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Enteroviral Infections and Development of Type 1 Diabetes: *The Brothers Karamazov* Within the CVBs

Diabetes 2014;63:384–386 | DOI: 10.2337/db13-1441

COMMENTARY

Type 1 diabetes (T1D) is the result of a selective autoimmune destruction of pancreatic islet β-cells, occurring in genetically predisposed subjects, possibly triggered or accelerated by environmental agents (1). Both innate (2) and adaptive (3) immune responses are involved in islet inflammation in T1D. The role of environmental factors has become increasingly relevant, as indicated by the marked recent rise of incidence (4), impossible to explain based on genetic changes alone. One of the environmental risk factors identified by several independent studies in man and in animal models (5) is represented by enteroviral infections, which have been epidemiologically associated to T1D development (6). Enteroviruses may contribute to the pathological events leading to β-cell damage by several different mechanisms, such as virus-induced cytolysis or islet inflammation leading to subclinical β-cell destruction (7). However, it should also be taken into account that in specific settings viral infections may also protect from diabetes development (8).

In this issue, two closely related articles written by Oikarinen et al. (9) and Laitinen et al. (10) provide important information on the potential roles of enteroviruses, and more specifically of group B coxsackieviruses (CVB), in modulating susceptibility to T1D development. Neutralizing antibodies against CVBs have been measured in a longitudinal sample series from a large prospective birth cohort in Finland (9) as well as cross-sectionally in children with newly diagnosed T1D and control subjects (10) matched according to sampling time, gender, age, and country, recruited in Finland, Sweden, England, France, and Greece. Results showed that CVB B1 (CVB1) was associated with an increased risk of β-cell autoimmunity. This risk was strongest when infection occurred a few months before autoantibodies appeared and it was attenuated by the presence of maternal antibodies against the virus. Two other CVB types, B3 and B6, were associated with a reduced T1D risk.

The finding that the three serotypes identified are closely related phylogenetically is of sure significance. As a matter of fact, close clustering is indeed what would be expected for serotypes that could be either causative or protective. It has been shown that CVB1 can infect human pancreatic islets in vitro, being one of the most cytolytic enterovirus serotypes in this model (7). In addition, insulitis and islet cell damage have been described in infants who have died of CVB1 infection.

On the other hand, the two studies (9,10) also revealed that infections by CVB3 and CVB6 were associated with a decreased risk of β-cell autoimmunity. This phenomenon may be explained by immunological cross-protection induced by CVB3 and CVB6 against the diabetogenic effect(s) of CVB1 (Fig. 1). Specifically, CVB1 infection, mediated by the expression of viral receptors, coxsackie adenovirus receptor and decay accelerating factor or CD55, may elicit a cell-mediated antiviral response. The highly cytopathic properties of CVB1 may thus lead to β-cell damage, possibly triggering or enhancing islet-specific autoimmune reaction. When CVB1 infection is preceded by CVB3 or CVB6 infection, this results in protection from diabetogenic effects of CVB1, possibly due to the development of CVB3/6-specific T cells, which, cross-reacting with CVB1, induce protection from a subsequent CVB1 infection, thus limiting its deleterious effects on β-cells. Cross-protection is also supported by the increased CVB1-related risk in children who were infected by CVB1 but not by the protective serotypes.

As for the cellular and molecular mechanisms that may be responsible for the "non-diabetogenic" effects of

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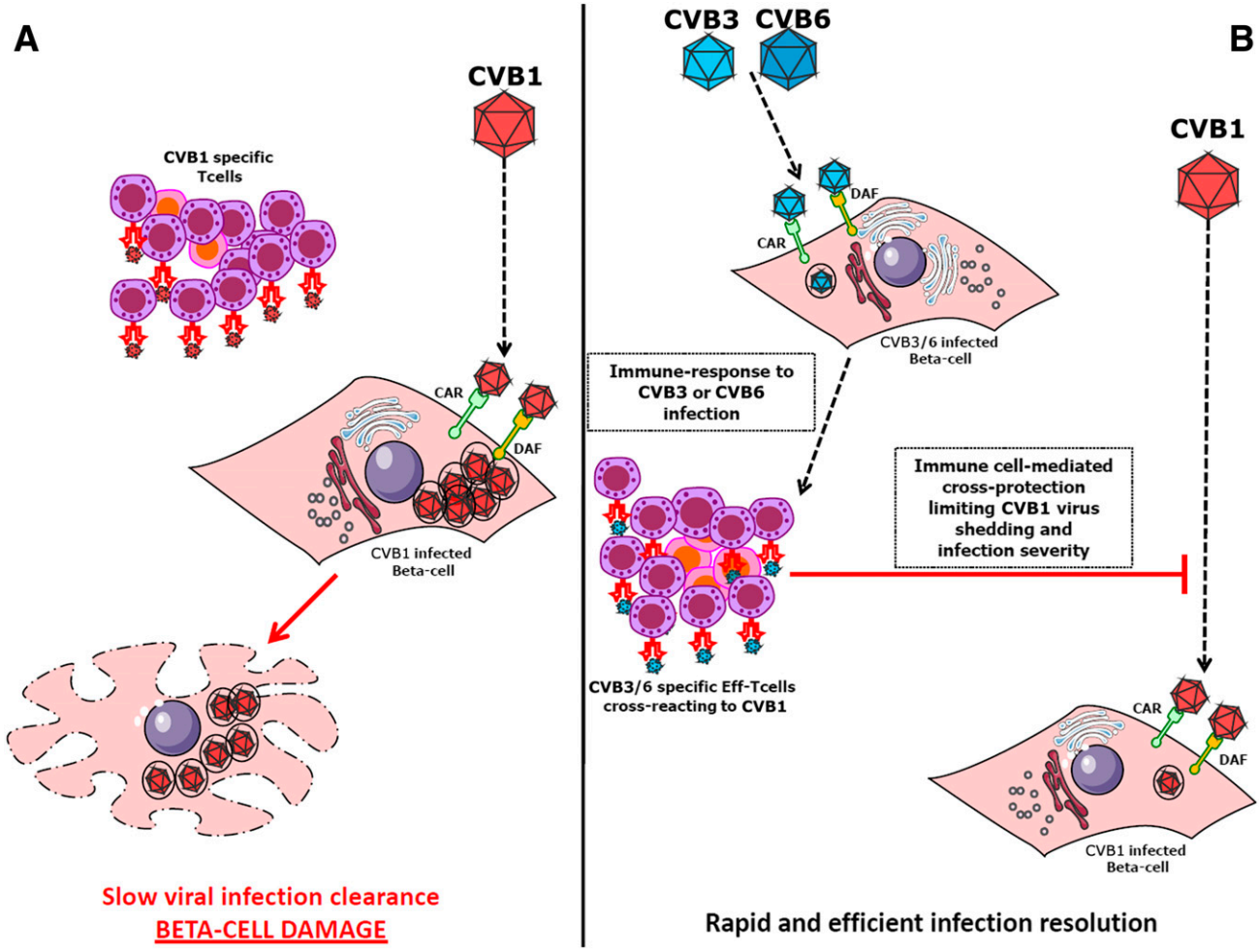

