Viruses in Type 1 Diabetes: Brief Review

Ji-Won Yoon and Hee-Sook Jun

Abstract

Type 1 diabetes results from the progressive destruction of insulin-producing pancreatic beta cells. Although the etiology of type 1 diabetes is believed to have a major genetic component, studies on the risk of developing type 1 diabetes suggest that environmental factors, such as viruses, may be important etiological determinants. Among the viruses, the most clear and unequivocal evidence that a virus induces type 1 diabetes in animals comes from studies on the D variant of encephalomyocarditis (EMC-D) virus in mice and Kilham rat virus (KRV) in rats. A high titer of EMC-D viral infection results in the development of diabetes within 3 days, primarily due to the rapid destruction of beta cells by viral replication within the cells. A low titer of EMC-D viral infection results in the recruitment of macrophages to the islets. Soluble mediators produced by the activated macrophages such as interleukin-1β, tumor necrosis factor-α, and nitric oxide play a critical role in the destruction of residual beta cells. KRV causes autoimmune type 1 diabetes in diabetes resistant-BioBreeding rats by breakdown of immune balance, including the preferential activation of effector T cells, such as Th1-like $CD45RC^+CD4^+$ T cells and $CD8^+$ T cells, and down-regulation of Th2-like $CD45RC^-CD4^+$ and $CD4^+CD25^+$ T cells, rather than by direct infection of pancreatic beta cells.

Key Words: beta cell-specific autoimmunity; encephalomyocarditis virus; immune balance; Kilham rat virus; macrophages; molecular mimicry; T cells; type 1 diabetes

More than 10 viruses have been reported to be associated with the development of type 1 diabetes-like syndromes in animals. They are coxsackie B viruses in mice and/or nonhuman primates, encephalomyocarditis (EMC[1]) virus in mice, mengo virus in mice, foot-and-mouth disease virus in pigs and/or cattle, retrovirus in mice, rubella virus in hamsters and rabbits, bovine viral diarrhea-mucosal disease virus in cattle, reovirus in mice, Kilham rat virus (KRV[1]) in rats, and cytomegalovirus in the Degu (Jun and Yoon 2001, 2003; Yoon and Jun 2002). Among those viruses, the most clear and unequivocal evidence that a virus induces type 1 diabetes in animals comes from studies on EMC virus in mice (Craighead and McLane 1968; Yoon et al. 1976, 1980, 1982) and KRV in rats (Chung et al. 1997, 2000; Ellerman et al. 1996; Guberski et al. 1991; Zipris et al. 2003). EMC virus is considered to be a primary agent that is selectively injurious to pancreatic beta cells, whereas KRV is considered to be a triggering agent of beta cell-specific autoimmunity without infection of beta cells. In this brief review that precedes our full review titled "A New Look at Virus in Type 1 Diabetes," we would like to highlight these two distinct pathogenic mechanisms for virus-induced diabetes in animals.

Pathogenic Mechanisms of EMC Virus-induced Diabetes

EMC virus has been the most thoroughly studied diabetogenic virus in animals. In genetically susceptible strains of mice, the M variant of EMC (EMC-M) virus induces a diabetes-like syndrome by selectively infecting pancreatic beta cells (Stefan et al. 1978; Yoon and Notkins 1976). However, the development of diabetes in mice after EMC-M viral injection was not consistent (Onodera et al. 1978; Ross et al. 1976; Yoon et al. 1977). Plaque purification of the virus resulted in the isolation of two stable, antigenically indistinguishable variants, a diabetogenic EMC-D virus and a nondiabetogenic EMC-B virus (Yoon et al. 1980). Separation of the EMC-M virus into EMC-D and EMC-B variants has made it possible to identify diabetogenic viral genes (Bae and Yoon 1993; Bae et al. 1989a,b, 1990, 1993; Eun et al. 1988; Jun et al. 1977, 1998; Yoon et al. 1988), to study the long-term complications (Rodrigues et al. 1983; Yoon and Reddi 1984; Yoon et al. 1982), and to elucidate pathogenic mechanisms (Baek and Yoon 1990, 1991; Hirasawa et al. 1997; Yoon et al. 1985).

