Harnessing immune cells to enhance β-cell mass in type 1 diabetes

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ABSTRACT

Type 1 diabetes is characterized by early β-cell loss leading to insulin dependence in virtually all patients with the disease in order to maintain glucose homeostasis. Most studies over the past few decades have focused on limiting the autoimmune attack on the β cells. However, emerging data from patients with long-standing diabetes who continue to harbor functional insulin-producing cells in their diseased pancreas have prompted scientists to examine whether proliferation of existing β cells can be enhanced to promote better glycemic control. In support of this concept, several studies indicate that mononuclear cells that infiltrate the islets have the capacity to trigger proliferation of islet cells including β cells. These observations indicate the exciting possibility of identifying those mononuclear cell types and their soluble factors and harnessing their ability to promote β-cell growth concomitant with autoimmune therapy to prevent the onset and/or halt the progression of the disease.

INTRODUCTION

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by destruction of most insulin-producing β cells, leading to absolute insulin deficiency.[1] The classical model for the pathogenesis of T1D is based on the premise that genetically susceptible individuals are affected by environmental factors leading to an aggressive invasion of the islet by autoreactive cytotoxic T cells. These cells produce and secrete cytokines that activate apoptotic pathways leading to a critical decrease in β-cell mass that is unable to maintain glucose homeostasis. While considerable progress has been made in understanding the processes that underlie rodent β-cell growth, the mechanisms and signaling proteins that promote the proliferation of human β cells are still poorly understood and warrant investigation.[2–4]

Immune cell infiltration in islets is associated with β-cell proliferation

Existing treatment options for T1D are primarily focused on insulin supplementation to counter the deleterious effects of hyperglycemia. However, the life-threatening side effects of poor insulin management coupled with a failure to restore normal glucose control in the face of immune suppression have prompted consideration of alternative therapies that include approaches to concomitantly preserve and/or enhance β-cell mass.[5] For instance, pancreatic islet transplantation and islet encapsulation have been considered to preserve β cells as safer and less invasive methods, but their treatment efficacy has been limited by graft malfunction and failure.[6] In this context, ex vivo genetic engineering of β cells has been used to prolong islet graft survival.[7] While in vitro, differentiation of embryonic and adult stem/progenitor cells to efficiently generate insulin-producing cells continues to evolve, few reports provide confidence that sufficient numbers of glucose-responsive β cells can be generated to effectively overcome hyperglycemia in vivo in mouse models of diabetes and potentially for human therapy.[8] An emerging alternative to cell replacement therapy is the concept of stimulating in vivo regeneration to replenish the loss of β-cell mass. Among the different processes described to promote the generation of new β cells,[9] neogenesis and replication from pre-existing β cells have both been explored.[9 10] Multiple studies in rodent models support a clear increase in β-cell proliferation during physiological and pathophysiological conditions.[10 11] In addition, hormones, growth factors, adipokines, and lactogens have all been shown to regulate β-cell proliferation.[12] Recent studies in patients with long-standing T1D provide evidence for the existence of functional β cells[13] that respond to mixed meals by increasing endogenous insulin levels.[14] These data have prompted approaches to consider promoting proliferation of β cells to enhance functional β-cell mass coupled with limiting autoimmunity to maintain glycemia.

The lack of availability of pancreatic material at different stages of the disease from humans with T1D continues to be a major challenge in the detailed investigation of its pathophysiology. Studies using the non-obese diabetic (NOD) mouse, a commonly used model of T1D which is characterized by the spontaneous development of diabetes, have shown that cells that comprise the mononuclear immune cell infiltrate have the capacity to stimulate β-cell replication.[15 16] Indeed, earlier studies have reported that β-cell regeneration is increased in pancreatic islets that are infiltrated with mononuclear immune cells in pre-diabetic NOD animals.[15] The concept of immune cells and β-cell regeneration is further supported by studies by Sherry *et al*[16] who were able to reverse the immune assault by anti-CD3

To cite: Dirice E, Kulkarni RN. *J Investig Med* 2016;**64**:14–20.

