Chapter 6
Enteroviruses in the Mouse Model of Type 1 Diabetes

Nora M. Chapman

Introduction

Findings of pancreatitis in mice after infection with the human enteroviruses coxsackievirus B (CVB) suggested a relationship to the onset of type 1 diabetes (Coleman et al. 1973), a correlation that had been suggested by studies that had variably found a serologic relationship of CVB4 to recent onset diabetic patients (Gamble et al. 1969). Although other enteroviruses may well be involved in induction of pancreatitis and type 1 diabetes (Tracy et al. 2010), the ability of CVBs to use the murine homolog of the coxsackievirus and adenovirus receptor CAR (Bergelson et al. 1997; Carson et al. 1997; Tomko et al. 1997; Bergelson et al. 1998) makes CVB-induced murine pancreatitis and diabetes a model for the human disease.

Properties of Diabetogenic CVBs

A CVB4 isolate from a human diabetic patient was capable of inducing insulitis and diabetes in SJL mice (Yoon et al. 1979), but, in general, most diabetogenic strains of CVB have had one or more passages in mice or murine pancreas or islets in culture (Al-Hello et al. 2005; Yoon et al. 1978a, b; Webb et al. 1976). As these enteroviruses evolve rapidly in selective cultures, passage in murine pancreatic cells is likely to increase the extent to which these viruses can infect and induce diabetes in

N.M. Chapman, Ph.D. (✉)
Department of Pathology & Microbiology, University of Nebraska Medical Center, 986495 Nebraska Medical Center, Omaha, NE 68198-6495, USA
e-mail: nchapman@unmc.edu

K.W. Taylor, H. Hyoty, A. Toniolo, A.J. Zuckerman (eds.), *Diabetes and Viruses*,
DOI 10.1007/978-1-4614-4051-2_6,

mice. Sequence analysis of pancreotropic and diabetogenic strains has identified sites of variation in the 5′ nontranslated region (5′NTR), the capsid, and the nonstructural proteins (Al-Hello et al. 2005; Kang et al. 1994; Caggana et al. 1993; Titchener et al. 1994; Yin et al. 2002). Chimeras of the 5′NTR of CVB3 strains demonstrate that attenuating determinants are present in this region of the CVB3/GA strain (Chapman et al. 1994) for replication in a murine β cell line, MIN6, and for replication in the murine pancreas in vivo (Kanno et al. 2006). Variations in the capsid proteins may alter sites which control interaction with the CAR receptor or with a co-receptor, the decay accelerating factor (DAF) which may play a role in CVB virus entry (Coyne and Bergelson 2006) and in the immune response to the virus (Huber and Rincon 2008; Huber et al. 2006). Some identified sites in CVB4 VP2 and VP3 (Kang et al. 1994) align close to sites shown to be important for DAF binding in CVB3 (Pan et al. 2011), although the relatively nonpancreovirulent CVB3/GA does not differ from the pancreovirulent CVB3/28 at these sites (Chapman et al. 1994). As the extent of replication in the pancreas is related to the extent of acceleration of diabetes (Kanno et al. 2006), several of these variations may be due to selection to match the murine receptor(s) and host cell factors. Selection by growth in pancreatic islets or pancreas is likely to generate strains capable of a high rate of replication in the pancreas in vivo, but as most CVB strains have some degree of pancreovirulence in mice (Tracy et al. 2000), a virulent CVB may cause murine pancreatitis, but not diabetes, without passage in mice.