EMC-D virus produces type 1 diabetes in more than 90% of infected animals, whereas mice inoculated with EMC-B virus do not acquire diabetes. Despite this important difference in pathological action, the D and B variants could not be distinguished antigenically by a sensitive plaque neutralization assay, a competitive radioimmunoassay, or molecular hybridization studies with radiolabeled

Ji-Won Yoon, Ph.D., and Hee-Sook Jun, Ph.D., are Professor and Director, and Associate Professor, respectively, at the Center for Immunologic Research, Bligh Cancer Research Laboratories, and the Department of Microbiology and Immunology, The Chicago Medical School, North Chicago, Illinois.

[1]Abbreviations used in this article: EMC, encephalomyocarditis; iNOS, inducible nitric oxide synthase; IL, interleukin; KRV, Kilham rat virus; NK, natural killer; NO, nitric oxide; PFU, plaque-forming unit; rVV, recombinant vaccinia viruses.

DNA complementary to EMC-D and EMC-B RNAs (Ray et al. 1983; Yoon et al. 1980).

EMC is a small, single-stranded RNA naked virus with a genome size of about 7.8 Kb. The capsid is composed of four polypeptides; VP1, VP2, VP3, and VP4. When the complete nucleotide sequence of both variants was analyzed, it was revealed that EMC-D (7829 bases) differs from EMC-B (7825 bases) by only 14 nucleotides (Bae et al. 1989a). The differences consist of two deletions of five nucleotides, one base insertion, and eight point mutations. The first deletion of three nucleotides and the second deletion of two nucleotides are located in the 5′-poly(C) tract and the 3′-end polyadenylation site, respectively. One base insertion in EMC-B occurs in the 5′-noncoding region. The eight point mutations are located in the polyprotein coding region. Two of these are silent, whereas the remaining six mutations, one located on the L gene and five on the VP1 gene, introduce amino acid changes. Additional studies of 21 different nondiabetogenic and 15 different diabetogenic viruses derived from stocks of the EMC-B and EMC-D variants revealed that only the 776th amino acid, alanine (Ala-776), of the EMC virus polyprotein, located on the major capsid protein VP1, is common to all diabetogenic variants. In contrast, threonine in this position (Thr-776) is common to all nondiabetogenic variants (Bae and Yoon 1993).

To determine whether Ala-776 plays a critical role in the development of type 1 diabetes in EMC virus-infected mice, we changed the amino acid at nucleotide position 3,155 [Ala (GCC)-776 on the polyprotein] by point mutation from alanine to various other amino acids, including threonine (ACC), serine (TCC), proline (CCC), aspartic acid (GAC) or valine (GTC). We found that alanine at amino acid position 776 of the EMC virus polyprotein results in viral diabetogenicity, whereas the substitution of other amino acids results in a loss of diabetogenicity (Jun et al. 1997). As revealed by three-dimensional molecular modeling, the van der Waals interactions are greater and the residues surrounding position 776 of the EMC virus polyprotein are more closely packed in the recombinant chimeric viruses that contain threonine in position 776 than in the recombinant chimeric viruses that contain alanine in this position. These changes result in more accessible surface areas surrounding alanine at position 776, which increases the number of binding sites for viral attachment to beta-cell receptors and promotes viral infection, the destruction of beta cells, and subsequently the development of type 1 diabetes (Jun et al. 1998).