Figure 1—CVB3 and CVB6 induce protection from diabetogenic CVB1 strain infection. *A*: CVB1 infection is mediated by the expression of viral receptors coxsackie adenovirus receptor (CAR) and decay accelerating factor (DAF) or CD55, which establish a specific CVB tropism for β-cells. The cell-mediated immune response to CVB1 infection and highly lytic properties of CVB1 lead to β-cell damage, possibly triggering or enhancing islet-specific autoimmune reaction. *B*: Specific CVB3 or CVB6 infection, when preceding CVB1 infection, induces protection from diabetogenic effects of CVB1. Specific immune response triggered by CVB3/6 infection, without induction of massive β-cell damage or diabetogenic effects, leads to the development of CVB3/6-specific effector (Eff) T cells, which may cross-react with and protect from a subsequent CVB1 infection, thus limiting its deleterious effects on β-cells.

CVB3, it is of interest that Kemball et al. (11) demonstrated that CVB3 is able to inhibit antigen presentation in vivo, exerting a profound and selective effect on the major histocompatibility complex class I pathway. In addition, Mukherjee et al. (12) showed that the $3C^{pro}$ cysteine protease of CVB3 cleaves the innate immune adaptor molecules mitochondrial antiviral signaling protein and Toll/interleukin 1 receptor domain-containing adaptor inducing interferon-β as a mechanism to escape host immunity, thus suggesting that CVB3 has evolved mechanisms to suppress host antiviral signal propagation by directly cleaving two key adaptor molecules associated with innate immune recognition.

Of note, it is now clear that some viruses can modulate β-cell function (13). As for CVB3, in vivo studies performed in CBS/j mice have shown (14) that infection with CVB3 virus (Nancy strain) does not affect glucose tolerance, in contrast, for example, with some CVB4 strains. It should be pointed out that the two studies (9,10) did not observe association with other recognized "diabetes-associated" enteroviruses (e.g., CVB4, some echoviruses) with robust in vitro and ex vivo evidence of links to T1D or to islet autoimmunity (15,16). This may be also due to the experimental strategy, which was based on a seroepidemological approach with no virus isolation or sequencing.

The overall scenario of the complex relationship between enteroviruses, the pancreatic β-cell, and T1D development (17) somehow recalls the plot of Fyodor Dostoyevsky's novel *The Brothers Karamazov*, in which, when the father is killed, one of his three sons is formally charged with patricide and then sentenced as guilty, since

all of the evidence points against him. However, this was a "judicial error," as the killer was another son considered physically incapable of committing a murder. Similarly, over the years, several viruses have been blamed of being responsible for β-cell killing; in some cases, this was probably a judicial error. Studies like Oikarinen et al. (9) and Laitinen et al. (10) that have been properly designed and conducted should minimize the risk of judicial error and should be encouraged, as those multicenter initiatives like the JDRF International–funded nPOD-Viral Work Group or the European Commission project PEVNET in which a network of investigators with different expertise collaborate and develop synergies to tackle key questions relevant to T1D pathogenesis, such as the molecular diabetogenic characteristics of viruses of interest and how these viruses may cause persistent infection.

Acknowledgments. The help of M. Prencipe (Fondazione Umberto Di Mario ONLUS) in editing the manuscript has been greatly appreciated.

Funding. F.D. is supported by grants from the European Commission (collaborative projects NAIMIT and PEVNET in the Framework Program 7 [FP7]), by JDRF International, by the Italian Ministry of Research, and by the Tuscany Region.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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