36 Karaghiosoff, M., Steinborn, R., Kovarik, P. *et al.* 003. Central role for type I interferons and Tyk2 in lipopolysaccharide-induced endotoxin shock. *Nat. Immunol.* 4:471.
37 Tokumasa, N., Suto, A., Kagami, S. *et al.* 2007. Expression of Tyk2 in dendritic cells is required for IL-12, IL-23, and IFN-γ production and the induction of Th1 cell differentiation. *Blood* 110:553.
38 Weinblatt, M. E., Kremer, J. M., Bankhurst, A. D. *et al.* 1999. A trial of etanercept, a recombinant tumor necrosis factor receptor: fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N. Engl. J. Med.* 340:253.
39 Edwards, J. C., Szczepanski, L., Szechinski, J. *et al.* 2004. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N. Engl. J. Med.* 350:2572.
40 Genovese, M. C., Becker, J. C., Schiff, M. *et al.* 2005. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N. Engl. J. Med.* 353:1114.

Chapter 5
Encephalomyocarditis Virus

[AU1] Seiho Nagafuchi, Hironori Kurisaki, and Hitoshi Katsuta

Introduction

Accumulating evidence has suggested a viral origin of type 1 diabetes development. Historical study has indicated the presence of certain viruses including coxsackie virus, cyotomegalovirus, varicella-zoster virus, and rubella virus in patients with fatal viral infections associated with pancreatic islet cell damage (Jenson et al. 1980), suggesting that many viruses have potent diabetes inducers. Recent advances in the field have focused on the enteroviral infection as the most possible candidate virus to induce type 1 diabetes associated with immunopathologic reaction (Clements et al. 1995; Hanafusa and Imagawa 2008; Tauriainen et al. 2011; Richardson et al. 2011).

Encephalomyocarditis (EMC) virus has provided the most useful animal model for virus-induced type 1 diabetes (Jun and Yoon 2001). Development of diabetes depends on many factors including virus strain, challenge dose, host factors such as sex, immunoprotective function, inflammatory responses with macrophages, cytokines, chemokines and chemical mediators (Jun and Yoon 2001). Autoimmunity induction is not likely to operate in this model, though a hit-and-run event cannot be excluded. The clarification of the pathogenesis of EMC virus-induced diabetes will not only promote a better understanding of the mechanisms of virus-induced diabetes, but also enhance the protection strategy against virus-induced diabetes. The history and pathogenesis of EMC virus-induced diabetes are described, and the future aspects of the significance of this animal model of virus-induced diabetes are discussed.

S. Nagafuchi, M.D., Ph.D. (✉) • H. Kurisaki • H. Katsuta
Department of Medical Science and Technology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan
e-mail: nagafuchi@shs.kyushu-u.ac.jp

A. Toniolo (ed.), *Diabetes and Viruses*, DOI 10.1007/978-1-4614-4051-2_5,

The EMC Virus

Encephalomyocarditis virus belongs to the Picornaviridae family, as enteroviruses including coxsackie virus, genus cardiovirus, unenveloped, icosahedral structure (Fig. 5.1a) consisting of four capsid proteins (VP1~4), surrounding a core of ssRNA, moderately resistant to acidic pH (Racaniello 2007). The virion contains one molecule of positive sense, ssRNA, about 7.8 kb in size, containing a single open reading frame (ORF) (Fig. 5.1b) (Racaniello 2007). The virus can be grown in a tissue culture well with a one step replication time of about 8 h (Fig. 5.1c), and can infect rodents, usually producing systemic infection representing encephalitis and myocarditis. The virus rarely infects humans. Craighead and McLane (1968) first found that the M variant of the EMC virus certainly induced diabetes in several susceptible strains of male mice. Later, Yoon et al. (1980) isolated the highly diabetogenic D variant of EMC virus and the non-diabetogenic B strain of the EMC virus, by the plaque clone purification method. EMC-D virus produces diabetes in over 90% of infected susceptible strain of mice, while EMC-B virus did not induce diabetes in any strain of mice (Yoon et al. 1980). The susceptibility depends on the strain of mice and sex, namely only male mice are susceptible to the virus (Ross et al. 1975; Yoon et al. 1980; Huber et al. 1985). These findings accelerated the study of viral genetic factors to enhance the induction of diabetes in susceptible animals as well as research on pathogenesis (Fig. 5.2). [AU2]