The D variant of the EMC virus, in the absence of the B variant, produces much more severe and prolonged diabetes than the original M variant of the EMC virus, with many complications similar to those seen in human patients with type 1 diabetes. The kidneys of mice that had been diabetic for 6 mo showed both diffuse and nodular types of glomerulosclerosis, and electron microscopy revealed a two- to four-fold increase in the thickness of the glomerular basement membrane (Rodrigues et al. 1983; Yoon et al. 1982). These findings were typical of those seen in humans with the Kimmelstiel-Wilson type of diabetic glomerulosclerosis. The diabetic animals also showed changes in the cornea and retinal vessels (e.g., a decrease in the number of pericytes) similar to those found in patients with diabetes mellitus. Furthermore, there was a four- to six-fold increase in mortality rate of the diabetic animals compared with the controls. A decrease in bone formation and mineralization was also seen in diabetic mice 30 to 180 days after infection with EMC-D virus. These endochondral bone changes were not the result of virus-induced tissue damage, but rather of chronic metabolic changes (Yoon and Reddi 1984). Thus, this animal model is valid in the sense that the virus can produce both early metabolic changes and at least some of the long-term complications of diabetes.

Our earlier studies showed that infection of a genetically susceptible mouse strain (i.e. SJL/J) with a high titer (5×10^5 plaque-forming units [PFUs[1]]/mouse) of EMC-D virus results in the development of diabetes within 4 days due to the acute destruction of beta cells by viral replication within the cells (Yoon et al. 1977). To determine whether T cell suppression had any effect on EMC-D virus-induced diabetes, we treated SJL/J mice with anti-L3T4 (anti-CD4) antibody or anti-Lyt2 (anti-CD8) antibody or both before and after EMC-D virus-infection. There was no significant difference in the incidence of diabetes between the anti-T cell antibody-treated and untreated animals (Baek and Yoon 1990; Yoon et al. 1985). To determine whether macrophages play any role in the destruction of beta cells in EMC-D virus-infected mice, we treated SJL/J mice with anti-macrophage antibody (anti-Mac-2) before and after EMC-D viral infection. We found that the incidence of virus-induced diabetes in mice treated with anti-macrophage antibody was significantly lower than that in the untreated mice, indicating that macrophages play a significant role in the process of beta cell destruction in the EMC-D virus-infected SJL/J mice (Baek and Yoon 1990). However, natural viral infections in animals and humans generally involve exposure to relatively low numbers of virus; exposure to the high viral titers used in the experiments described above would be unlikely in nature. Thus, we established another animal model to study the immune mechanisms involved in the destruction of beta cells, in which mice were infected with a low dose (50-100 PFUs/mouse) of EMC-D virus (Baek and Yoon 1991).

In mice infected with a low dose of EMC-D virus, macrophages play a central role in the destruction of pancreatic beta cells, because activation of macrophages before viral infection results in a significant increase in the incidence of diabetes and inactivation of macrophages before viral infection completely prevents EMC-D virus-induced diabetes (Baek and Yoon 1991). Our 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 of other immunocytes including T cells, natural killer (NK[1]) cells, and B cells (Baek and Yoon 1990). Although these studies demonstrate that mac-

rophages are involved in the destruction of pancreatic beta cells, the role played by macrophages in the destruction of beta cells is not fully understood. Because macrophages are known to produce soluble mediators—interleukin (IL[1])-1β, tumor necrosis factor (TNF[1])-α, and nitric oxide (NO[1])—we recently investigated whether these mediators are involved in the destruction of beta cells in mice infected with a low dose of EMC-D virus. We found that the destruction of beta cells in mice infected with a low dose of EMC-D virus was due to macrophage-derived soluble mediators such as IL-1β, TNF-α, and NO (Hirasawa et al. 1997). However, the molecular mechanism involved in the destruction of beta cells by these soluble mediators is not known. IL-1β induces apoptosis through the induction of inducible nitric oxide synthase (iNOS[1]) expression and NO production in rat islet cells (Fehsel et al. 1993; Kaneto et al. 1995; Rabinovitch et al. 1994). IL-1β and TNF-α increase the expression of Fas, which is involved in apoptosis (Firestein et al. 1995). Therefore, IL-1β and TNF-α, produced by activated macrophages in the pancreatic islets after the infection of mice with a low dose of EMC-D virus, may induce iNOS expression and NO production and contribute to beta cell death through apoptosis.