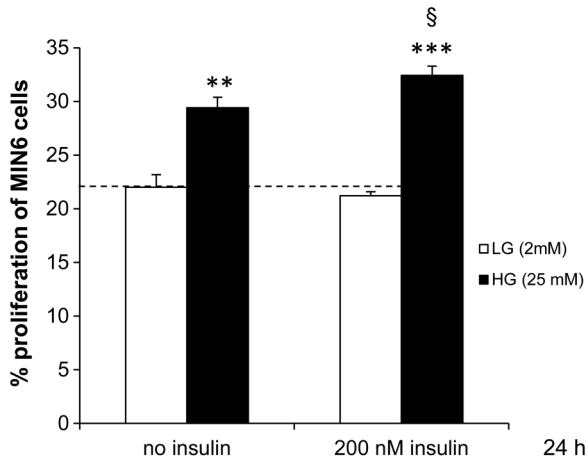

Figure 1 Exogenous insulin enhances β-cell proliferation. MIN6 insulinoma cells were treated under low (2 mM, white bars) or high (25 mM, black bars) glucose conditions in the presence or absence of 200 nM insulin for 24 h. *p<0.05, **p<0.01, ***p<0.001. Comparison: *, low (LG) versus high glucose (HG); §, no insulin versus insulin treated. Data are representative of two independent experiments and presented as mean±SEM.

monoclonal antibody or regulatory T-cell therapy with a consequent reduced β-cell replication. Recently, we reported a linear correlation between immune-cell infiltration and β-cell proliferation when total splenocytes (SPLs) derived from female new-onset diabetic NOD mice were transferred into immunodeficient NOD-recombination activation gene-1 null (NOD.RAG1$^{-/-}$) mice.[17] Our study demonstrates that adoptive transfer of diabetogenic CD4+ and CD8+ T-cells, but not B cells, induces β-cell proliferation in vivo. This effect was independent of the effects secondary to apoptosis, blood glucose or circulating insulin levels. Furthermore, the co-culture of diabetogenic CD4+ and CD8+ T cells with NOD.RAG1$^{-/-}$ islets in an in vitro transwell system led to increased β-cell proliferation due to the stimulatory effects dependent on soluble factors secreted by CD4+ and/or CD8+ T-cell subtypes. The ability of T cells to promote proliferation of its target cells has been reported earlier in aortic smooth muscle cells[18] and orbital fibroblasts.[19] Among cytokines, low levels of interleukin (IL)-1β have been reported to induce β-cell replication and enhance β-cell secretory function via the Fas-FLICE-like inhibitory protein (FLIP) pathway, an effect facilitated by the IL-1 receptor agonist (IL-1Ra) production.[20]