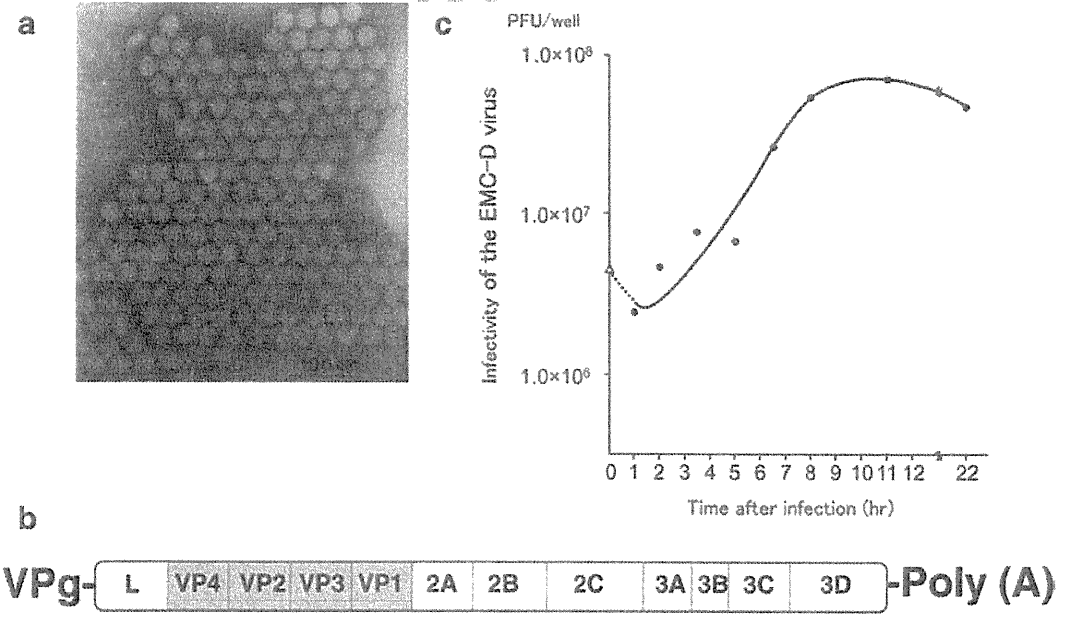

Fig. 5.1 Characteristics of encephalomyocarditis. (**a**) Negative stain of EMC-D virus. Courtesy of Dr. Yuji Ueki and Emeritus Professor Kazunobu Amako, Kyushu University. (**b**) Growth curve of EMC-D virus in mouse embryonic fibroblasts. (**c**) Genomic structure of EMC virus