We further studied whether EMC-D viral infection of macrophages directly induced the expression of these soluble mediators. We isolated macrophages from DBA/2 mice, infected the macrophages with EMC-D virus, and measured the expression of soluble mediators in vitro. We found a significantly higher level of expression of iNOS than of either IL-1β or TNF-α in the cultured macrophages activated by EMC-D virus. There was also a high level of NO production in the cultured macrophages activated by EMC-D virus (Hirasawa et al. 1999). We next determined whether a tyrosine kinase signaling pathway might be involved in the activation of macrophages by EMC-D virus infection, resulting in the production of these soluble mediators, and whether tyrosine kinase inhibitors might abrogate EMC-D virus-induced diabetes in mice. We found that a tyrosine kinase signaling pathway is clearly involved in the EMC-D virus-induced activation of macrophages, because inactivation of tyrosine kinase activity by treatment of EMC-D virus-infected mice with a tyrosine kinase inhibitor, tyrphostin AG126, prevented macrophage activation, resulting in the protection from beta cell destruction (Hirasawa et al. 1999). However, we did not know which family of tyrosine kinases was involved in macrophage activation. Our subsequent studies revealed that the Src family of kinases, p59/p56^{hck}, plays a major role in the activation of macrophages, which subsequently produce TNF-α and NO, leading to the destruction of pancreatic beta cells. Blocking this pathway by treatment with the Src kinase inhibitor, PP2, results in the inhibition of macrophage activation and the subsequent prevention of TNF-α and NO production, leading to the prevention of EMC-D virus-induced diabetes in mice (Choi et al. 2001).

On the basis of these observations, we conclude that the infection of genetically susceptible strains of mice with EMC-D virus initially results in the replication of the virus in pancreatic beta cells, because EMC-D virus is beta cell-tropic. This initial replication results in the recruitment of macrophages that are activated by the EMC-D virus into the pancreatic islets. The activated macrophages produce soluble mediators such as IL-1β, TNF-α, and NO in the islets, which induce apoptosis in the beta cells. The tyrosine kinase signaling pathway, particularly the Src family of kinases, p59/p56^{hck} play a major role in the activation of macrophages, resulting in the production of TNF-α and NO and leading to the destruction of beta cells and subsequent development of diabetes in mice.

Pathogenic Mechanisms of KRV-induced Diabetes

KRV is a small DNA virus that can induce diabetes by provoking autoimmune responses against the beta cell, rather than by direct beta cell cytolysis, in diabetes-resistant-BioBreeding (DR-BB[1]) rats. These rats are derived from diabetes-prone progenitors, but they do not normally develop the disease. When infected with KRV at 3 wk of age, approximately 30% of DR-BB rats develop autoimmune diabetes within 2 to 4 wk, and a further 30% show insulitis without diabetes (Guberski et al. 1991). However, the incidence of diabetes can be increased to between 80 and 100% if DR-BB rats are given injections of poly (I:C) along with KRV (Chung et al. 1997, 2000; Ellerman et al. 1996; Guberski et al. 1991; Zipris et al. 2003).

It is not clear how KRV causes the destruction of beta cells in DR-BB rats without infection of these cells. Molecular mimicry, such as the existence of a common epitope between a KRV-specific peptide and a beta cell autoantigen, has been suggested as a mechanism for the initiation of beta cell-specific autoimmune diabetes (Chung et al. 1997; Guberski et al. 1991). If molecular mimicry is involved in the initiation of beta cell-specific autoimmunity, then KRV antigen-specific T cells generated by KRV peptides might cross-react with beta cells and attack them, resulting in the development of insulitis and, subsequently, diabetes. To induce KRV antigen-specific T cells, recombinant vaccinia viruses (rVVs[1]) expressing KRV proteins were used, because previous work showed that rVVs were successful in inducing cell-mediated immune responses against a target protein (Hany et al. 1989; Moss 1996; Sutter et al. 1994). In addition, the wild-type strain of vaccinia virus does not induce insulitis or diabetes in DR-BB rats (Guberski et al. 1991). When DR-BB rats were infected with these rVVs expressing the KRV peptides (VP1, VP2 [completely overlapped by VP3], or nonstructural proteins 1 or 2), it was found that each viral peptide was clearly expressed in the infected DR-BB rats, viral peptide-specific T cells were generated, and antibodies against the KRV peptides were also induced. However, none of the DR-BB rats developed insulitis or diabetes (Chung et al. 2000). This result suggests

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.

