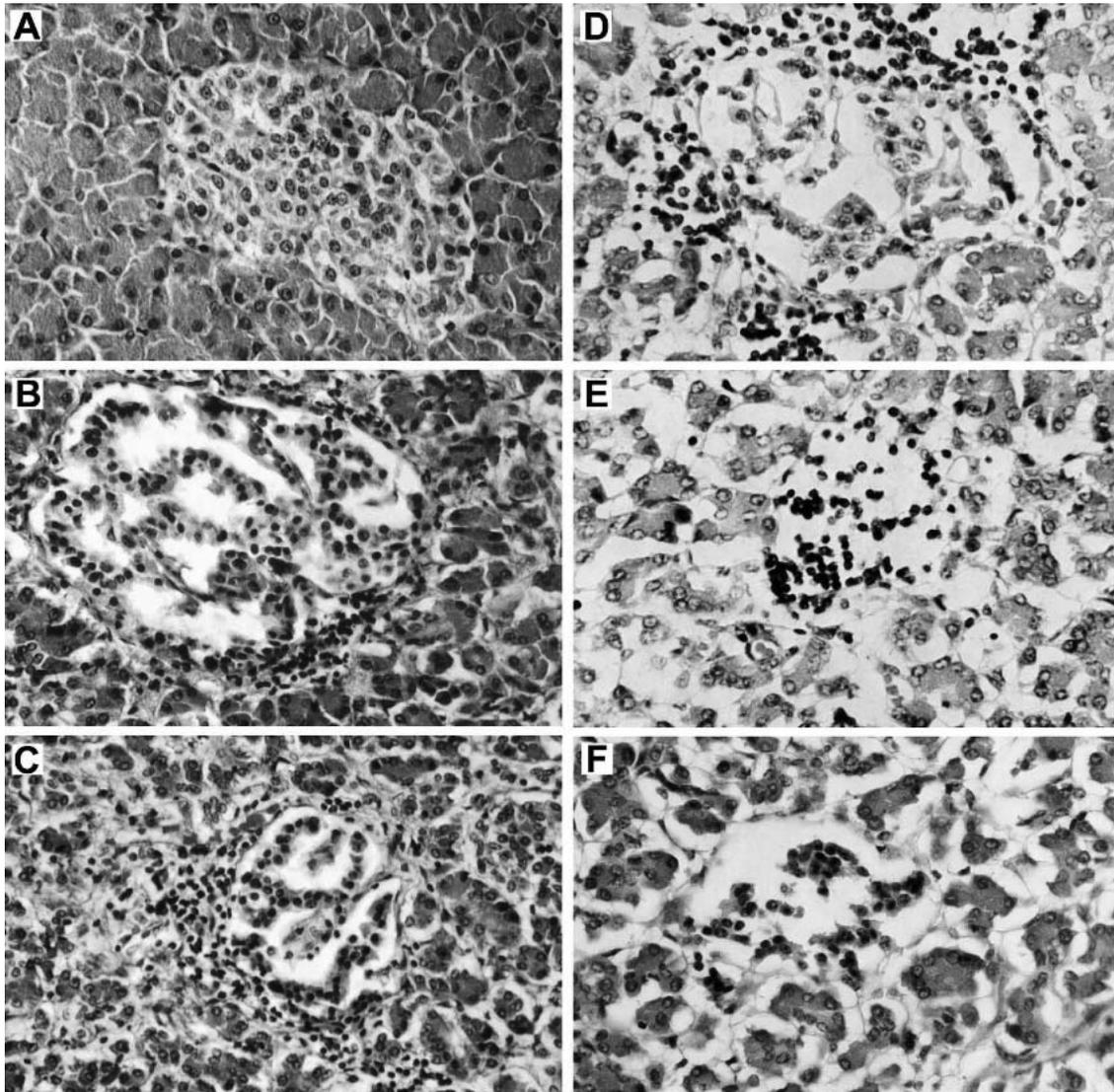


Figure 7 Pancreatic sections from a non-diabetic subject and a diabetic, Cocksackie B4 virus-infected patient. (A) Section of a pancreas from a non-diabetic subject, showing a single intact islet of Langerhans surrounded by acinar cells ($\times 160$). (B-F) Sections from different locations of a pancreas from a Cocksackie B4 virus-infected 10 year-old boy who died after acute onset of IDDM. (B) Moderate accumulation of inflammatory cells within the islet ($\times 230$). (C) Severe accumulation of inflammatory infiltrate in the atrophied islet ($\times 160$). (D) Lymphocytic infiltration in the periphery of the islet. (E) Extensive inflammatory infiltrate, loss of islet architecture and severe islet destruction. (F) Severe necrosis of the beta cells with only a few lymphocytes remaining in the islet.