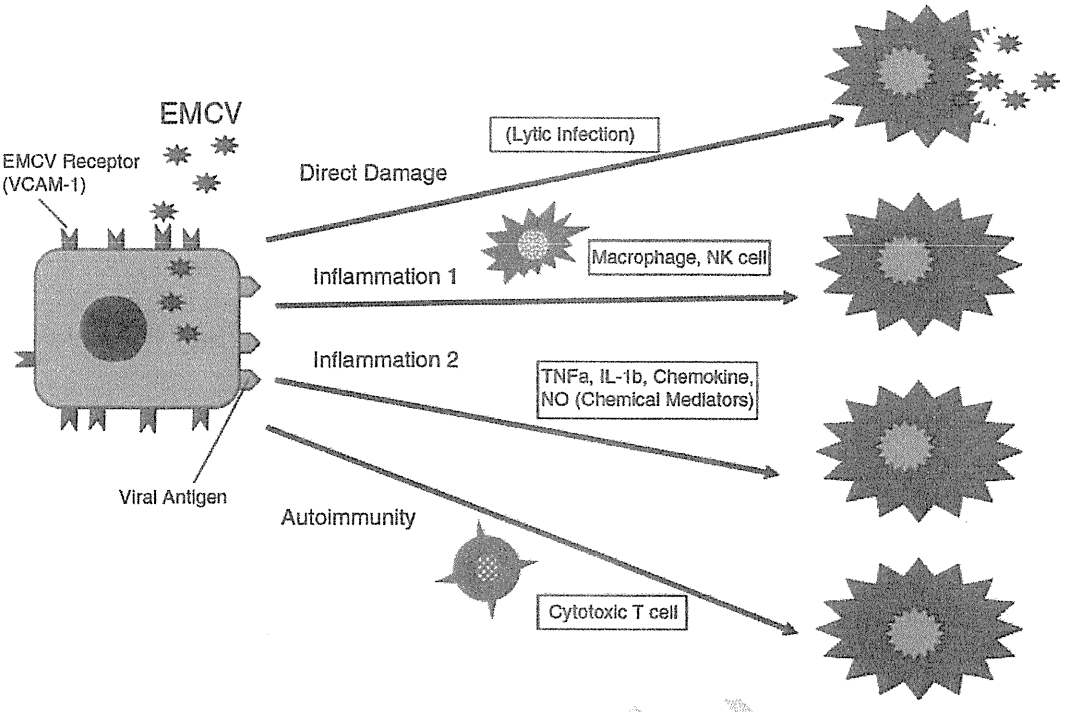

Fig. 5.2 Mechanisms of pancreatic β-cell damage due to EMCV infection

Pathogenesis of EMC Virus-Induced Diabetes

EMC Virus

The differing diabetogenicity among EMC viruses has been noted to be dependent on the genetic variation. First, the M variant of EMC virus was obtained as highly diabetogenic, and later the diabetogenic EMC-D virus and the non-diabetogenic B variant were isolated, respectively (Craighead and MacLane 1964; Yoon et al. 1980). Although EMC-D virus and EMC-B virus could not be distinguished by either neutralization assay or competitive radioimmunoassay (Yoon et al. 1980), examination of the complete nucleotide sequences of the genomes of both variants showed that they were different in only 14 nucleotide positions (Eun et al. 1988; Bae et al. 1989). Further molecular analysis by generating mutant viruses revealed that a "G" base at position 3155 ([GCC] Ala-776) is common to all diabetogenic variants, while an "A" base at the same position ([ACC] Thr-776) is common to all non-diabetogenic variants (Bae and Yoon 1993). Therefore, only one amino acid, alanine (776th amino acid on the polyprotein), is essential for the diabetogenicity of the EMC virus (Bae and Yoon 1993). These beautiful studies revealed that the single point mutation of "A" to "G" at position 3155 (Thr-776 to Ala-776) are critical to operate as the diabetogenic EMC virus (Jun et al. 1997). It was found that a change from Thr-776 to Ala-776 reduced the hydrophilicity of the region by 37%,

which may increase the efficiency of viral attachment to pancreatic beta cells (Kang and Yoon 1993; Jun et al. 1997, 1998), suggesting that the significance of the genetic difference had been supposed to influence the effectiveness of the attachment for beta cells. A challenge dose is not critical for inducing diabetes; however, it has been indicated that a high dose (10^5) PFU challenge destroys directly the pancreatic beta cells, while low dose (10^2) challenge will induce inflammatory responses which may damage beta cells (Yoon et al. 1980; Huber et al. 1985).

Protection

Innate Immunity

Since the EMC virus-induced diabetes develops within 3 days after infection, innate immunity, such as macrophages, interferons, and early inflammatory responses, is likely to be most important for determining the outcome after EMC virus infection (Yoon et al. 1980). Recent advances in the immunology of innate immunity found the significance of pattern recognition receptors (PRRs) directed against pathogen-associated molecular patterns (PAMPs) (Takeuchi and Akira 2009). They include toll-like receptors (TLR), and intracellular helicase such as melanocyte differentiation antigen (MDA) 5 for picornavirus retinoic acid inducible gene (RIG) I, and interferon induced with the helicase C domain 1 (IFIH1) for paramyxovirus. It was reported that polymorphism of the IFIH1 gene is associated with type 1 diabetes (Smyth et al. 2006), although it remains uncertain whether this may be associated with the viral infection. In EMC virus infection, TLR 3, 7, 8, and MDA-5 function as receptors, mediating the signal transduction pathway, inducing cellular activation including interferon production (Takeuchi and Akira 2009). McCartney et al. (2011) reported that MDA5 and TLR3 are both required to prevent diabetes in mice infected with EMC-D virus. Infection of *Tlr3*$^{-/-}$ mice caused diabetes due to impaired IFN-I responses and virus-induced β-cell damage rather than T-cell-mediated autoimmunity (McCartney et al. 2011). Mice lacking just one copy of MDA5 developed transient hyperglycemia when infected with EMCV-D, whereas homozygous *MDA5*$^{-/-}$ mice developed severe cardiac pathology (McCartney et al. 2011). TLR3 and MDA5 controlled EMC-D virus infection and diabetes by acting in hematopoietic and stromal cells, respectively, inducing IFN-I responses at kinetically distinct time points (McCartney et al. 2011). They conclude that optimal functioning of viral sensors and prompt IFN-I responses are required to prevent diabetes in this animal model, suggesting the significance of PRR-dependent innate immunity activation (McCartney et al. 2011).

Regarding the role of interferon, conflicting data in this model have been reported (Kaptur et al. 1989; Hirasawa et al. 1995). One study reported that interferon may worsen the EMC-D virus-induced diabetes (Kaptur et al. 1989), and others point to