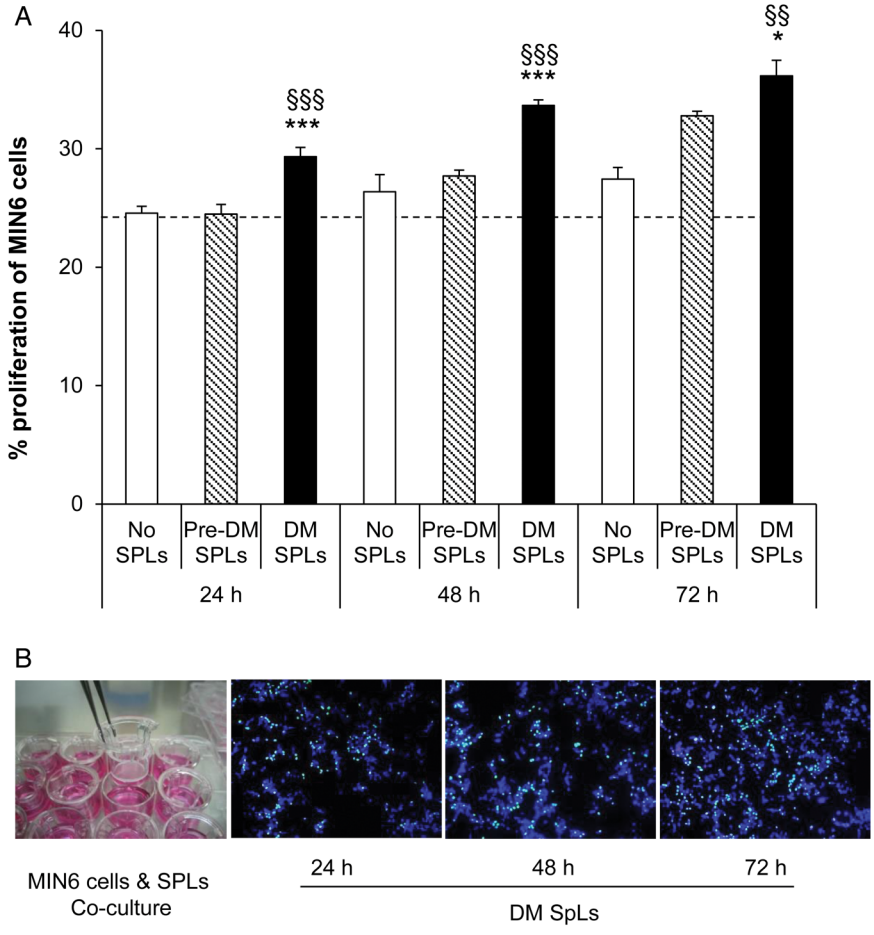

Figure 2 Diabetogenic splenocytes (SPLs) stimulate β-cell proliferation. (A) MIN6 cells were cultured either alone or co-cultured with SPLs, isolated either from pre-DM or DM, for 24, 48, or 72 h in a transwell system. *p<0.05, **p<0.01, ***p<0.001. Comparison: *, diabetic versus pre-diabetic; §, diabetic versus no SPL. (error bars, SEM). (B) Representative image showing a transwell used for separating the MIN6 cell grown on the plate surface from SPLs. Immunofluorescent images showing proliferating cells. BrdU is shown in green and nuclear staining by DAPI (4′,6-diamidino-2-phenylindole) is shown in blue. Data are expressed as mean±SEM.

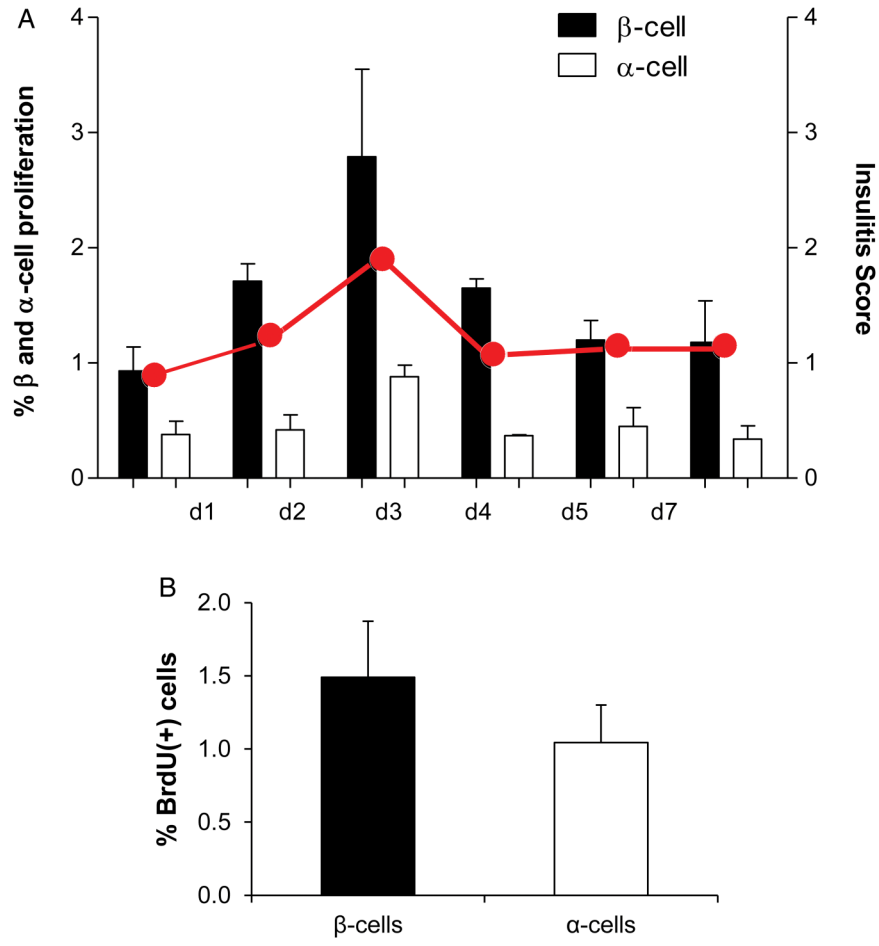

Figure 3 Immune cell infiltration stimulates β-cell and α-cell proliferation. (A) Quantification of β-cell and α-cell proliferation and insulitis scores in streptozotocin (STZ)-injected non-obese diabetic (NOD) mouse islets. (A) Three mice for each time point were analyzed post-STZ administration. (B) Quantification of β-cell and α-cell proliferation in pancreatic sections derived from total splenocyte (SPL)-injected diabetic mice. Data are expressed as mean±SEM.