Typically, inoculation of mice with a CVB will result in much more extensive pathology of the acinar tissue than the islets (Harrison et al. 1972). Inoculation of 6-week-old SJL mice at dosages of 10^5 PFU of either the standard CVB4 serotype strain, JVB, or the E2 diabetogenic strain resulted in extensive acinar cell death (Yap et al. 2003). Infection of Swiss Albino mice with the prototypic CVB3, Nancy, was able to generate infection of the pancreas with pathology of the acinar tissue (Bopegamage et al. 2005). Survival of the islets during infection has been attributed to the relative expression of CAR (Mena et al. 2000). Studies have demonstrated CAR expression in islets of infected mice (Drescher et al. 2004) as well as viral RNA (Yap et al. 2003). Although very low level expression of CAR may limit virus infection in cultures, almost indetectable levels of CAR still allow virus replication (Carson et al. 2007), but the low level expression is likely to limit the degree of infection of the islets. Components of the innate immune response provide more antiviral protection to the murine exocrine tissue than the islets. Islets of mouse strains knockout of RNase L and the double-stranded RNA-activated protein kinase, PKR, are more resistant to infection than the acinar tissue in vivo, despite increased mortality due to CVB4 infection (Flodström-Tullberg et al. 2005). Knockouts of interferon α and β receptors, melanoma differentiation-associated protein-5 (MDA-5) and its signaling adaptor, mitochondrial antiviral signaling (MAVS) did not enhance infection of pancreatic islets after CVB4 infection (Hühn et al. 2010; Wang et al. 2010), although there was more extensive pathology of the exocrine pancreas. As MDA-5 is degraded during the course of enterovirus cell infection (Barral et al. 2007) and another viral sensor, retinoic acid-induced gene 1 (RIG-I), does not affect susceptibility to picornavirus infection (Kato et al. 2006), CVBs are likely to have

evolved means of avoiding reduction of virus replication through the innate immune response to some degree.

Diabetogenic virus infections resulted in reduced neogenesis of islets postinfection indicating that there may be a lasting effect of the infection with the diabetogenic viruses (Yap et al. 2003). In studies in which RT-PCR was employed to detect viral RNA, persistence of CVB RNA is noted after loss of detection of virus by cytopathic assays (Bopegamage et al. 2005; Yap et al. 2003). In the heart, CVBs can persist in the form of a defective virus (Kim et al. 2005). The defect results in reduced levels of positive strand RNA which results in reduced levels of virus replication and cytolysis (Kim et al. 2005). These defective viruses tend to be selected in quiescent cells in culture (Kim et al. 2008) or in adult hearts (Kim et al. 2005; Chapman et al. 2008). The presence of viral antigens in islets without obvious cellular necrosis suggests the selection of defective virus in islet cells. As these genomes produce viral proteins (although at a reduced rate), the potential for alterations of function of these cells remains despite their reduced replication rate. Part of the apparent resistance to CVB infection of the islets may be due to the resistance of nondividing cells to replication of CVBs (Feuer et al. 2002; Chapman and Kim 2008), as most of the islet cells in a non-regenerating islet in vivo are quiescent (Salpeter et al. 2010). Regeneration or neogenesis of islets necessarily involves dividing cells which are more susceptible to viral infection and, consequently, can be eliminated in a pancreas with a persisting infection.

Nonobese diabetic (NOD) female mice (Atkinson and Leiter 1999; Kikutani and Makino 1992) develop spontaneously autoimmunity to pancreatic antigens, insulitis, and diabetes by 12 weeks of age. As in other mice, inoculation of NOD mice prior to 8 weeks of age results in less extensive infection of the islets than the exocrine tissue even with diabetogenic virus strains (Serreze et al. 2000; Tracy et al. 2002; Drescher et al. 2004). Infection of NOD mice with diabetogenic and nondiabetogenic CVBs at the stage in which insulitis is beginning to be manifested (8–12 weeks of age) results in the infection of islets and accelerated development of diabetes (Serreze et al. 2000; Drescher et al. 2004). Increasing dosage of a less virulent CVB can increase the extent of conversion to diabetes indicating the extent of replication in the pancreas correlates with induction of diabetes (Kanno et al. 2006). Knockouts of interleukin-4 (IL-4) do not alter the conversion to diabetes in NOD mice by CVB4 infection, whereas loss of interferon-γ (IFN-γ) does delay the onset of diabetes (Serreze et al. 2005). As CVBs can induce IFN-γ (Nair et al. 2010), the finding that higher levels of replication correlate with accelerated onset of diabetes may increase the exposure of islets to IFN-γ. As transforming growth factor-β (TGF-β) can reduce the expression of the CVB receptor (Lacher et al. 2006; Shi et al. 2010), one effect of the expression of TGF-β by beta cells may be to lower the expression of the receptor necessary for infection of beta cells (Richer et al. 2008; Peng et al. 2004). In CVB3-induced myocarditis, adoptive transfer of T regulatory cells increased TGF-β expression, decreased CAR expression, and lowered CVB3 replication in the heart (Shi et al. 2010) indicating that one protective effect of T regulatory cells may be lowering CVB replication by reducing the expression of the CVB receptor.