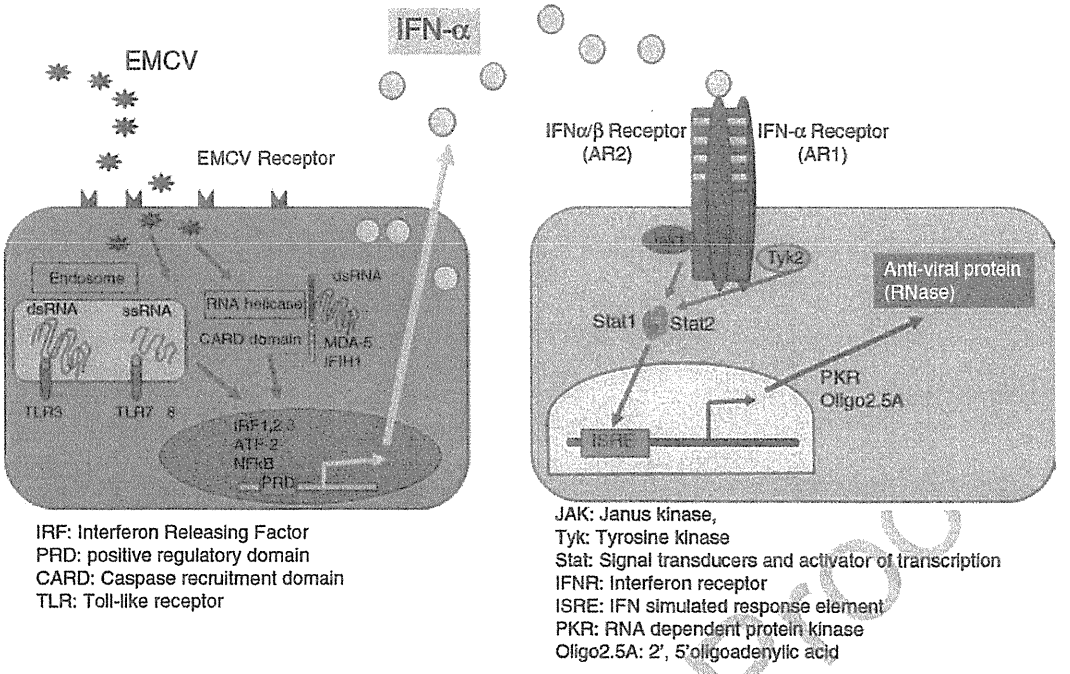

Fig. 5.3 Interferon production following EMCV infection and induction of anti-viral substances by interferon receptor signaling pathway

the significance of protective role of interferon (Hirasawa et al. 1995; McCartney et al. 2011). Possibly the challenge dose of EMC-D virus may alter the protective and/or pathogenic role of interferon in this model (Fig. 5.3).

Adaptive Immunity

Because EMC virus-induced diabetes develops as early as 3–5 days after infection, acquired and/or adaptive immunity did not likely play an important role (Yoon et al. 1980; Kounoue et al. 2008). Susceptibility to EMC-D virus-induced diabetes is not controlled by the MHC type, and passive transfer of lymphocytes from mice made diabetic mice with EMC-D virus into normal mice failed to produce diabetes (Yoon et al. 1985). In addition, T-cell-deficient nude mice, B-cell-deficient (muMT) mice, or both T-cell- and B-cell-deficient Rag-2 knockout mice could resist against EMC-D virus-induced diabetes (Kounoue et al. 2008), indicating that adaptive immunity did not affect the outcome of virus-induced diabetes. On the other hand, adoptively transferred antibody to the EMC virus was effective when given before and within 36 h after infection (Kounoue et al. 2008), suggesting that early adoptive antibody transfer or vaccination before infection may work to protect against EMC virus-induced diabetes.

[AU4]

Accelerating Factors

Inflammatory Cells

EMC virus belongs to the group of cytolytic viruses, and therefore a large challenge dose with the virus destroys pancreatic β-cells extensively enough to lead to diabetes (Yoon et al. 1980; Jun and Yoon 2001). At lower doses of infection, pancreatic β-cell damage is rather minimal. However, induced inflammatory response including infiltrated macrophages and produced cytokines and chemical mediators may damage further the β-cells to a reduced level of insulin secretion, leading to hyperglycemia (Baek and Yoon 1990, 1991; Hirasawa et al. 1997). Indeed histopathologic study of EMCV-induced animals developing insulitis with infiltration of macrophages to the islets showed that they were associated with the extensive destruction of pancreatic β-cells (Baek and Yoon 1990, 1991).

Cytokines, Chemokines, and Chemical Mediators

Interleukin-1 and tumor necrosis factor (TNF) α were suggested to function as key mediators of pancreatic beta-cell destruction, inducing DNA fragmentation (Hirasawa et al. 1997; Rabinovitch et al. 1994). Nitric oxide may work as a damaging factor to worsen the deterioration of pancreatic β-cell function (Fehsel et al. 1993). Infiltrated macrophages may be responsible to produce those cytotoxic mediators (Hirasawa et al. 1997). Recently, it was reported that the CXCR3 ligand CXCL10 was produced by enterovirus-infected pancreatic β-cells, attracting cytotoxic T cells and macrophages, expressing CXCR3, associated with the induction of insulitis, leading to β-cell damage (Tanaka et al. 2009).