Cytokines and chemokines promote β-cell proliferation

In addition to growth factors[21 22] and hormones,[22–25] it is now evident that cytokines and chemokines both appear to effectively increase the number of proliferating β cells in vitro.[17 26] For example, proliferation in the commonly used MIN6 insulinoma cell line can be significantly increased from a basal value of 22% by growth factor (eg, insulin) treatment (figure 1). Immune cell-derived soluble molecules have proliferation effects of a similar magnitude when β cells, separated by a transwell, are cultured with or without SPLs derived either from pre-diabetic mice (pre-DM) or diabetic mice (DM) for 24, 48, or 72 h (figure 2A,B). Thus, while β-cell proliferation after 24 h co-culture with pre-DM SPLs or without SPLs remained at basal levels (pre-DM SPL; 24.5%±0.81 and No-SPLs; 24.6%±0.58), β cells cultured with SPLs obtained from mice with new-onset diabetes reached levels that were comparable to those observed when MIN6 cells were treated with insulin (DM-SPLs; 29.3%±0.79). The increase in β-cell proliferation is obvious after co-culturing β cells for 48 (DM SPLs; 33.7%±0.47) or 72 (DM SPLs; 36.2% ±1.32) h in DM SPLs compared to co-cultures with SPLs from pre-DM mice or those cultured without SPLs.

Among other cytokines, Ellingsgaard *et al*[26] reported that IL-6 is necessary for the expansion of pancreatic α cells in response to a high-fat diet and suggested that this expansion is necessary for functional β-cell compensation to increased metabolic demand. Using islet-SPL co-culture studies, we observed that IL-6 and other cytokines and chemokines (eg, IL-2, IL-10, MIP1α, and RANTES) promoted β-cell proliferation.[17] Interestingly, β-cell proliferation was increased in streptozotocin (STZ)-induced diabetic mice and NOD.RAG1$^{-/-}$ mice that received total diabetogenic SPLs and, strikingly, the extent of mononuclear cell infiltration correlated positively with the β-cell proliferation (figure 3A).[17] To examine whether soluble factors derived from mononuclear immune cells also increase replication of non-β-endocrine cells (eg, α cells), we analyzed α-cell replication in these two in vivo mouse models. While α-cell mitosis was significantly increased 3 days after STZ treatment, in parallel with the insulitis score, it did not reach similar levels of β-cell proliferation (figure 3A). In addition, injection of total SPLs obtained from new-onset diabetic NOD mice showed attenuated levels of α-cell proliferation 3 weeks after transfer when compared to β-cell proliferation (figure 3B). A similar finding was reported by Cechin