tibility and host response to Cocksackie B4 virus [204,205] and was associated with an impaired humoral response to Cocksackie B4 viral infection as infected mice did not develop an adequate level of anti-Cocksackie B4 IgM and IgG antibodies [206,207]. The animals were also found to be deficient in absolute and relative numbers of splenic lymphocyte subsets [208]. It has also been reported that Cocksackie B4 viral infection alters thymic, splenic, and peripheral lymphocyte repertoire before the onset of hyperglycaemia in mice [209].

With respect to the Cocksackie B4 viral genome, the amino acid residue responsible for the virulence of the virus has been identified [210,211]. We sequenced the entire ge-

nome of the diabetogenic E2 strain of Cocksackie B4 virus [210] and compared it with the published sequence of the prototype non-diabetogenic JVB strain [211]. We found 111 amino acids that were different between the E2 and JVB strains. Another group passaged Cocksackie B4 JVB strain in murine pancreatic beta cells to obtain a beta cell-tropic variant of the Cocksackie B4 JVB virus. They sequenced the entire genome of this variant and compared it with the nucleotide sequence of the prototype strain. They found only 7 amino acids that were different between the two strains [212]. The identification of critical sites (nucleotides or amino acids) responsible for the diabetogenicity of Cocksackie B4 virus remains to be determined.

