Enterovirus Infection of Human β-Cells Activates Dendritic Cells and Triggers Innate Antiviral Responses: Are Enteroviruses Convicted Now?

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Despite a large number of epidemiologic associations, clinical studies, and animal models, a direct involvement of pathogens in the etiology of human type 1 diabetes is still a matter of critical debate. Human enteroviruses (HEV), such as coxsackievirus B (CVB) and echoviruses (EV), have been associated with type 1 diabetes since the late 1960s (1) and have been found in pancreatic isolates of type 1 diabetic patients (2–4) (rev. in 5). However, there are several difficulties hampering the conviction of HEV as inducers of type 1 diabetes: *1*) pancreatic biopsies are not done on a regular basis in type 1 diabetic patients; *2*) HEV infections are frequently found in nondiabetic individuals and often remain asymptomatic; *3*) the clinical manifestation of type 1 diabetes may occur years to decades after infection (virus infection as a "hit-and-run" event); *4*) type 1 diabetic patients as well as healthy individuals undergo multiple virus infections throughout their lifetime, some of which might even protect them from rather than induce autoimmune disease; and *5*) genetic predispositions might cover possible environmental (i.e., virus) factors.

In this issue of *Diabetes*, Schulte et al. (6) address the mechanism by which HEV infection of human islets might influence type 1 diabetes onset and/or progression. They found that monocyte-derived dendritic cells (DCs) efficiently phagocytosed CVB3-infected human and porcine islets. Phagocytosis of CVB3-infected islets induced the expression of several interferon-stimulated genes (ISGs), such the retinoic acid–inducible gene (RIG)-I–like helicases, RIG-I and melanoma differentiation–associated gene 5 (Mda5), in DCs. Further experiments with the murine insulinoma cell line, Min6, revealed that CVB3-infected cells induced an antiviral state that protected ISG-expressing DCs from subsequent CVB3-infection (Fig. 1). The antiviral state of protected DCs was found to be dependent on the presence of intracellular viral RNA, required (unlike lipopolysaccharides-induced responses) endosomal acidification, and the expression of type I interferons (6). Thus, DCs are being reprogrammed upon phagocytosis of CVB3-infected islets and might therefore influence the balance of effector (aggressive) and regulatory T-cells (T_{reg}). The question is, however, in which direction the immune balance is being shifted by enterovirus infection. Interestingly, in mouse models different enterovirus strains have been demonstrated to have opposite effects on the outcome of type 1 diabetes. Several studies demonstrate that CVB4 promotes type 1 diabetes in nonobese diabetic (NOD) mice (7–9). In contrast, CVB3 seems to have dual effect on the outcome of type 1 diabetes in NOD mice by either promoting the disease or, under certain circumstances, significantly protecting from type 1 diabetes (10). However, CVB3-mediated protection of NOD mice was dependent on the viral dose, the CVB3 substrain, and the time of administration in relation to the immunopathogenic process (5). However, there is no epidemiological evidence suggesting that CVB4 strains are more involved in human type 1 diabetes than other CVB serotypes (5). Recently, it has been reported that in both NOD as well as rat insulin promoter–lymphocytic choriomeningitis virus (RIP-LCMV) mice infection with CVB3 resulted in the upregulation of programmed death 1 ligand 1 (PD-L1) on lymphoid cells and an enhancement of CD4+CD25+ regulatory T-cells (11). In concert, PD-L1 upregulation and T_{reg} expansion protected CVB3-infected mice from type 1 diabetes, indicating that CVB3 can indeed display protective properties under certain conditions in animal models. Such a protective effect would support the hygiene theory, which postulates that allergies and autoimmune diseases are less frequent in regions with a poor hygienic status (12). An association of the incidence of type 1 diabetes and enterovirus infections has been found in several European populations, indicating that countries with a low infection rate of enterovirus, such as Finland or Sweden, have the highest incidences of type 1 diabetes (13). Further, a comparison between Finland and Russian Karelia, which have a similar genetic backgrounds but different rates of enterovirus infection, revealed an inverse correlation between the presence of anti-enterovirus antibodies and type 1 diabetes incidence (14). The concept of the hygiene theory has been verified in several animal models including the RIP-LCMV model for type 1 diabetes, which is dependent on virus infection to induce disease (15). It was found that secondary virus infection at a remote location when the destruction of the β-cells was already ongoing might recruit aggressive T-cells away from the pancreas and subsequently reverse the ongoing auto-destructive process in the islets of Langerhans (16). Since enhanced T-cell apoptosis has been found within the infected tissue, such a remote location can be considered a filter for potentially auto-aggressive T-cells (17). However, one has to consider that secondary infection with another virus (Pichinde virus) has been demonstrated to accelerate type 1 diabetes in the same mouse model (18).