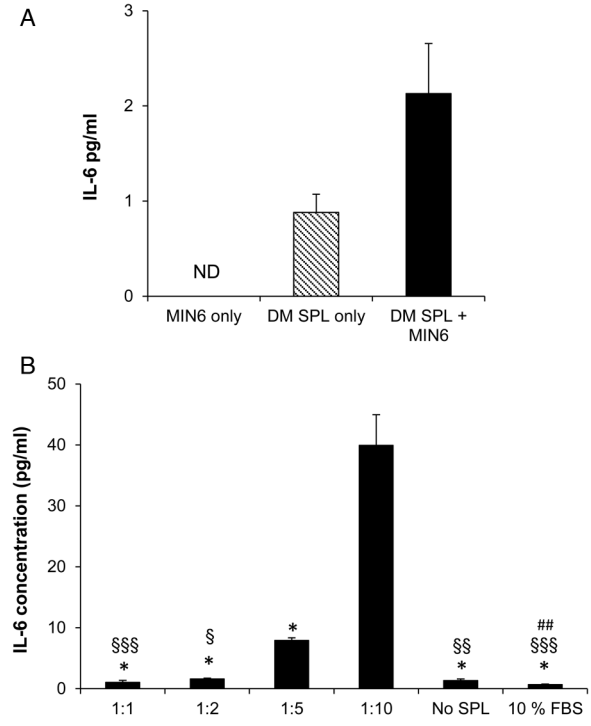

Figure 4 Interleukin (IL) 6 concentrations are elevated in human islets co-cultured with diabetogenic splenocytes (SPLs). (A) IL-6 levels in culture media from MIN6 only, diabetic mice (DM) SPL only, or MIN6 with DM SPLs. (B) IL-6 concentrations from human islets co-cultured with total SPLs from diabetic NOD mice at an islet cell:SPL ratio of 1:1, 1:2, 1:5, 1:10. Also shown are no SPL and 10% FBS-treated conditions. *p<0.05, **p<0.01, ***p<0.001. Comparison: #, vs 1:2 ratio; §, vs 1:5 ratio; *, vs 1:10 ratio. Data are expressed as mean±SEM.

et al.[27] Together, these data suggest that immune cell-derived soluble factors provide preferential stimulatory effects favoring β cells.

Analyses of culture media obtained from co-culture studies revealed a trend toward elevated IL-6 levels when β cells were co-cultured with DM SPLs compared to β cells cultured with pre-DM SPLs (figure 4A). IL-6 was not detected in β-cell cultures without immune cells (ie, cultures of MIN6 cells only), suggesting that co-existence of β cells (a target for cytotoxic T cells) along with immune cells is necessary to trigger IL-6 secretion. Furthermore, co-culture of human islets with SPLs, from new-onset NOD mice, in an increasing islet cell:SPL ratio (1:1, 1:2, 1:5, 1:10) showed a significant increase in IL-6 levels in the culture media at the 1:5 and 1:10 ratios ($p \leq 0.05$) compared to 1:1, 1:2, or controls (cultures lacking SPL treatment or cultures treated with 10% fetal bovine serum (FBS) groups, figure 4B). These data confirm the mouse findings and indicate that IL-6 has significant effects on islet-cell proliferation in addition to a pro-inflammatory role in immune cells.

Another class of cytokine-chemokine compounds that showed an effect on proliferation includes the chemoattractant molecules (eg, eotaxin (CCL11), IP-10 (CXCL-10), and MCP-1) that were also increased in the co-culture studies.[17] Indeed, increased concentrations of these molecules were dependent on the number of SPLs and were significantly