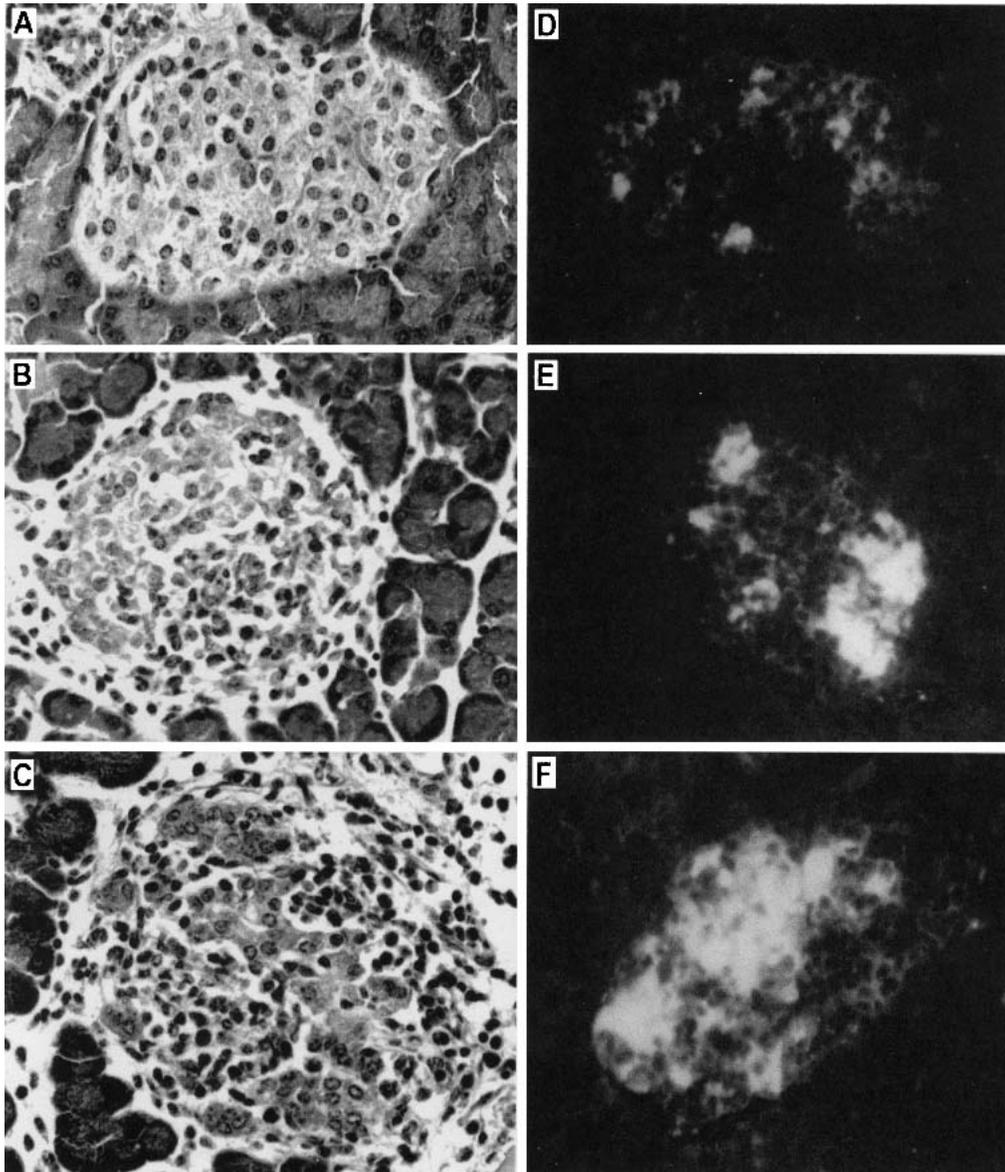


Figure 8 Histopathologic changes and detection of viral-specific antigens in the islets after infection with Coxsackie virus B4 in SJL/J mice. (A) Section of pancreas from an uninfected mouse showing a normal islet. Sections of pancreas 5 days after infection showing: (B) moderate infiltration of the islet with mononuclear cells and (C) extensive infiltration of the islet with mononuclear cells. Sections of pancreas stained with fluorescein-labeled antibody to Coxsackie B4 virus showing islets with: (D) scattered cells containing viral antigens in the cytoplasm, (E) focal areas of infection and (F) viral antigens in most of the cells.

Nonhuman primates

We infected several different species of monkeys, including rhesus, cynomolgus, cebus and patas with monkey β -cell-passaged Coxsackie B4 virus [213]. Glucose tolerance tests were performed before and after infection, and an elevation of the glucose tolerance curve and marked depression of the insulin secretion curve were seen only in the Coxsackie B4 virus-infected patas monkeys. The rhesus, cynomolgus, and cebus monkeys did not show any changes in insulin or blood glucose levels after Coxsackie B4 viral infection. As

Coxsackie B4 virus produced abnormalities in glucose tolerance tests and impaired insulin secretion in only the patas monkey, genetic factors must therefore be critical for the development of diabetes in monkeys infected with Coxsackie B4 virus. However, EMC-D virus did not induce diabetes in any non-human primates tested [213].

From the results of the above research on humans and animals, it is speculated that Coxsackie B viruses, especially the B4 serotype, may play a role in the development of T1D, either by initiating the development of the disease or by operating as the final insult to beta cells in individuals

where ongoing autoimmune beta cell destruction has already been taking place. Whatever the mechanism, evidence from studies on mice, non-human primates, and humans indicates that Coxsackie B viruses affect glucose homeostasis. Research on Coxsackie B4 virus has demonstrated that antigenic changes at the epitope level occur at a frequency greater than 1/100 [189,214]. This suggests that even within the same virus pool, there may be many antigenic variants with different tissue tropisms and different physiological properties, which would account for the wide spectrum of clinical disease produced by the Coxsackie B viruses. Only rare variants may be diabetogenic, explaining why T1D appears to be associated with Coxsackie B viral infection in infrequent isolated cases [25].

Coxsackie A Viruses and T1D

There is some indirect evidence that Coxsackie A virus may also be associated with T1D [215]. In addition, it was found that 36 out of 108 recent-onset T1D patients had IgM antibodies that reacted only with enteroviral procapsids, indicating a recent Coxsackie A and/or echovirus infection [216].