Because the KRV proteins failed to induce autoimmune diabetes in DR-BB rats, we hypothesized that KRV infection disturbs the finely tuned immune balance and activates autoreactive T cells that are cytotoxic to beta cells, resulting in T cell-mediated autoimmune diabetes similar to that seen in DP-BB rats. 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. When KRV-infected DR-BB rats were treated with OX-8 (anti-CD8) monoclonal antibody, the incidence of diabetes in these rats was significantly decreased. Taken together, these results indicate that $CD8^+$ T cells are clearly involved in the destruction of beta cells, although the possibility of the involvement of NK cells cannot be absolutely excluded, because OX-8 monoclonal antibody also depletes NK cells. However, it has been reported that the treatment of DP-BB rats with anti-NK cell antibody failed to prevent diabetes (Ellerman et al. 1993), indicating that $CD8^+$ T cells play a major role in KRV-induced diabetes.

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 part in humoral immune responses (Fowell et al. 1991). It has been suggested that the immune balance between Th1- and Th2-type cells plays an important role in the maintenance of peripheral tolerance. The dominance of Th1 cells over Th2 cells is associated with the development of autoimmune type 1 diabetes, whereas the dominance of Th2 cells over Th1 cells is associated with the prevention of type 1 diabetes (Delovitch and Singh 1997; Liblau et al. 1995; Rabinovitch 1994). It was previously found that KRV infection in DR-BB rats increased the expression of Th1-type cytokines in the splenocytes and pancreatic infiltrates (Chung et al. 1997). Therefore, it is possible that the proportions of Th1 and Th2 cells are altered during KRV infection in DR-BB rats. As expected, 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 compared with PBS-treated controls (Chung et al. 2000).

It seems clear that the infection of DR-BB rats with KRV results in the selective activation of Th1-like $CD45RC^+CD4^+$ T cells and $CD8^+$ T cells. Thus, we questioned whether the selectively activated Th1-like $CD45RC^+CD4^+$ and $CD8^+$ T cells could induce autoimmune diabetes in young DP-BB rats. Th1-like $CD45RC^+CD4^+$ and $CD8^+$ T cells were isolated from DR-BB rats after infection with KRV, stimulated with ConA, and transferred to young DP-BB rats. A total of 88% of the recipients of both $CD45RC^+CD4^+$ and $CD8^+$ T cells developed autoimmune diabetes, indicating 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 decreased compared with the incidence in rats that received a combination of $CD45RC^+CD4^+$ and $CD8^+$ T cells. A combination of $CD45RC^+CD4^+$ T cells from infected rats and $CD8^+$ T cells from uninfected rats or a combination of $CD8^+$ T cells from infected rats and $CD45RC^+CD4^+$ T cells from uninfected rats did not change the incidence of diabetes (Chung et al. 2000). These results indicate that Th1-like $CD4^+$ and $CD8^+$ T cells from KRV-infected rats work synergistically to destroy beta cells. In contrast, none of the recipients of both $CD45RC^-CD4^+$ and $CD8^+$ T cells developed diabetes, indicating that $CD45RC^-CD4^+$ T cells play a part as regulatory T cells. Recent studies also clearly showed that the development of KRV-induced diabetes could be due to the failure to maintain the function of regulatory T cells ($CD4^+CD25^+$ T cells) (Zipris et al. 2003). On the basis of these observations, we conclude that the infection of DR-BB rats with KRV results in the preferential activation of effector T cells, such as Th1-like $CD45RC^+CD4^+$ T cells and $CD8^+$ T cells and the down-regulation of Th2-like $CD45RC^-CD4^+$ and $CD4^+CD25^+$ T cells, and that the activated effector T cells kill the beta cells, similar to the case in DP-BB rats.