Other Chemicals

Streptozoin and alloxan are well-known diabetogenic substances, inducing DNA strand breads and poly ADP ribose synthetase, which lead to the lack of ATP in pancreatic β-cells, resulting in extensive β-cell damage (Yamamoto et al. 1981). Some possible chemicals exist in the environment such as streptozoin, which is a compound of nitrourea and glucose; ta streptoxocin-like substance may be generated reacting with nitrosamines in food and water, and glucose. In addition, alloxan can be derived from uric acid in the purine metabolism pathway by oxidation with superoxide substance (Santos et al. 1999). The produced alloxan would possibly work as a β-cell-specific cytotoxic chemical. Although these possible chemicals to induce β-cell damage have not been proved to operate in the environment, it should be noticed that they may work as a risk factor in addition to viral infection. [AU5]

Accumulation of Environmental Insults

An interesting report has established the significance of the accumulation of environmental insults such as viruses and chemicals (Toniolo et al. 1980). The concept may also be applicable to type 2 diabetes, where accumulation of risk factors such as diet, obesity, aging, genetic risk, and little exercise together lead to impaired insulin action, resulting in the development of diabetes. Viral infection may contribute to the development of type 2 diabetes when viral infection alone is not sufficient to induce diabetes, but the damage to β-cells may reduce β-cell function to some extent. In this sense, viral infection may serve as another risk factor for the development of type 2 diabetes, although direct evidence is lacking.

Autoimmunity

The EMC virus is a cytolytic virus but does not cause persistent infection, and therefore infected cells are not likely to be attacked by cytotoxic T cells similar to the autoimmune reaction. However, infiltration of T cells with a restricted T-cell receptor repertoire has been reported, suggesting a pathogen and/or autoantigen-directed reaction (Kawagishi et al. 2003). Indeed, lysed cells after infection with the EMC virus would release self-antigens and thus may possibly trigger autoimmunity to pancreatic β-cells as well as virus antigen-directed protective immune response (Flodström et al. 2002; Christen et al. 2010). The hit-and-run theory is hard to confirm and/or disclaim with evidence for or against paradigm. In addition, interferon production induced by viral infection may play a role in the development of autoreactivity to pancreatic β-cells (Fig. 5.4) (Devendra and Eisenbarth 2004). Recent advances in controlling organ-specific autoimmune diseases often associated with autoimmune type 1 diabetes, the significance of AIRE being in the thymus and Treg in the peripheral immunoregulation system have been extensively described in addition to microbial environment engagement (Nagamine et al. 1997; Kogawa et al.2002; Eisenbarth and Gottlieb 2004; Sakaguchi 2005). Programmed death factor, suppressor of cytokine signaling (SOCS), and B lymphocytes may also contribute to the prevention of autoimmunity to pancreatic β-cells (Chervonsky 2010; Yoshimura, et al. 2007; Pescovitz et al. 2009; Nagafuchi et al. 2010).

Long-Term Complications

Susceptible mice infected with diabetogenic EMC-D virus develop hyperglycemia and develop characteristic diabetic complications similar to those in humans such as glomerulosclerosis, retinal vessel involvement, and decrease in bone formation and mineralization. The diabetic mice with glomerulosclerosis after 6 months' duration

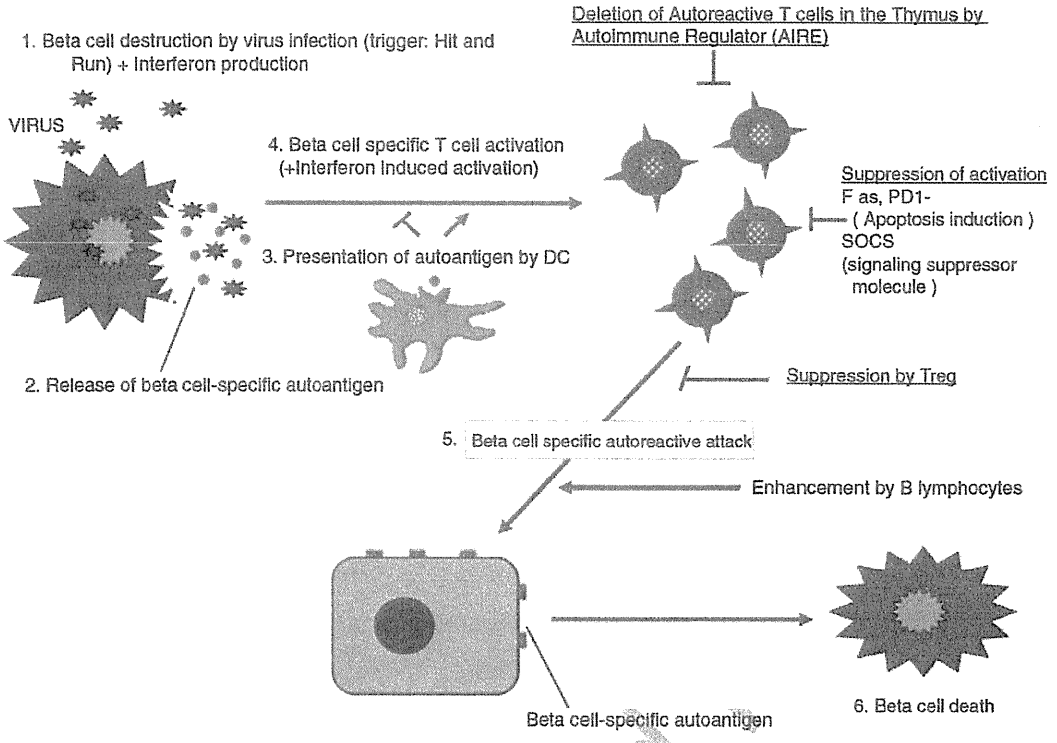

Fig. 5.4 Hypothesis: possible induction of autoreactivity to pancreatic β cells triggered by viral infection

revealed the two- to threefold 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 2–6 months after infection (Yoon et al. 1982).