On the other hand, infection of NOD mice at 3–4 weeks of age with CVBs (an age at which non-NOD mice are more susceptible to pancreatitis) results in delayed onset and decreased incidence of diabetes from uninfected NOD mice (Tracy et al. 2002; Serreze et al. 2000; Filippi et al. 2009). It is known that infection or treatment with a number of agents will decrease or delay the onset of diabetes in the NOD mouse (Atkinson and Leiter 1999). Neither IL-4 nor IFN-γ are required for the delay or decrease in the development of type 1 diabetes in the NOD mouse due to CVB4 infection prior to insulitis (Serreze et al. 2005). Activation or supplementation of invariant natural killer cells (iNKT cells) in NOD mice leads to later onset or less conversion to diabetes in the NOD mouse (Lehuen et al. 1998; Naumov et al. 2001; Sharif et al. 2001), an effect requiring $CD4^+$ $CD25^+$ T regulatory cells (Ly et al. 2006). As CVB infections in the context of virus-induced expression of tumor necrosis factor-α (TNF-α) have been shown to upregulate CD1d (Huber and Sartini 2005), it is possible that the very active CVB infection of the exocrine pancreas produces an environment in which iNKT cell activation is more likely. CVB3-infected murine dendritic cells do not produce cytopathic virus but are stimulated to produce interferons, interleukins, and chemokines (Weinzierl et al. 2008). A.BY/SnJ mice susceptible to CVB3-induced chronic myocarditis produce dendritic cells (DC) which, upon infection, have similar levels of positive and negative CVB3 RNA, produce less IL-10 than those from C57BL/6 mice (resistant to CVB3-induced chronic myocarditis), and have a later peak of IL-6 and TNF-α (Weinzierl et al. 2008). It is possible that CVB-infected DCs may interact with iNKT differently resulting in changes to the level to which pancreatic antigen-specific T regulatory cells are generated via murine DCs.

As discussed above, there is evidence of persistent infection of the pancreas after inoculation of mice with diabetogenic CVBs (Yap et al. 2003; Bopegamage et al. 2005). In the heart, CVBs can persist in the form of a defective virus (Kim et al. 2005). As this slower replicating defective virus is capable of persisting without rapid cytolysis, DCs and other cells infected with defective viruses may persist so that an environment for the activation of T regulatory cells specific for pancreatic antigens may be long-lasting.

Is this relevant for human disease? CVBs are human pathogens which target the pancreas and produce pathogenic immune responses. As these viruses have also been shown to have an ability to persist after an acute infection in a form capable of replicating and producing viral proteins, but with reduced cytolysis, they are good candidates to alter the complex immune responses involved in autoimmune pathogenesis. The ability to study these human viruses in a murine model of type 1 diabetes allows an analysis of mechanisms for this disease. Enterovirus infections are common in the human population, but their frequency may be decreasing in the populations in which type 1 diabetes is increasing (Viskari et al. 2005; Tracy et al. 2010). This may have the resulting double defect of decreasing regulation of pancreatic autoimmunity in those prone to this disease, and increasing the chance that when an enterovirus infection occurs it will be at an age in which autoimmunity has made islets susceptible to infection.

References

Al-Hello H, Davydova B, Smura T, Kaialainen S, Ylipaasto P, Saario E, Hovi T, Rieder E, Roivainen M (2005) Phenotypic and genetic changes in coxsackievirus B5 following repeated passage in mouse pancreas in vivo. J Med Virol 75:566–574

Atkinson MA, Leiter EH (1999) The NOD mouse model of type 1 diabetes: as good as it gets? Nat Med 5:601–604

Barral PM, Morrison JM, Drahos J, Gupta P, Sarkar D, Fisher PB, Racaniello VR (2007) MDA-5 is cleaved in poliovirus-infected cells. J Virol 81:3677–3684

Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW (1997) Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 275:1320–1323

Bergelson JM, Krithivas A, Celi L, Droguett G, Horwitz MS, Wickham T, Crowell RL, Finberg RW (1998) The murine CAR homolog is a receptor for coxsackie B viruses and adenoviruses. J Virol 72:415–419

Bopegamage S, Kovacova J, Vargova A, Motusova J, Petrovicova A, Benkovicova M, Gomolcak P, Bakkers J, van Kuppeveld F, Melchers WJG, Galama JM (2005) Coxsackie B virus infection of mice: inoculation by the oral route protects the pancreas from damage, but not from infection. J Gen Virol 86:3271–3280