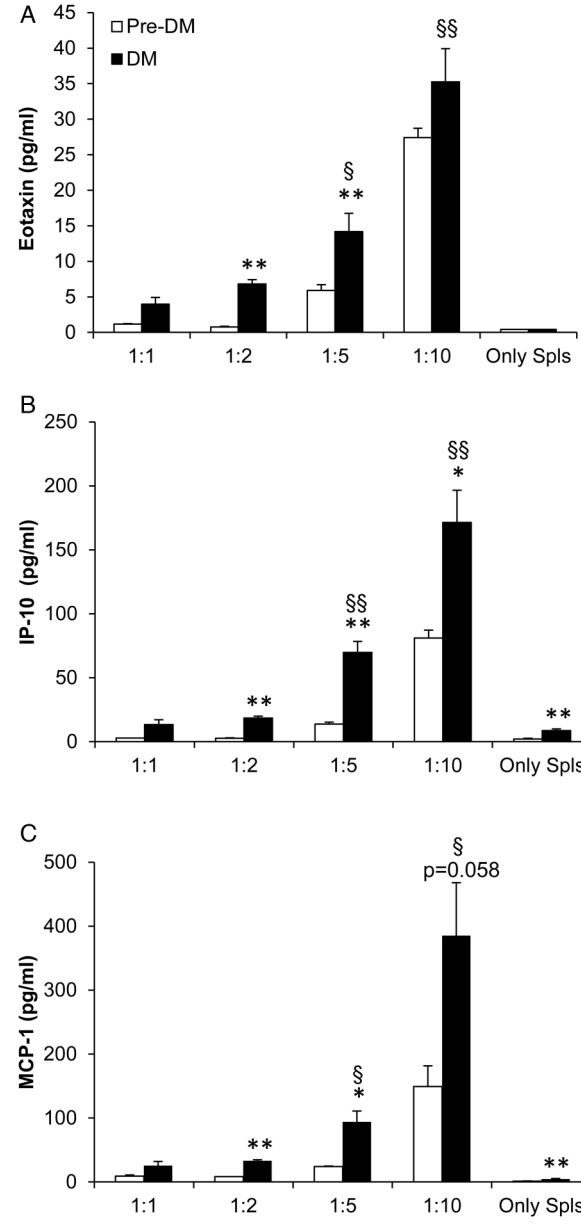

Figure 5 Chemoattractant molecules are increased in islet cell–splenocyte (SPL) co-culture. Luminex assay results from culture media obtained 48 h after co-culturing the RAG1$^{-/-}$ islet with NOD SPLs (pre-diabetic or diabetic) at an islet cell:SPL ratio of 1:1, 1:2, 1:5, 1:10 or only SPLs at 10× for eotaxin (A), IP-10 (B), and MCP-1 (C). *p<0.05, **p<0.01. Comparison: §, vs 1:1 diabetic SPLs; *, pre-diabetic versus diabetic SPL treatment. Data are expressed as mean±SEM (DM, diabetic mice).

higher in islet co-cultures treated with diabetogenic SPLs compared to pre-diabetic SPL treatment (figure 5A–C). The absence of these molecules in culture media when either pre-DM SPLs or DM-SPLs were cultured alone indicates that eotaxin, IP-10, or MCP-1 are secreted by β cells most likely in the presence of inflammation.

Macrophages are involved in β-cell proliferation

Recently, it has been shown that transforming growth factor (TGF) β1 and epidermal growth factor (EGF), secreted by M2 macrophages, stimulate β-cell

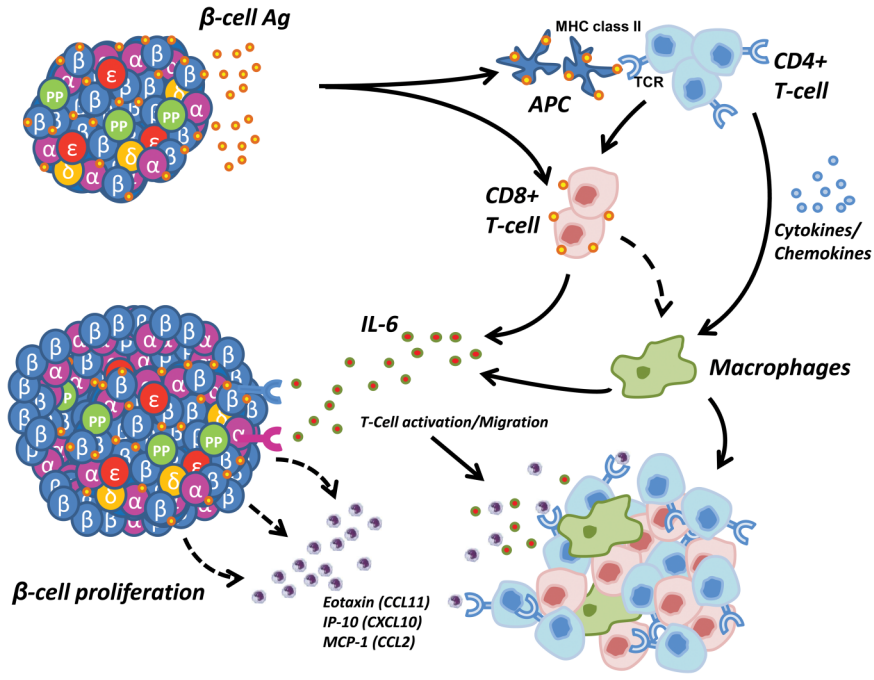

Figure 6 A schematic showing β-cell proliferation mediated by cytokines and chemokines secreted by infiltrating immune cells. See Conclusion section for an explanation.

proliferation.[28] M2 macrophages appear following the activation of M1 macrophages to mediate humoral immunity and tissue repair[29] and secrete a wide range of chemokines, enzymes, and growth factors.[30] In the study from Xiao *et al*, TGFβ1 secreted from M2 macrophages has been shown to induce SMAD7 in pancreatic β cells. SMAD7 activates cell cycle activators and induces the nuclear exclusion of cell cycle inhibitors to promote β-cell proliferation.[28] Most recently, Riley *et al*[31] demonstrated the importance of macrophages for connective tissue growth factor (CTGF)-mediated β-cell proliferation. In this study, following partial β-cell ablation, induction of CTGF in β cells increases the number of pancreatic macrophages and T cells and depletion of macrophages completely abrogates CTGF-mediated increases in β-cell replication after injury. Brissova *et al*[32] have reported that bone marrow macrophages recruited to sites of β-cell injury are important for the proliferation response and act independent of the effects of glucose.