Host Factors

Even diabetogenic EMC-D virus induced diabetes only in a few strains of mice such as SJL/J, SWR, DBA/1J, DBA/2J, while others such as C57BL/6, CBA/J, AKR, C3H/HeJ. A/J mice are all resistant to EMC-D virus-induced diabetes (Ross et al. 1975; Yoon et al. 1980; Huber et al. 1985). Onodera and others have reported that F1 hybrid mice between susceptible SWR and resistant C57BL/6 mice were resistant to virus-induced diabetes, while the next F1 and SWR mating showed that 50% of those mice exhibited the susceptibility to the virus, thus indicating that a single autosomal recessive gene, which is inherited in a Mendelian fashion, controls the susceptibility to the virus (Onodera et al. 1978). It was indicated that the susceptibility gene may modulate the expression of virus receptors on β-cells in susceptible mice (Kang and Yoon 1993); however the exact controlling gene has remain to be identified.

Prevention

Several preventive strategies have been reported to be effective. As described in the pathogenesis section, Nitrate oxide inhibitors and chemokine suppression may reduce the diabetogenic effect of EMC virus infection. Since the antibody is very effective to prevent the EMC virus infection, vaccination and every early phase of the disease may be effective in preventing the EMC virus-induced diabetes (Kounoue et al. 2008). Immunostimulants such as BCG, *Corynebacterium parvum*, and PolyIC treatment have been shown to be effective in preventing EMD-D virus-induced diabetes (Kounoue et al. 1987; Choi et al. 2000).

Perspectives

EMC virus has contributed greatly to better understanding of the pathogenesis of virus-induced diabetes. The data described above show the development of diabetes even under the diabetogenic virus challenge, with the outcome being influenced by many factors, such as genetic background, sex, protective immunity, inflammatory cells, cytotoxic mediators, and also perhaps the regenerative activity of pancreatic β-cells (Hover and Sauter 2010). The elevation of blood glucose level may be due to the accumulation of such "risk factors," leading to the development of virus-induced diabetes. Moreover, since even susceptible strains of inbred mice develop virus-induced diabetes in a rather variable but not homogeneous fashion, the "stochastic process" in this model and/or human virus-induced diabetes should therefore be recognized. In order to acquire a better understanding of the pathogenesis of virus-induced diabetes, the assay system for the diabetogenicity of the virus together with clarification of host genetic risk factors should be exploited, as these may lead to the identification of the "diabetogenic" virus. Those studies will hopefully lead to the development of a vaccination strategy to the "diabetogenic" virus, which will in turn not only prevent the development of the virus-induced type 1 diabetes but may also contribute to reduce the risk for the development of type 2 diabetes.

Summary

- EMC virus has provided the most useful animal model for virus-induced type 1 diabetes. Development of diabetes depends on many factors including virus strain, challenge dose, host factors such as genetic background, sex, immunoprotective function, inflammatory responses with macrophages, cytokines, chemokines, and chemical mediators.
- Autoimmunity induction is not likely to be a factor in this model, though a hit-and-run event cannot be excluded.

- Most importantly, the difference between the diabetogenic strain D (EMC-D) and the non-diabetogenic strain B (EMC-B) virus depends on only one amino acid change due to single point mutation of "A" to "G" at position 3155 (Thr-776 to Ala-776), suggesting that possible acquisition of diabetogenicity may occur often among environmental "non-diabetogenic" viruses.
- Although susceptibility to the EMC-D virus-induced diabetes depends on the genetic background of mice, the genetic determinants of the host remain to be elucidated.

Clarification of the pathogenesis of EMC virus-induced diabetes will not only promote a better understanding of the mechanisms of virus-induced diabetes in general, but will also contribute to the development of new protective strategies against viral diabetes.

Acknowledgments The authors thank Professor Takehiko Sasazuki for his helpful advice. We also thank Dr. Chiri Nagatsuka and Mrs Arisa Moroishi for their help in the preparation of the manuscript. Financial support by Ministry of Health, Labour and Welfare of Japan is acknowledged.