In this brief review of virus-induced diabetes, we have discussed how two distinct viruses, a small RNA virus (EMC-D virus) and a small DNA parvovirus (KRV), can kill pancreatic beta cells in mice and rats, resulting in type 1 diabetes. EMC-D virus is a beta cell trophic virus. Infection of genetically susceptible strains of mice (SJL/J, SWR, DBA/2) with a high dose of EMC-D virus results in the development of diabetes by the acute destruction of beta cells through rapid replication of the virus within the beta cells. However, infection of genetically susceptible mouse strains with a low dose of EMC-D virus results in the initial replication of the virus in beta cells, followed by recruitment of macrophages to the infected pancreatic islets. These activated macrophages secrete soluble mediators, such as cytokines (IL-1, TNF-α, IFN-γ) and oxygen free radicals (NO), which destroy the residual beta cells by apoptosis. In contrast to EMC-D-virus-induced diabetes, KRV can cause type 1 diabetes by provoking autoimmune responses against pancreatic beta cells, rather than by direct infection of beta cells. Infection of DR-BB rats with KRV results in the breakdown of immune tolerance by the down-regulation of the Th2-type immune response, including a decrease in the regulatory T cell population, and/or the up-regulation of the Th1-type immune response, including an increase in the autoreactive effector T cell population, leading to autoimmune type 1 diabetes.

References

Bae YS, Eun HM, Pon RT, Yoon JW. 1990. Two amino acids, Phe16 and Ala776, on the polyprotein are most likely to be responsible for the diabetogenicity of EMC virus. J Gen Virol 71:639-645.

Bae YS, Eun HM, Yoon JW. 1989a. Genomic differences between the diabetogenic and nondiabetogenic variants of encephalomyocarditis virus. Virology 170:282-287.

Bae YS, Eun HM, Yoon JW. 1989b. Molecular identification of viral gene. Diabetes 38:316-320.

Bae YS, Kang Y, Ohtsuka E, Yoon JW. 1993. Development of a recombinant RNA technique for the construction of chimeric RNA with a long poly(C) tract. Nucl Acids Res 21:2703-2708.

Bae YS, Yoon JW. 1993. Determination of diabetogenicity attributable to a single amino acid, Ala-776, on the polyprotein of encephalomyocarditis virus. Diabetes 42:435-443.

Baek HS, Yoon JW. 1991. Direct involvement of macrophages in destruction of β-cells leading to development of diabetes in virus-infected mice. Diabetes 40:1586-1597.

Baek HS, Yoon JW. 1990. Role of macrophages in the pathogenesis of encephalomyocarditis virus-induced diabetes in mice. J Virol 64:5708-5715.

Choi KS, Jun HS, Kim HN, Park HJ, Eom YW, Noh HL, Kim HM, Yoon JW. 2001. Role of hck in the pathogenesis of encephalomyocarditis virus-induced diabetes in mice. J Virol 75:1949-1957.

Chung YH, Jun HS, Hirasawa K, Lee BR, van Rooijen N, Yoon JW. 1997. Role of macrophages and macrophage-derived cytokines in the pathogenesis of Kilham rat virus-induced autoimmune diabetes in diabetes-resistant BB rats. J Immunol 159:466-471.

Chung YH, Jun HS, Son M, Bao M, Bae HY, Kang Y, Yoon JW. 2000. Cellular and molecular mechanism for Kilham rat virus-induced autoimmune diabetes in DR-BB rats. J Immunol 165:2866-2876.

Craighead JE, McLane MF. 1968. Diabetes mellitus: Induction in mice by encephalomyocarditis virus. Science 162:913-915.

Delovitch T, Singh B. 1997. The nonobese diabetic mouse as a model of autoimmune disease: Immune dysregulation gets the NOD. Immunity 7:727-738.

