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New Insight Into Early Events in Type 1 Diabetes: Role for Islet Stem Cell Exosomes

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Type 1 diabetes (T1D) is an autoimmune disease in which an inappropriate self-directed immune response affects and destroys insulin-producing β-cells in pancreatic islets leading to dysregulated blood glucose levels. T1D may affect people of any age, its clinical presentation is highly variable, and its incidence is increasing worldwide (1). The initial triggering events of T1D are unknown and their elucidation is of pivotal importance. Several factors might lead to the breakdown of β-cell–specific T-cell tolerance, including genetics, exogenous infectious pathogen, noninfectious environment agents, endogenous superantigens, or physiological stress events (2).

The hallmark of autoimmune diabetes is insulitis, which progresses to a destruction of β-cells that results in clinical T1D. An altered balance between proinflammatory T-helper type 1 (Th1)/Th17 cells (γ-interferon [IFN-γ], interleukin [IL]-17) and Th2 immune response (IL-4, IL-10) leads to T1D (3,4). Evidence also suggests that both genetic and environmental factors may induce local inflammatory response, where activated intraislet dendritic cells (DCs) prevent peripheral T-cell tolerance (5). Moreover, it has been recently demonstrated that the development of destructive insulitis is partly due to impaired islet-resident $Foxp3^+$ regulatory T cells (6,7).

Several animal experimental models have been used for the investigation of the pathogenesis of T1D and it appears that a NOD mouse is the model of choice. NOD mice spontaneously develop early peri-insulitis and later intraislet insulitis caused by autoreactive T cells, but the reasons for the loss of tolerance to islets antigens are largely unknown. Prior to islets infiltration by autoreactive lymphocytes in the NOD strain, the abnormalities such as vascular pathology, increased β-cell endoplasmic reticulum stress, and upregulation of inflammatory cytokines may exist, which may result in β-cell death and release of the islet antigens (8).

In this issue, Rahman et al. (9) use this experimental model to report that exosomes (EXOs) released from fibroblast-like, fast-replicating cells that express mesenchymal stem cell (MSC) markers in the islet may act as a trigger of the autoimmune response. It has already been established that both EXOs and MSCs may play a role in modulating autoimmunity, including T1D (10). Namely, insulinoma-released EXOs can stimulate the autoimmune responses in NOD mice by their ability to induce proinflammatory cytokines, including IL-6 and tumor necrosis factor-α, the effect that was Toll-like receptor–mediated as MyD88 was required for this adjuvant effect. Of importance, the candidate islet antigen, GAD65 kDa, was demonstrated to be expressed in these EXOs (11). However, a study by Rahman et al. (9) provides the first evidence that primary islets contain MSCs able to activate primed B and T autoreactive cells. The underlying mechanism of activation of autoreactive lymphocytes appears to be the release of EXOs, which promote expansion of diabetogenic T cells and accelerate the effect of T-cell–mediated destruction of the islets. It has been debated that the use of stem cells (SCs) holds a promise for the treatment of T1D due to their documented immunological and regenerative effects (12). SCs are undifferentiated cells capable of giving rise to virtually any tissue cells and have been categorized as embryonic SCs, cord blood SCs, and adult SCs. Adult SCs are undifferentiated multipotent cells present in all tissues. Bone marrow–derived MSCs are the most frequently used cells in investigation of T1D and have been reported to induce immunological tolerance toward β-cells through inhibition of autoaggressive T cells (13,14). Immunomodulatory function of MSCs was shown in