Caggana M, Chan P, Ramsingh A (1993) Identification of a single amino acid residue in the capsid protein VP1 of coxsackievirus B4 that determines the virulent phenotype. J Virol 67: 4797–4803

Carson SD, Kim KS, Pirruccello SJ, Tracy S, Chapman NM (2007) Endogenous low-level expression of the coxsackievirus and adenovirus receptor enables coxsackievirus B3 infection of RD cells. J Gen Virol 88:3031–3038

Carson SD, Chapman NM, Tracy SM (1997) Purification of the putative coxsackievirus B receptor from HeLa cells. Biochem Biophys Res Commun 233:325–328

Chapman NM, Kim KS (2008) Persistent coxsackievirus infection: enterovirus persistence in chronic myocarditis and dilated cardiomyopathy. Curr Top Microbiol Immunol 323:275–292

Chapman NM, Kim KS, Drescher KM, Oka K, Tracy S (2008) 5′ terminal deletions in the genome of a coxsackievirus B2 strain occurred naturally in human heart. Virology 375:480–491

Chapman NM, Tu Z, Tracy S, Gauntt CJ (1994) An infectious cDNA copy of the genome of a non-cardiovirulent coxsackievirus B3 strain: its complete sequence analysis and comparison to the genomes of cardiovirulent coxsackieviruses. Arch Virol 135:115–130

Coleman TJ, Gamble DR, Taylor KW (1973) Diabetes in mice after coxsackie B4 virus infection. Br Med J 3:25–27

Coyne CB, Bergelson JM (2006) Virus-induced Abl and Fyn kinase signals permit coxsackievirus entry through epithelial tight junctions. Cell 124:119–131

Drescher KM, Kono K, Bopegamage S, Carson SD, Tracy S (2004) Coxsackievirus B3 infection and type 1 diabetes development in NOD mice: insulitis determines susceptibility of pancreatic islets to virus infection. Virology 329:381–394

Feuer R, Mena I, Pagarigan R, Slifka MK, Whitton JL (2002) Cell cycle status affects coxsackievirus replication, persistence, and reactivation in vitro. J Virol 76:4430–4440

Filippi CM, Estes EA, Oldham JE, von Herrath MG (2009) Immunoregulatory mechanisms triggered by viral infections protect from type 1 diabetes in mice. J Clin Invest 119:1515–1523

Flodström-Tullberg M, Hultcrantz M, Stotland A, Maday A, Tsai D, Fine C, Williams B, Silverman R, Sarvetnick N (2005) RNase L and double-stranded RNA-dependent protein kinase exert complementary roles in islet cell defense during coxsackievirus infection. J Immunol 174:1171–1177

Gamble DR, Kinsley ML, FitzGerald MG, Bolton R, Taylor KW (1969) Viral antibodies in diabetes mellitus. Br Med J 3:627–630

Harrison AK, Bauer SP, Murphy FA (1972) Viral pancreatitis: ultrastructural pathological effects of coxsackievirus B3 infection in newborn mouse pancreas. Exp Mol Pathol 17:206–219

Huber S, Song WC, Sartini D (2006) Decay-accelerating factor (CD55) promotes CD1d expression and Vgamma4+ T-cell activation in coxsackievirus B3-induced myocarditis. Viral Immunol 19:156–166

Huber SA, Sartini D (2005) Roles of tumor necrosis factor alpha (TNF-alpha) and the p55 TNF receptor in CD1d induction and coxsackievirus B3-induced myocarditis. J Virol 79:2659–2665

Huber SA, Rincon M (2008) Coxsackievirus B3 induction of NFAT: requirement for myocarditis susceptibility. Virology 381:155–160

Hühn MH, McCartney SA, Lind K, Svedin E, Colonna M, Flodström-Tullberg M (2010) Melanoma differentiation-associated protein-5 (MDA-5) limits early viral replication but is not essential for the induction of type 1 interferons after coxsackievirus infection. Virology 401:42–48

Kang Y, Chatterjee NK, Nodwell MJ, Yoon JW (1994) Complete nucleotide sequence of a strain of coxsackie B4 virus of human origin that induces diabetes in mice and its comparison with nondiabetogenic coxsackie B4 JBV strain. J Med Virol 44:353–361