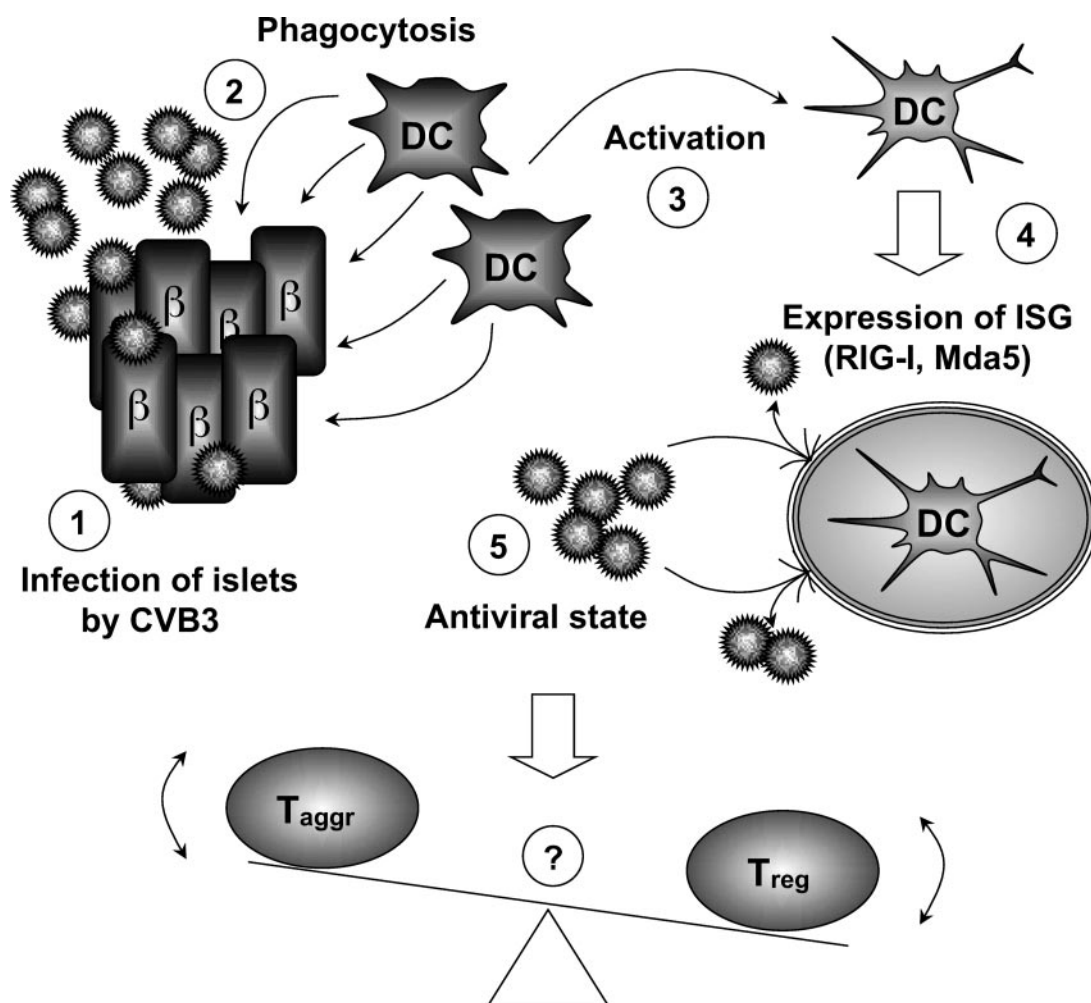The finding by Schulte et al. (6) that innate immune

FIG. 1. CVB3-infected human islets of Langerhans (*1*) are being phagocytosed by human monocyte-derived DCs (*2*). Activated DCs (*3*) express ISGs, such as RIG-I and Mda5 (*4*), and reprogram the DCs to an antiviral state (*5*), which might influence the immune balance between aggression and regulation.

responses, such as expression of ISGs, are being induced in DCs upon phagocytosis of CVB3-infected human islets provides insight into the mechanisms involved in the antiviral defense. The data fit well with previous results of the same group demonstrating that uptake of enterovirus-infected Vero cells induce an antiviral state in DCs (19). However, the work neither implies initiation nor protection from type 1 diabetes. In order to establish an inducing or protective role for such antiviral DCs, a detailed assessment of their functional properties and the mechanistic interactions with either aggressive or regulatory T-cells will be necessary. Of special interest is the balance of islet antigen–specific aggressive versus regulatory T-cells locally within the islets of Langerhans. It has been recently demonstrated in a T-cell receptor–transgenic NOD mouse model that the vast majority of islet infiltrating T-cells are indeed antigen-specific and that the entry into the islet of Langerhans is regulated in an antigen-dependent manner (20). Attraction of nonspecific bystander lymphocytes seems to play only a minor role in the destruction of β-cells. Direct infection of islet cells and subsequent activation of DCs within the islets of Langerhans might therefore directly influence the balance of antigen-specific T-cells within the islets.

Thus, the question of whether enteroviruses are now convicted as inducers of type 1 diabetes remains unanswered. The present study identifies a mechanism by which enterovirus-infected islets influence the local immune balance. However, it is not clear whether such a change will lead to induction of type 1 diabetes or, on the contrary, might even protect the islets from autoimmune destruction.

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