References

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

Bae Y, Yoon JW (1993) Determination of diabetogenicity attributable to a single amino acid Ala776, on the polyprotein of encephalomyocarditis virus. Diabetes 42:435–443

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

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

Choi BK, Cho SH, Bai GH, Kim SJ, Hyun BH, Choe YK, Bae YS (2000) Prevention of encephalomyocarditis virus-induced diabetes by live recombinant mycobacterium bovis Bacillus Calmette-Guerin in susceptible mice. Diabetes 49:1459–1467

Chervonsky AV (2010) Influence of microbial environment on autoimmunity. Nat Immunol 11:28–33

Christen U, Hintermann E, Holdener M, von Herrath MG (2010) Viral triggers for autoimmunity: is the 'glass of molecular mimicry' half full or half empty? J Autoimmun 34:38–44

Clements GB, Galbraith DN, Taylor KW (1995) Coxsackie B virus infection and onset of child hood diabetes. Lancet 346:221–223

Craighead JE, McLane MF (1968) Diabetes mellitus induction in mice by encephalomyocarditis virus. Science 162:913–914

Devendra D, Eisenbarth GS (2004) Interferon alpha—a potential link in the pathogenesis of viral-induced type 1 diabetes and autoimmunity. Clin Immunol 111:225–233

Eisenbarth GS, Gottlieb PA (2004) Autoimmune polyendocrine syndromes. N Engl J Med 350:2068–2079

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

Fehsel AK, Jalowy A, Qi S, Burkart V, Hartmann B, Kolb H (1993) Islet cell DNA is a target of inflammatory attack by nitric oxide. Diabetes 42:496–500

Flodström M, Maday A, Balakrishna D, Cleary MM, Yoshimura A, Sarvetnick N (2002) Target cell defense prevents the development of diabetes after viral infection. Nat Immunol 3:373–382

Hanafusa T, Imagawa A (2008) Insulitis in human type 1 diabetes. Ann NY Acad Sci 1150:297–299

Hirasawa K, Ogiso Y, Takeda M, Lee MJ, Itagaki S, Doi K (1995) Protective effects of macrophage-derived interferon against encephalomyocarditis virus-induced diabetes mellitus in mice. Lab Anim Sci 45:652–656

Hirasawa K, Jun HS, Maeda K, Kawaguchi Y, 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

Hover D, Sauter P (2010) Pathogenesis of type 1 diabetes mellitus: interplay between enterovirus and host. Nat Rev Endocrinol 6:279–289

Huber SA, Babu PG, Craighead JE (1985) Genetic influences on the immunologic pathogenesis of encephalomyocarditis (EMC) virus-induced diabetes mellitus. Diabetes 34:1186–1190

Jenson AB, Rosenberg HS, Notkins AL (1980) Pancreatic islet-cell damage in children with fatal viral infections. Lancet 316:354–358

Jun HS, Kang Y, Notkins AL, Yoon JW (1997) Gain or loss of diabetogenicity result in 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 (2001) The role of viruses in type I diabetes: two distinct cellular and molecular pathogenic mechanisms of virus-induced diabetes in animals. Diabetologia 44:271–285

Kang Y, Yoon JW (1993) A genetically determined host factor controlling susceptibility to encephalomyocarditis virus-induce diabetes in mice. J Gen Virol 74:1207–1213

Kaptur PE, Thomas DC, Giron DJ (1989) The role of interferon in the protection of ICR Swiss male mice by the nondiabetogenic variant of encephalomyocarditis virus against virus-induced diabetes mellitus. J Interferon Res 9:671–678

Kawagishi A, Kubosaki A, Takeyama N, Sakudo A, Saeki K, Matsumoto Y, Hayashi T, Onodera T (2003) Analysis of T-cell receptor Vβ gene from infiltrating T cells in insulitis and myocarditis in encephalomyocarditis virus-infected BALB/C mice. Biochem Biophys Res Commun 310:791–795

Kogawa K, Kudoh J, Nagafuchi S, Ohga S, Katsuta H, Ishibashi H, Harada M, Hara T, Shimizu N (2002) Distinct clinical phenotype and immunoreactivity in Japanese siblings with autoimmune polyglandular syndrome type 1 (APS-1) associates with compound heterozygous novel AIRE gene mutations. Clin Immunol 103:277–283

Kounoue E, Nagafuchi S, Nakamura M, Nakano S, Koga T, Nakayama M, Mitsuyama M, Niho Y, Takaki R (1987) Encephalomyocarditis (EMC) virus-induced diabetes mellitus prevented by Corynebacterium parvum in mice. Experientia 43:430–431

Kounoue E, Izumi K, Ogawa S, Kondo S, Katsuta H, Akashi T, Niho Y, Harada M, Tamiya S, Kurisaki H, Nagafuchi S (2008) The significance of T cells, B cells, antibodies, and macrophages against encephalomyocarditis (EMC)-D virus-induced diabetes in mice. Arch Virol 153:1223–1231

McCartney SA, Vermi W, Lonardi S, Rossini C, Otero K, Calderon B, Gilfillan S, Diamond MS, Unanue ER, Colonna M (2011) RNA sensor-induced type 1 IFN prevents diabetes caused by a β-cell tropic virus in mice. J Clin Invest 121:1497–1507