Possible Pathogenic Mechanisms

Coxsackie B viruses may induce diabetes through several mechanisms. The virus has cytolytic activity, and in animals, may directly destroy enough of the beta cell-mass to cause T1D [48,203]. Autoimmune mechanisms may also be involved, perhaps because the immune response raised against the virus crossreacts with specific beta cell antigens. P2-C, a non-capsid protein of Coxsackie B4, has sequence homology with glutamic acid decarboxylase (GAD), which is expressed by beta cells and is a putative autoantigen [217]. Moreover, infection with the virus increases expression of GAD by beta cells [218]. Antibodies have been detected in T1D patients that react with both P2-C and GAD [219], suggesting that this “molecular mimicry” could underlie the autoimmune damage to the beta cells. However, this hypothesis is not supported by studies that characterized antibodies produced by lymphocytes isolated from a newly diagnosed T1D patient. Four of six antibodies studied recognized and bound to the region of GAD65 that is homologous to P2-C, but none cross-reacted with P2-C itself or with any other Coxsackie B4 viral proteins. The lack of crossreactivity between these two proteins may be due to differences in secondary or tertiary structure between the two proteins [220]. On the other hand, the capacity of murine T lymphocytes to cross-react with P2-C and GAD is associated with a diabetes susceptibility allele; crossreactive T-cell recognition of GAD65 may therefore contribute to the initiation or amplification of autoimmune responses against the beta cell, and perhaps to the association of T1D with certain HLA alleles [221].

Coxsackie B virus may also induce diabetes by bystander activation of autoreactive T cells against islet anti-

gens. Mice with susceptible MHC alleles had no viral acceleration of diabetes, but mice with a T cell receptor transgene specific for a different islet autoantigen rapidly developed diabetes. This suggests that Coxsackie B virus induced diabetes by a direct local infection leading to inflammation, tissue damage and the release of sequestered islet antigens resulting in the re-stimulation of resting autoreactive T cells [222].

A further possibility is that a defective Coxsackie B virus, lacking the usual high lytic activity, could cause persistent infection of beta cells, resulting in autoimmune beta cell destruction [223]. This hypothesis would be consistent with evidence of continuing Coxsackie B viral infection in other diseases. IFN- α associated with hyperexpression of HLA type I antigens, only in the islets was found in three out of four children who died from Coxsackie B pancreatitis [224]. In addition, it was reported that the level of IFN- α was elevated in the plasma of T1D patients, and this was associated with Coxsackie B virus infection [225]. On the basis of studies by us and others on Coxsackie B virus-induced diabetes, we propose that persistent infection of beta cells with Coxsackie B viruses may result in expression of IFN- α , which in turn could induce HLA class I antigen hyperexpression and expression of chemokines that recruit and activate macrophages and T cells. These activated immunocytes could kill beta cells, resulting in T1D (Figure 9).

Finally, Coxsackie viral infections may be involved in the pathogenesis of T1D by acting as the terminal insult in individuals who have already lost substantial beta cell mass through ongoing autoimmune damage. Destruction of a critical number of residual cells would result in the clinical onset of T1D.

Prevention of T1D by Viruses

Studies with animal models indicates that under some circumstances, viral infection can actually prevent the development of diabetes. One possible mechanism involves the generation of neutralizing antibodies. EMC-D virus-induced diabetes can be prevented in genetically susceptible mice by vaccination using the non-diabetogenic EMC-B variant [191]. Since the two variants are virtually antigenically identical, antibodies generated against one variant will cross-react with the other.

Viral infection may affect the immune system, resulting in the induction of immunoregulatory cells or the Th2 immune response. In spontaneously diabetic animals, such as the NOD mouse and BB rat, viral infection sometimes paradoxically prevents the development of T1D. This is the case with EMC-D viral infection of NOD mice, which has been shown to prevent the development of autoimmune diabetes or to specifically lessen the immune process in EMC-D virus-infected animals [226]. The development of diabetes in NOD mice can also be prevented by immunization with retroviral proteins, such as the major envelope glycoprotein of C-type retrovirus (gp70) or group-specific antigen of A-