Kanno T, Kim K, Kono K, Drescher KM, Chapman NM, Tracy S (2006) Group B coxsackievirus diabetogenic phenotype correlates with replication efficiency. J Virol 80:5637–5643

Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh C-S, Reis E, Sousa C, Matsuura Y, Fujita T, Akira S (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441:101–105

Kikutani H, Makino S (1992) The murine autoimmune diabetes model: NOD and related strains. Adv Immunol 51:285–322

Kim K-S, Tracy S, Tapprich W, Bailey J, Lee C-K, Kim K, Barry WH, Chapman NM (2005) 5'-Terminal deletions occur in coxsackievirus B3 during replication in murine hearts and cardiac myocyte cultures and correlate with encapsidation of negative-strand viral RNA. J Virol 79:7024–7041

Kim KS, Chapman NM, Tracy S (2008) Replication of coxsackievirus B3 in primary cell cultures generates novel viral genome deletions. J Virol 82:2033–2037

Lacher MD, Tiirikainen MI, Saunier EF, Christian C, Anders M, Oft M, Baimain A, Akhurst RJ, Korn WM (2006) Transforming growth factor-beta receptor inhibition enhances adenoviral infectability of carcinoma cells via up-regulation of coxsackie and adenovirus receptor in conjunction with reversal of epithelial-mesenchymal transition. Cancer Res 66:1648–1657

Lehuen A, Lantz O, Beaudoin L, Laloux V, Carnaud C, Bendelac A, Bach J-F, Monteiro RC (1998) Overexpression of natural killer T cells protects Valpha14-Jalpha281 transgenic nonobese diabetic mice against diabetes. J Exp Med 188:1831–1839

Ly D, Mi QS, Hussain S, Delovitch TL (2006) Protection from type 1 diabetes by invariant NK T cells requires the activity of CD4+ CD25+ regulatory T cells. J Immunol 177:3695–3670

Mena I, Fischer C, Gebhard JR, Perry CM, Harkins S, Whitton JL (2000) Coxsackievirus infection of the pancreas: evaluation of receptor expression, pathogenesis, and immunopathology. Virology 271:276–288

Nair S, Leung KC, Rawlinson WD, Naing Z, Craig ME (2010) Enterovirus infection induces cytokine and chemokine expression in insulin-producing cells. J Med Virol 82:1950–1957

Naumov YN, Bahjat KS, Gausling R, Abraham R, Exley MA, Koezuka Y, Balk SB, Strominger JL, Clare-Salzer M, Wilson SB (2001) Activation of CD1d-restricted T cells protects NOD mice from developing diabetes by regulating dendritic cell subsets. Proc Natl Acad Sci USA 98:13838–13843

Pan J, Narayanan B, Shah S, Yoder JD, Cifuente JO, Hafenstein S, Bergelson JM (2011) Single amino acid changes in the virus capsid permit coxsackievirus B3 to bind decay-accelerating factor. J Virol 85:7436–7443

Peng Y, Laouar Y, Li MO, Green EA, Flavell RA (2004) TGF-beta regulates in vivo expansion of Foxp3-expressing CD4+ CD25+ regulatory T cells responsible for protection against diabetes. Proc Natl Acad Sci USA 101:4572–4577

Richer MJ, Straka N, Fang D, Shanina I, Horwitz MS (2008) Regulatory T-cells protect from type 1 diabetes after induction by coxsackievirus infection in the context of transforming growth factor-beta. Diabetes 57:1302–1311

Salpeter SJ, Klein AM, Huangfu D, Grimsby J, Dor Y (2010) Glucose and aging control the quiescence period that follows pancreatic beta cell replication. Development 137:3205–3213

Serreze DV, Wasserfall C, Ottendorfer EW, Stalvey M, Pierce MA, Gauntt C, O'Donnell B, Flanagan JB, Campbell-Thompson M, Ellis TM, Atkinson MA (2005) Diabetes acceleration or prevention by a coxsackievirus B4 infection: critical requirements for both interleukin-4 and gamma interferon. J Virol 79:1045–1052

Serreze DV, Ottendorfer EW, Ellis TM, Gauntt CJ, Atkinson MA (2000) Acceleration of type 1 diabetes by a coxsackievirus infection requires a preexisting critical mass of autoreactive T-cells in pancreatic islets. Diabetes 49:708–711