Nagafuchi S, Katsuta H, Anzai K (2010) Rituximab, B-lymphocyte depletion, and beta-cell function. N Engl J Med 362:761

Nagamine K, Peterson P, Scott HD, Kudoh J, Minoshima S, Heino M, Krohn KJE, Lalioti MD, Mullis PE, Antonarakis SE, Kawasaki K, Asakawa S, Ito F, Shimizu N (1997) Positional cloning of the APECD gene. Nat Genet 17:393–398

Onodera AT, Yoon JW, Brown ES, Notkins AL (1978) Evidence for a single locus controlling susceptibility to virus-induced diabetes mellitus. Nature 274:693–696

338 Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, Gottlieb
339 PA, Marks JB, McGee PF, Moran AM, Raskin P, Rodriguez H, Schatz DA, Wherrett D, Wilson
340 DM, Lachin JM, Skyler JS (2009) Rituximab, B-lymphocyte depletion, and preservation of
341 beta-cell function. N Engl J Med 361:2143–2152
342 Rabinovitch A, Suarez-Pinzon WL, Shi Y, Morgan AR, Bleackley RC (1994) DNA fragmentation
343 is an early event in cytokine-induced islet beta-cell destruction. Diabetologia 37:733–738
344 Racaniello VR (2007) Picornaviridae: the viruses and their replication. In: Knipe DM, Howley
345 PM, Griffin DE, Lamb RA, Straus SE, Martin MA, Roizman B (eds) Fields virology, 5th edn.
346 Lippincott Williams & Wilkins, Philadelphia, pp 795–838
347 Richardson AJ, Willcox A, Bone AJ, Morgan N, Foulis AK (2011) Immunopathology of the human
348 pancreas in type-I diabetes. Semin Immunopathol 33:9–21
349 Ross ME, Onodera T, Brown KS, Notkins AL (1975) Virus-induced diabetes mellitus. IV. Genetic
350 and environmental factors influencing the development of diabetes after infection with the M
351 variant of encephalomyocarditis virus. Diabetes 25:190–197
352 Sakaguchi S (2005) Naturally arising Foxp3-expressiong $CD25^+$ $CD4^+$ regulatory T cells in immu-
353 nological tolerance to self and non-self. Nat Immunol 6:345–398
354 Santos CX, Anjos EI, Augusto O (1999) Uric acid oxidation by peroxynitrate: multiple reaction,
355 free radical formation, and amplification of lipid oxidation. Arch Biochem Biophys
356 372:285–294
357 Smyth DJ, Cooper JD, Bailey R, Field S, Burren O, Smink LJ, Guja C, Ionescu-Tirgoviste C,
358 Widmer B, Dunger DB, Savage DA, Walker NM, Clayton DG, Todd JA (2006) A genome-wide
359 association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-
360 induced helicase (IFIH1) region. Nat Genet 38:617–619
361 Takeuchi O, Akira S (2009) Innate immunity to virus infection. Immunol Rev 227:75–86
362 Tanaka S, Nishida Y, Aida K, Maruyama T, Shimada A, Suzuki M, Shimura H, Takizawa S,
363 Takahashi M, Akiyama D, Arai-Yamashita S, Furuya F, Kawaguchi A, Kaneshige M, Katoh R,
364 Endo T, Kobayashi T (2009) Enterovirus infection CXC chemokine ligand 10 (CXCL10), and
365 CXCR3 circuit. Diabetes 58:2285–2291
366 Tauriainen S, Oikarinen S, Oikarinen M, Hyöty H (2011) Enteroviruses in the pathogenesis of type
367 1 diabetes. Semin Immunopathol 33:45–55
368 Toniolo A, Onodear T, Yoon JW, Notkins AL (1980) Induction of cumulative environmental insults
369 from viruses and chemicals. Nature 288:383–385
370 Yamamoto H, Uchigata Y, Okamoto H (1981) Streptozocin and alloxan induced DNA strand
371 breaks and poly ADP-ribose synthetase in pancreatic islets. Nature 294:284–286
372 Yoon JW, McLintock PR, Onodera T, Notkins AL (1980) Virus-induced diabetes mellitus. XVIII.
373 Inhibition by a nondiabetogenic variant of encephalomyocariditis virus. J Exp Med
374 152:878–892
375 Yoon JW, Rodrigues MM, Currier C, Notkins AL (1982) Long-term complications of virus-induced
376 diabetes mellitus in mice. Nature 296:566–569
377 Yoon JW, McClintock PR, Bachurski CJ, Longstreth JD, Notkins AL (1985) Virus-induced diabe-
378 tes mellitus. No evidence for immune mechanisms in the destruction of β-cell by the D-variant
379 of encephalomyocarditis virus. Diabetes 34:922–923
380 Yoshimura A, Naka T, Kubo M (2007) SOCS proteins, cytokine signaling and immune regulation.
381 Nat Rev Immunol 7:454–465