Ellerman KE, Richards CA, Guberski DL, Shek WR, Like AA. 1996. Kilham rat virus triggers T-cell-dependent autoimmune diabetes in multiple strains of rat. Diabetes 45:557-562.

Ellerman K, Wrobleski M, Rabinovitch A, Like A. 1993. Natural killer cell depletion and diabetes mellitus in the BB/Wor rat (revisited). Diabetologia 36:596-601.

Eun HM, Bae YS, Yoon JW. 1988. Amino acid differences in capsid protein, VP1, between diabetogenic and nondiabetogenic variants of encephalomyocarditis virus. Virology 163:369-373.

Fehsel K, Jalowy A, Sun Q, Burkart V, Hartmann B, Kolb H. 1993. Islet cell DNA is a target of inflammatory attack by nitric oxide. Diabetes 42:496-500.

Firestein GS, Yeo M, Zvaifler NJ. 1995. Apoptosis in rheumatoid arthritis synovium. J Clin Invest 96:1631-1638.

Fowell D, McKnight AJ, Powrie F, Dyke R, Mason DW. 1991. Subsets of $CD4^+$ T cells and their roles in the induction and prevention of autoimmunity. Immunol Rev 123:37-64.

Guberski DL, Thomas VA, Shek WR, Like AA, Handler ES, Rossini AA, Wallace JE, Welsh RM. 1991. Induction of type I diabetes by Kilham's rat virus in diabetes-resistant BB/Wor rats. Science 254:1010-1013.

Hany M, Oehen S, Schulz M. 1989. Anti-viral protection and prevention of lymphocytic choriomeningitis or of the local footpad swelling reaction in mice by immunization with vaccinia-recombinant virus expressing LCMV-WE nucleoprotein or glycoprotein. Eur J Immunol 19:417-424.

Hirasawa K, Jun HS, Han HS, Zhang ML, Hollenberg MD, Yoon JW. 1999. Prevention of encephalomyocarditis virus-induced diabetes in mice by inhibition of the tyrosine kinase signalling pathway and subsequent suppression of nitric oxide production in macrophages. J Virol 73:8541-8548.

Hirasawa K, Jun HS, Maeda K, Kawaguchi, Itagaki S, Mikami T, Baek HS, Doi K, Yoon JW. 1997. Possible role of macrophage-derived soluble mediators in the pathogenesis of encephalomyocarditis virus-induced diabetes in mice. J Virol 71:4024-4031.

Jun HS, Kang Y, Notkins AL, Yoon JW. 1997. Gain or loss of diabetogenicity resulting from a single point mutation in recombinant encephalomyocarditis virus. J Virol 71:9782-9785.

Jun HS, Kang Y, Yoon HS Kim KH, Notkins AL, Yoon JW. 1998. Determination of encephalomyocarditis viral diabetogenicity by a putative binding site of the viral capsid protein. Diabetes 47:576-582.

Jun HS, Yoon JW. 2003. A new look at viruses in type 1 diabetes. Diabet Metab Res Rev 19:8-31.

Jun HS, Yoon JW. 2001. Role of viruses in type 1 diabetes: Two distinct cellular and molecular pathogenic mechanisms of virus-induced diabetes in animals. Diabetologia 44:271-285.

Kaneto H, Fujii J, Seo HG, Suzuki K, Matsuoka T, Nakamura M, Tatsumi H, Yumasaki Y, Kamada T, Taniguchi N. 1995. Apoptotic cell death triggered by nitric oxide in pancreatic β-cells. Diabetes 44:733-738.

Liblau RS, Singer SM, McDevitt HO. 1995. Th1 and Th2 $CD4^+$ T cells in the pathogenesis of organ-specific autoimmune disease. Immunol Today 16:34-38.

Moss B. 1996. Genetically engineered poxvirus for recombinant gene expression, vaccination, and safety. Proc Natl Acad Sci U S A 93:11341-11348.