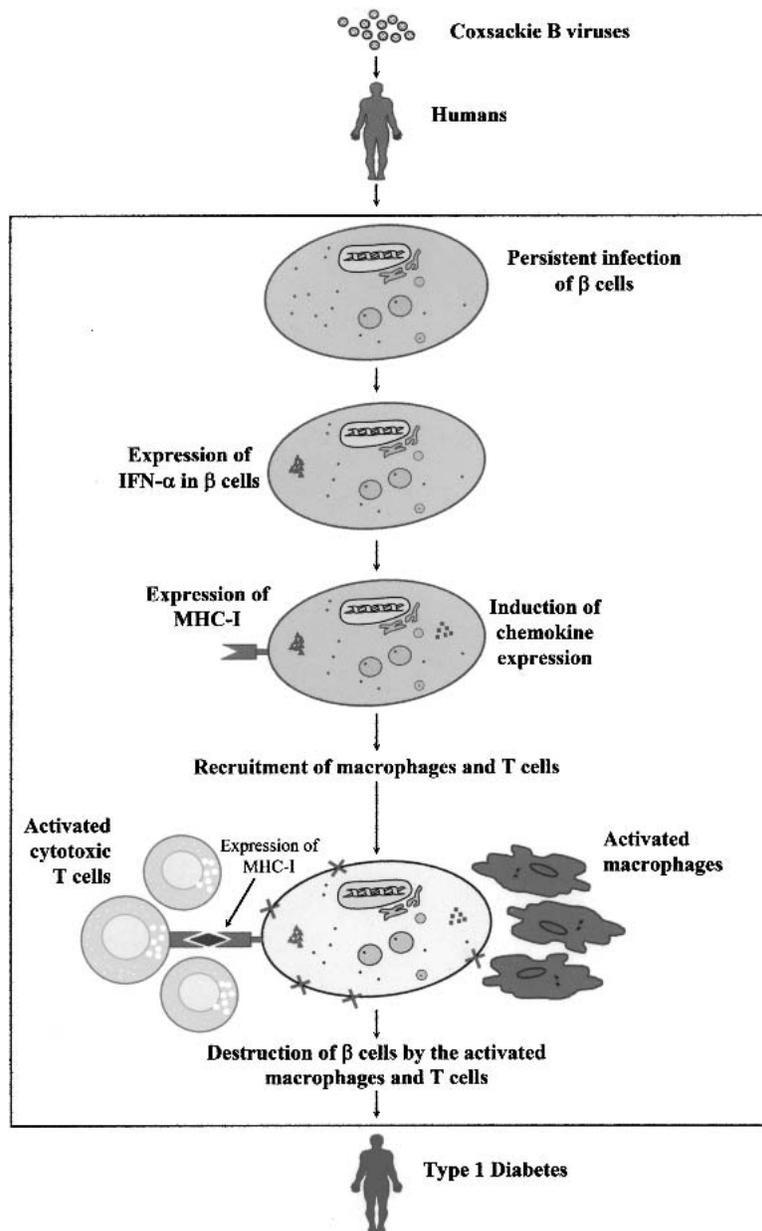


Figure 9 Hypothetical scheme of a possible mechanism of Coxsackie B virus-induced diabetes by persistent infection. Coxsackie B4 virus may persistently infect pancreatic beta cells and induce the expression of IFN- α . IFN- α can induce the expression of chemokines and the expression of MHC-I on the beta cells. The expressed chemokines can recruit macrophages and T cells to the pancreatic islets. The recruited macrophages and T cells are activated and may kill beta cells in conjunction with the hyperexpressed MHC class I molecules, resulting in the development of type 1 diabetes.

type retrovirus (p73) [227]. In addition, mouse hepatitis virus has been shown to lessen the incidence of diabetes in NOD mice [58].

Inoculation of newborn or 6-week-old NOD mice with LCMV can also prevent or decrease the incidence of diabetes (0-6%) [56,228]. It is thought that LCMV may infect and deplete a subpopulation of CD4⁺ T cells since selective suppression of some CD4⁺ T cells has been observed during LCMV infection [228]. Further studies showed that various strains of LCMV, including Armstrong 53b, Traub, WE,

and Pasteur, can prevent diabetes in NOD mice, while other strains of LCMV do not [229]. A strain of LCMV that established a persistent infection which lasted for the lifespan of the animals was not able to prevent T1D [229]. Further investigation using recombinant strains of LCMV to infect NOD mice showed that the portion of the viral genome responsible for the prevention of T1D mapped to the small RNA segment of LCMV Pasteur [229].