Sharif S, Arreaza GA, Zucker P, Mi Q-S, Sondhi J, Naidenko OV, Kronenberg M, Koezuka Y, Delovitch TL, Gombert J-M, Leite-de-Moraes M, Gouarin C, Zhu R, Hameg A, Nakayama T, Taniguchi M, Lepault F, Lehuen A, Bach J-F, Herbelin A (2001) Activation of natural killer T cells by alpha-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. Nat Med 7:1057–1062

Shi Y, Fukuoka M, Li G, Liu Y, Chen M, Konviser M, Chen X, Opavsky MA, Liu PP (2010) Regulatory T cells protect mice against coxsackievirus-induced myocarditis through the transforming growth factor beta-coxsackie-adenovirus receptor pathway. Circulation 121:2624–2634

Titchener PA, Jenkins O, Szopa TM, Taylor KW, Almond JW (1994) Complete nucleotide sequence of a beta-cell tropic variant of coxsackievirus B4. J Med Virol 42:369–373

Tomko RP, Xu R, Philipson L (1997) HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. Proc Natl Acad Sci USA 94:3352–3356

Tracy S, Drescher KM, Chapman NM, Kim K-S, Carson SD, Pirruccello S, Lane PH, Romero JR, Leser JS (2002) Toward testing the hypothesis that group B coxsackieviruses (CVB) trigger insulin-dependent diabetes: inoculating nonobese diabetic mice with CVB markedly lowers diabetes incidence. J Virol 76:12097–12111

Tracy S, Höfling K, Pirruccello S, Lane PH, Reyna SM, Gauntt CJ (2000) Group B coxsackievirus myocarditis and pancreatitis: connection between viral virulence phenotypes in mice. J Med Virol 62:70–81

Tracy S, Drescher KM, Jackson JD, Kim K, Kono K (2010) Enteroviruses, type 1 diabetes and hygiene: a complex relationship. Rev Med Virol 20:106–116

Viskari H, Ludvigsson J, Uibo R, Salur L, Marciulionyte D, Hermann R, Soltesz G, Füchtenbusch M, Ziegler A-G, Kondrashova A, Romanov A, Kaplan B, Laron Z, Koskela P, Vesikari T, Huhtala H, Knip M, Hyöty H (2005) Relationship between the incidence of type 1 diabetes and maternal enterovirus antibodies: time trends and geographical variation. Diabetologia 48: 1280–1287

Wang JP, Cerny A, Asher DR, Kurt-Jones EA, Bronson RT, Finberg RW (2010) MDA5 and MAVS mediate type I interferon responses to coxsackie B virus. J Virol 84:254–260

Webb SR, Loria RM, Madge GE, Kibrick S (1976) Susceptibility of mice to group B coxsackie virus is influenced by the diabetic gene. J Exp Med 143:1239–1248

Weinzierl AO, Szalay G, Wolburg H, Sauter M, Rammensee H-G, Kandolf R, Stevanović S, Klingel K (2008) Effective chemokine secretion by dendritic cells and expansion of cross-presenting CD4−/CD8+ dendritic cells define a protective phenotype in the mouse model of coxsackievirus myocarditis. J Virol 82:8149–8160

Yap IS, Giddings G, Pocock E, Chantler JK (2003) Lack of islet neogenesis plays a key role in beta-cell depletion in mice infected with a diabetogenic variant of coxsackievirus B4. J Gen Virol 84:3051–3068

Yin H, Berg AK, Westman J, Hellerström C, Frisk G (2002) Complete nucleotide sequence of a coxsackievirus B-4 strain capable of establishing persistent infection in human pancreatic islet cells: effects on insulin release, proinsulin synthesis, and cell morphology. J Med Virol 68:544–557

Yoon JW, Austin M, Onodera T, Notkins AL (1979) Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. N Engl J Med 300:1173–1179

Yoon JW, Onodera T, Jenson AB, Notkins AL (1978a) Virus-induced diabetes mellitus. XI. Replication of coxsackie B3 virus in human pancreatic beta cell cultures. Diabetes 27: 778–781

Yoon JW, Onodera T, Notkins AL (1978b) Virus-induced diabetes mellitus. XV. Beta cell damage and insulin-dependent hyperglycemia in mice infected with coxsackie virus B4. J Exp Med 148:1068–1080