Onodera T, Yoon JW, Brown KS., Notkins AL. 1978. Evidence for a single locus controlling susceptibility to virus-induced diabetes mellitus. Nature 274:693-696.

Rabinovitch A. 1994. Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM: Therapeutic intervention by immunostimulation? Diabetes 43:613-621.

Rabinovitch A, Suarez-Pinzon WL, Shi Y, Morgan AR, Bleackley RC. 1994. DNA fragmentation is an early event in cytokine-induced islet beta-cell destruction. Diabetologia 37:733-738.

Ray UR, Aulakh GS, Schubert M, McClintock PR, Yoon JW, Notkins AL. 1983. Virus-induced diabetes mellitus: XXV. Difference in the RNA fingerprints of diabetogenic and non-diabetogenic variants of encephalomyocarditis virus. J Gen Virol 64:947-950.

Rodrigues M, Currier C, Yoon JW. 1983. Electron microscopy of renal and ocular changes in virus-induced diabetes mellitus in mice. Diabetologia 24:293-299.

Ross ME, Onodera T, Brown KS, Notkins AL. 1976. Virus-induced diabetes mellitus. IV. Genetic and environmental factors influencing the development of diabetes after infection with the M variant of encephalomyocarditis virus. Diabetes 25:190-197.

Stefan Y, Malaisse-Lagae F, Yoon JW, Notkins AL, Orci L. 1978. Virus-induced diabetes in mice: A quantitative evaluation of islet cell population by immunofluorescence technique. Diabetologia 15:395-401.

Sutter G, Wyatt LS, Foley PL, Bennick JR, Moss B. 1994. A recombinant vector derived from the host-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to influenza virus. Vaccine 12:1032-1040.

Yoon JW, Jun HS. 2002. Viruses in the pathogenesis of type 1 diabetes. In: Pickup J, Williams G, eds. Textbook of Diabetes. 3rd ed. London: Blackwell Scientific Publications. p 16.1-16.16.

Yoon JW, Lesniak MA, Fussganger R, Notkins AL. 1976. Genetic differences in susceptibility of pancreatic β-cells to virus-induced diabetes mellitus. Nature 264:178-180.

Yoon JW, McClintock PR, Bachurski CJ, Longstreth JD, Notkins AL. 1985. Virus-induced diabetes: No evidence for immune mechanisms in the destruction of beta cells by the D variant of encephalomyocarditis virus. Diabetes 34:922-925.

Yoon JW, McClintock PR, Onodera T, Notkins AL. 1980. Virus-induced diabetes mellitus. XVIII. Inhibition by a nondiabetogenic variant of encephalomyocarditis virus. J Exp Med 152:878-892.

Yoon JW, Notkins AL. 1976. Virus-induced diabetes mellitus. VI. Genetically determined host differences in the replication of encephalomyocarditis virus in pancreatic β-cells. J Exp Med 143:1170-1185.

Yoon JW, Onodera T, Notkins AL. 1977. Virus-induced diabetes mellitus. VIII. Passage of encephalomyocarditis virus and severity of diabetes in susceptible and resistant strains of mice. J Gen Virol 37:225-232.

Yoon JW, Reddi AH. 1984. Decreased bone formation and mineralization in virus-induced diabetes mellitus. Am J Physiol 246:C177-C179.

Yoon JW, Rodrigues MM, Currier C, Notkins A. 1982. Long-term complications of virus-induced diabetes mellitus in mice. Nature 296:566-569.

Yoon JW, Wong AK, Bae YS, Eun HM. 1988. An apparent deletion of an oligonucleotide detected by RNA fingerprint in the nondiabetogenic B variant of encephalo-myocarditis virus is caused by a point mutation. J Virol 62:637-650.

Zipris D, Hillebrands JL, Welsh RM, Rozing J, Xie JX, Mordes JP, Greiner DL, Rossini AA. 2003. Infections that induce autoimmune diabetes in BBDR rats modulate $CD4^+CD25^+$ T cell populations. J Immunol 170: 3592-3602.