Cytokines and Type 1 Diabetes: A Numbers Game

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Cytokines are protein mediators of intercellular stress communication with autocrine, paracrine, and endocrine modes of action. Hormones differ from cytokines by being produced in specialized glandular organs, by not all being proteins, and by their homeostatic properties. Virtually all nucleated cells produce and respond to cytokines under defined conditions, mainly in response to stress signals and in parallel to their differentiated functions. The cytokine group of molecules encompasses several hundred individual protein moieties, including 37 interleukins (ILs) and a multitude of chemokines. Many of these molecules have redundant functions whereas others elicit specific cellular reactions. It is debated whether cytokines also have homeostatic properties, i.e., contribute to the maintenance of normal cellular physiology.

Cytokines are central mediators of inflammation by controling innate and adaptive immune responses as well as tissue damage, defense, repair, and remodeling. Type 1 diabetes is an inflammatory disease of the pancreatic islet, in which insulin-producing β-cells are preferentially destroyed to varying degrees by the concerted action of autoreactive T-cells and monocytic cells (1). Despite huge research efforts over the past 40 years, no therapy targeting pathogenetic events in type 1 diabetes has attained routine clinical utility. A number of cytokines have been shown to be important for the development of type 1 diabetes both at the level of the immune system and at the level of the target β-cells (2,3). The actual mechanism of β-cell destruction is still unclear, and classical T-cell effector pathways as well as many proinflammatory cytokines have been proven dispensable in transgenic animal models. Only a few inflammatory proteins have been demonstrated to be critical for type 1 diabetes development in the NOD mouse, one of which is IL-21, discovered in 2000 by the Foster team at Zymogenetics. IL-21 is a member of the class I cytokine receptor-binding cytokine family including leptin, IL-2–7, IL-9, IL-11–13, and IL-15 (4) and signals via the JAK-STAT3 pathway to drive immunoglobulin production and proliferation of T- and B-cells as well as natural killer cells.

In 2008, Spolski et al. (5) showed near-complete prevention of insulitis and diabetes in IL-21R–deficient NOD mice associated with reduced numbers and function of the highly proinflammatory helper T_H17 cells implicated in type 1 diabetes development (6) as well as increased expression of regenerative genes in β-cells. Interestingly, T-cell numbers, regulatory T-cell activity and cytokine production were normal in these mice, suggesting that the effect was not a consequence of general immunosuppression as might be expected from the in vitro effects of IL-21 (4). These findings were confirmed and extended by Sutherland et al. (7) in 2009, who showed that β-cell overexpression of IL-21 caused inflammatory cytokine and chemokine induction, insulitis, β-cell destruction, and diabetes in nondiabetes-prone mice. Again, the lymphoid compartment was normal, but IL-21R–deficient splenocytes failed to adoptively transfer diabetes to NOD/SCID mice, indicating that IL-21 is essential for effector T-cell competence. Taken together, previous work in this field pointed to the potential use of anti–IL-21 as a preventive therapy in individuals at risk for type 1 diabetes, but did not address the clinically important questions of whether anti–IL-21 therapy reduces disease activity around the time of diabetes onset when detecting a reduction in first phase insulin or overt diabetes would allow a more targeted therapy.

This question was asked in the study of McGuire et al. (8), taking advantage of a neutralizing IL-21R fusion protein (IL-21R/Fc) to block IL-21 activity in the NOD mouse and of a model of islet allograft rejection in diabetic *IL-21r*$^{-/-}$ recipient mice. The article adds three important messages to current knowledge (Fig. 1): short-term anti–IL-21 therapy alone is effective only just before diabetes onset; the therapy together with a syngeneic islet graft but not alone reverts overt diabetes, an effect maintained after graft removal; and islet allograft rejection is significantly improved in IL-21 receptor–deficient recipients, an effect that was abolished in the presence of IL-21 receptor expressing and thereby responsive competent $CD8^+$ T-cells.

What are the mechanisms of action of IL-21 blockade in these mouse models? IL-21 neutralization decreased numbers of pancreatic and splenic T_H17 T-cells and numbers of activated $CD8^+$ and $CD4^+$ T-cells in the pancreas and pancreatic lymph nodes (5,8–10). The importance of reduced IL-17 production and regulatory T-cells is debated (5,7,8). McGuire et al. propose that a reduction in IL-21–induced IL-21 contribute to the sustained effects of short-term IL-21 neutralization (8). However, in *IL-21r*$^{-/-}$ mice, IL-21 production was sufficient to induce a CD8+ T-cell–mediated graft destruction in adoptive transfer experiments (8) indicating that a reduced IL-21 production per se is dispensable for the protective effects of anti–IL-21. Further, McGuire et al. did not examine β-cell expression of regenerative genes found enhanced in *IL-21r*$^{-/-}$ mice by others (5), but the adoptive transfer experiment (8) suggests that IL-21–induced graft destruction is not dependent on direct negative effects on β-cells, and in addition β-cells have not been found to express *IL-21r* (7). Thus, more research is needed to clarify the mechanisms of action, e.g., via which secondary mediator IL-21 inhibits β-cell *reg* gene expression.

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DOI: 10.2337/db10-1782

See accompanying original article, p. 867.