Inoculation of BB rats with LCMV (Armstrong strain) also reduced their incidence of diabetes and prevented

mononuclear cell infiltration into the islets, by somehow disordering particular lymphocyte subsets [57]. Viral antibody-free BB rats show an increased frequency and accelerated onset of diabetes, suggesting that infection may have a protective effect against the development of diabetes by these animals [230]. Thus, we speculate that infection or immune stimulation in humans may also reduce the penetrance of susceptibility genes, which could account for the low concordance rate between identical twins of less than 40% for the development of T1D [13].

Conclusion

While genetic predisposition appears to be a major component for the development of T1D, non-genetic factors also play an important role in the expression of the disease. Viruses, as one non-genetic factor, are implicated in the pathogenesis of T1D.

Viruses may directly infect and destroy pancreatic beta cells or may trigger or contribute to beta cell-specific autoimmunity with or without beta cell infection. Viruses such as EMC-D can induce T1D by directly infecting and destroying beta cells through cytolysis. Viruses such as retroviruses may infect beta cells and change existing beta cell antigens into immunogenic forms or may induce new antigens, leading to beta cell-specific autoimmunity. In addition, cellular proteins taken up by the retrovirus envelope can elicit autoimmune responses. Viruses such as Coxsackie virus can also infect beta cells and induce the expression of IFN- α and subsequently chemokines and cytokines, which results in the recruitment of immunocytes to the pancreatic beta cells leading to beta cell destruction (Figure 9). In addition, viruses such as KRV can activate lymphocytes, particularly Th1-like cells, and disrupt the immune balance, resulting in the development of beta cell-specific autoimmune responses in genetically susceptible animals. Systemic virus infection or infection of beta cells can induce the expression of cytokines such as IFN- γ and modulate the expression of MHC class I and class II molecules, leading to the initiation of beta cell-specific autoimmunity. Furthermore, viruses such as rubella and CMV can generate effector T cells that cross-react with beta cell antigens if homologies exist between the viral and beta cell antigens (molecular mimicry) (Figure 1).

In this review, we have attempted to summarize the possible role of viruses in the pathogenesis of T1D. Retrovirus, reovirus, KRV, BVD-MD virus, mumps virus, rubella virus, CMV and Epstein-Barr virus may be associated with the development of autoimmune diabetes, but with different mechanisms of action, in man or animals. The hypothesis that virus induced autoimmune diabetes is supported by studies using transgenic animal models, such as mice transgenic for LCMV and influenza viral proteins. IFN- γ expression in beta cells of RIP-LCMV transgenic mice resulted in the spontaneous development of diabetes. Double transgenic mice expressing HA and MHC class I-restricted HA-

specific TCR developed diabetes immediately after birth with a massive infiltration of CD8⁺ cytotoxic T cells in the pancreatic islets. Perforin/granzyme and fas/fas ligand pathways have been shown to be involved in the destruction of beta cells by CD8⁺ T cells in these transgenic mice.

In contrast to virus-induced autoimmune diabetes, the D variant of EMC virus can cause diabetes in genetically susceptible mice by infecting and destroying pancreatic beta cells. The infection of SJL/J male mice with a high dose of EMC-D virus results in the development of diabetes within 3 days of infection by acute destruction of beta cells, without the development of beta cell-specific autoimmunity. The development of diabetes in these infected mice is mainly due to the replication of the virus in the beta cells. In contrast, infection of DBA/2 mice with a very low dose of EMC-D virus results in the development of diabetes by activation of macrophage-derived soluble mediators. Tyrosine kinase signaling pathways, such as hck, are clearly involved in EMC-D virus-induced macrophage activation. Treatment of EMC-D virus-infected mice with a Src kinase inhibitor, PP2, resulted in the inhibition of hck activity, a decrease in the production of TNF- α and iNOS in the macrophages, and subsequent prevention of diabetes.

Viruses not only cause diabetes, but also prevent the disease in autoimmune diabetes-prone animals. Infection of young NOD mice or BB rats with LCMV results in the prevention of autoimmune diabetes by disordering particular subsets of lymphocytes.

Unequivocal evidence that viruses can cause diabetes comes from animal models such as EMC-D virus-induced diabetes in mice and KRV-induced autoimmune diabetes in DR-BB rats. However, there is no direct evidence that viruses can cause diabetes in humans. It is difficult to study the role of viruses in the development of human diabetes. A large prospective cohort study in prediabetic or genetically susceptible individuals as well as newly diabetic patients may help to understand viral etiology of T1D in humans.

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