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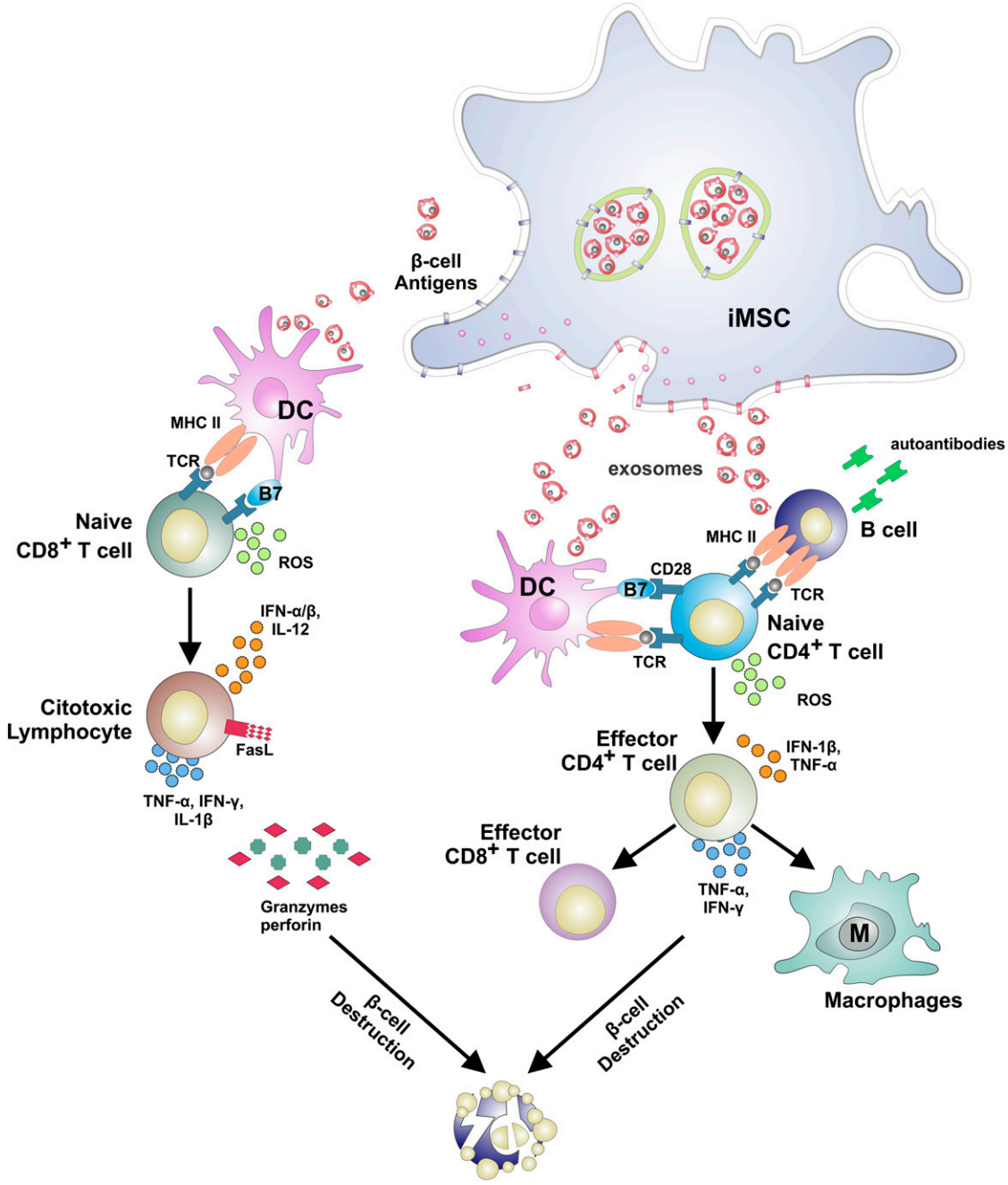

Figure 1—Proposed effects of EXOs released by islet MSCs. Although there is the possibility that EXOs directly stimulate T and B cells, it is likely that EXOs affect DCs that, in turn, initiate anti-β-cell–specific T-cell response. EXOs may lead to the maturation of inflammatory DCs or "feed" DCs with relevant islet antigens. B7, B7-1, B7-2; FasL, Fas ligand; MHC, major histocompatibility complex; ROS, reactive oxygen species; TCR, T-cell receptor; TNF, tumor necrosis factor.

NOD mice. MSCs derived from the bone marrow of Balb/c mice downregulated infiltration of lymphocytes and increased the number of regulatory Foxp3$^+$ T cells in the islets of NOD mice (15).

Thus, it was surprising in the study by Rahman et al. (9) that cells expressing MSC markers—CD105, SC antigen-1, CD90, and CD44—were negative for endothelial (CD31) and leukocyte (CD45) cell markers and were derived from the intraislet MSC (iMSC) release of EXOs that are highly immunostimulatory. The EXOs are nano-sized membrane-bound vesicles secreted by many hematopoietic and nonhematopoietic cells. EXOs are formed by inverse membrane building into the lumen of the endocyte compartment and by fusion with plasma membrane and are released in response to stress or tissue damage. Several lines of evidence suggest that EXOs may stimulate or downregulate immune response. For example, EXOs derived from IL-10–treated DCs can suppress

inflammation and downregulate collagen-induced arthritis (16). There is also evidence that antigen-specific $CD8^+$ T cells can suppress immune responses via the release of EXOs (17).

Rahman et al. (9) found that iMSCs are located at the periphery of normal islets in prediabetic NOD mice, but penetrate into β-cell areas as lymphocyte infiltration occurs. It appears that iMSCs lead rather than follow the development of insulitis. Immunostimulatory EXOs from these iMSCs develop in NOD mice prior to any evidence of insulitis. These EXOs are able to induce production of proinflammatory cytokines and may enhance the maturation of antigen-presenting cells. Interestingly, EXOs derived from iMSCs of NOD mice are more effective in inducing IFN-γ production than EXOs from C57BL/6J iMSCs, indicating that NOD EXOs may contain additional antigens that stimulate Th1 cells. In addition, by complementary approach in vivo and in vitro Rahman et al. (9) demonstrated that immunization with EXOs expand autoreactive T and B cells and enhanced susceptibility to passively transfer insulitis by effector cells from $Thy1^+$BDC2.5 transgenic mice. EXOs derived from iMSCs may therefore initiate local autoimmune response—data that might provide further insights into the triggering events that lead to T1D progression in NOD mice.

However, Rahman et al. (9) did not report development of diabetes in the recipient. It should be established whether EXOs from disease-susceptible and/or diabetic mice may induce disease when injected in naïve recipients. Finally, the role of antigen-presenting cells in the islet and pancreatic lymph nodes in the EXO-induced enhancement of insulitis should be further explored. It is possible that EXOs containing β-cell antigens "feed" DCs that, in turn, initiate destructive response in the islets (Fig. 1) or alternatively contribute to the maturation of inflammatory DCs that migrate to pancreatic lymph nodes to stimulate anti-β-cell–specific T-cell response.

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