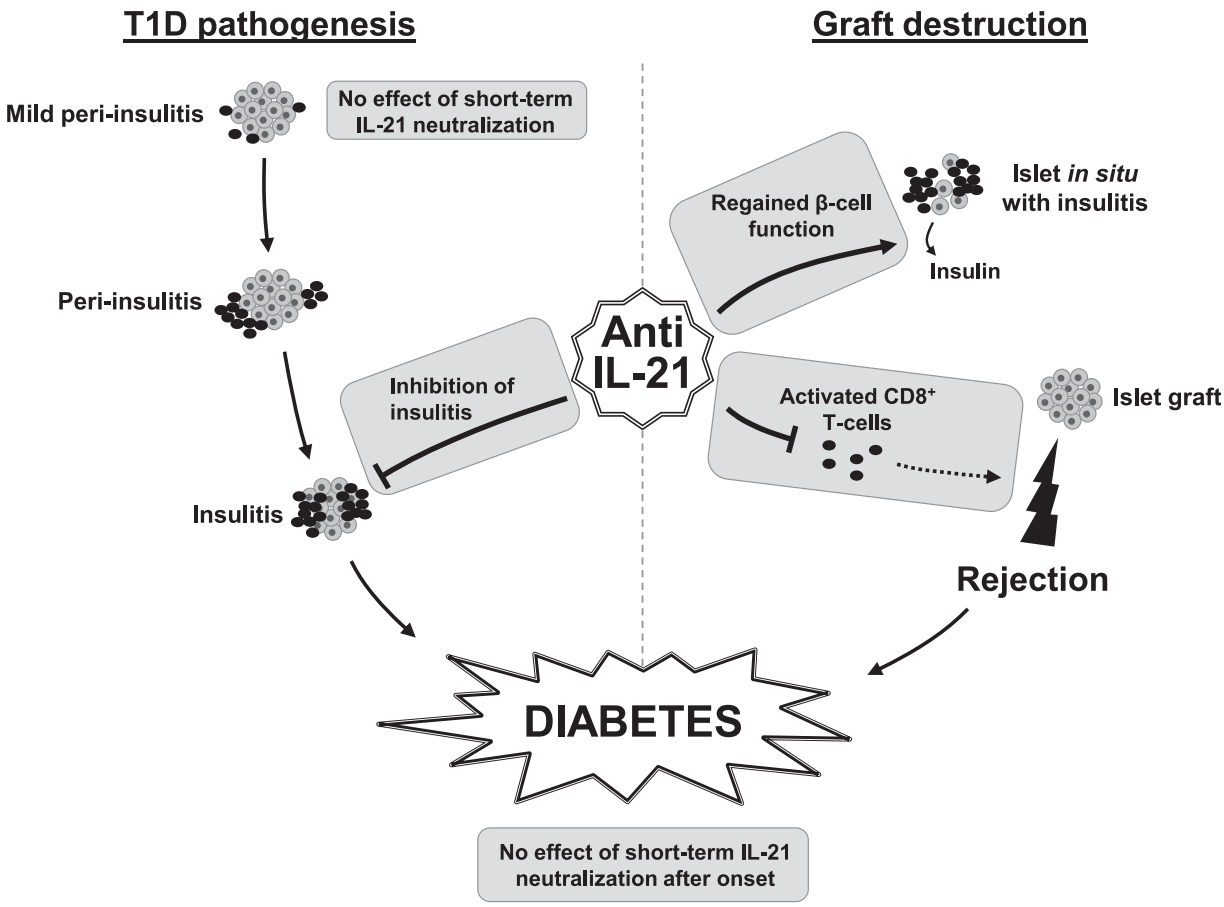

FIG. 1. Effects of anti–IL-21 treatment on type 1 diabetes progression and graft destruction. New knowledge provided by McGuire et al. (8) is highlighted in gray boxes. T1D, type 1 diabetes.

Lymphopenia has been suggested to precede diabetes onset and result in homeostatic expansion of tissue-destructive T-cells in the NOD mouse (11). IL-21 is important in this respect by inducing a rapid turnover and thereby replicative senescence of T-cells (10,11). However, lymphopenia is not found consistently in all NOD colonies (12) and $CD4^+$ and $CD8^+$ T-cell numbers were not affected in spleen and pancreatic lymph nodes of other *IL-21r*$^{-/-}$ NOD mice colonies (5,7). The study by McGuire et al. (8) does not directly address this issue. However, because IL-21 is important for homeostatic expansion of tissue-destructive T-cells preceding insulitis, the observation that IL-21 neutralization reverses insulitis (8) points to other important functions of IL-21 in the maintenance of islet inflammation.

Whereas the evidence reviewed above identifies IL-21 as a central cytokine in β-cell destruction in mouse models, the relevance of these promising findings for the application of anti–IL-21 therapy for type 1 diabetes in humans is yet to be proven. IL-21 is being evaluated as a therapy in metastatic melanoma and renal carcinoma, in non-Hodgkin's lymphoma and ovarian cancer, and anti–IL-21 is in phase I trials for rheumatoid arthritis (13). Considering the unknown long-term safety profile of immunomodulatory agents such as anti-CD3 (T-cell) or anti-CD20 (B-cell) antibodies and IL-21 neutralization as well as the transience of remission induced by some of these drugs, there is an urgent need for studies to define the clinical benefit of combining therapies targeting key pathways in the inflammatory pathogenesis of type 1 diabetes to increase safety and efficacy. McGuire et al. did not examine whether the adjuvant effect of islet transplantation to the action of IL-21R/Fc is mimicked by exogenous insulin. Thus, adding in treatments shown to protect β-cell function directly or indirectly such as intensive insulin treatment or anti–IL-1 may provide synergy with therapies targeting mainly the immune system. Future advances in this field may become a game of the number of the cytokines to be targeted as well as the number of therapies in the combination.

ACKNOWLEDGMENTS

T.M.P. is a part-time employee of Novo Nordisk and owns employee shares in Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

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