A β-cell proliferation paradigm to counter T1D

Evidence that patients with long-lasting T1D have the ability to produce insulin in response to meals from residual β cells found in their pancreas[13 33] has prompted consideration of enhancing β-cell proliferation as a means to improve functional β-cell mass to counter the disease.

While the traditional role of autoimmune cells in mediating the killing of β cells is accepted, the accumulation of data from several laboratories indicates that mononuclear cells (CD4+, CD8+, macrophages, and potentially dendritic cells) also have potential beneficial effects in the form of promoting β-cell proliferation. This may appear contradictory in that immune cells can activate both death and proliferation signals; however, it is likely that the effects occur during different phases of the disease. For example, it is possible that Tregs overpower and dampen the effector T-cell response and concomitantly the proliferation signals dominate when the β-cell mass drops below a critical threshold (eg, honeymoon phase).[34] Several studies, including our own,[15–17] indicate that SPLs from hyperglycemic DM mice significantly increase the proliferation of β cells. Indeed, the proliferation-promoting effects of autoimmune T cells are not limited to β cells but extend to α cells in a potential attempt to maintain the islet architecture. Some of these effects on α cells have been reported to be mediated by IL-6 in rodent and human islets.[26] A recent report indicates that new α cells are generated mainly by IL-6-dependent self-duplication and seldom as a consequence of reprogramming of β cells in the pancreatic-duct ligation (PDL)-injured mouse pancreas.[35] It will be worth exploring whether these data on IL-6 effects on islet cell proliferation are linked with the findings of elevated IL-6 in young adults with T1D.[36] The significance of elevated levels of chemoattractant molecules (eotaxin (CCL11), IP-10 (CXCL-10), and MCP-1) in culture media from islet-SPL co-cultures is not fully understood.[17] Notably, pancreatic β-cell-specific upregulation of eotaxin was reported in rats as early as 40 days of age with early pre-insulitis.[37] Further, Chao *et al*[38] reported that overexpression of eotaxin is under the control of a region in chromosome 12 that overlaps with a previously mapped T1D locus, *iddm30*, in the diabetes prone bio-breeding (BBDP) rat. The same group also provided evidence that this trait is associated not with increased susceptibility but rather with resistance to T1D and an increase in Th2 differentiation among T lymphocytes infiltrating the islets.[38]

Several other pieces of evidence point to chemokines being attracted to islet cells. For example, interferon

γ-induced protein 10 (IP10) has been reported to be produced in NOD mouse pancreatic islets just prior to and following immune infiltration.[39] In addition, serum IP-10 levels were observed to be elevated in patients with T1D diagnosed positive for either one or both glutamic acid decarboxylase (GAD) or IA-2 autoantibodies.[40] An important role ascribed to IP-10 is as a chemoattractant to monocyte/macrophages, T cells, natural killer cells, and dendritic cells.[41] MCP-1 is upregulated by cytokines in mouse and human β cells and β cells from NOD mice exhibit an age-related increase in MCP-1 gene expression peaking at 8 weeks.[42] Moreover, pancreatic islet expression of chemokine MCP-1 suppresses autoimmune diabetes via tolerogenic CD11c+ CD11b+ dendritic cells.[43] Together, these data indicate that multiple cytokines/chemokines are elevated in and near the islets prior to the onset and/or during the progression of T1D. Whether these candidates can be harnessed to magnify their beneficial effects at specific stages of the disease to either delay and/or reverse the progression of T1D by specifically enhancing β-cell mass concomitant with autoimmune suppression warrants further investigation.

CONCLUSION

We propose a model (figure 6) where increased IL-6 levels secreted by autoreactive immune cells lead to enhanced islet cell proliferation prior to the onset of diabetes. These cytokine/chemokines (in this example, IL-6), drive the upregulation of other cytokines/chemokines (eg, eotaxin, IP-10, and MCP-1) that enhances islet survival (eotaxin) and/or suppresses autoimmune diabetes (tolerogenic immune cells). While challenging, it will nevertheless be important to carefully design studies aimed at evaluating these effects in the context of human T1D.

Funding ED is supported by a JDRF advanced Post-Doctoral Fellowship grant # 3-APF-2014-220-A-N. RNK is supported by grants for the National Institutes of Health R01 Dk67536, R01 DK103215, and R01 55523.

Provenance and peer review Commissioned; internally peer reviewed.

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