OBJECTIVE—The contribution of innate immunity responsible for aggressive β-cell destruction in human type 1 diabetes is unclear.

RESEARCH DESIGN AND METHODS—Islet cell expression of Toll-like receptors (TLRs), cytoplasmic retinoic acid–inducible gene 3 (MDA5)-Initiated innate immunity linked with melanoma differentiation associated gene-5 was hyperexpressed in islet cells, including β- and α-cells. TLR3 and TLR4 were expressed in mononuclear cells that infiltrated islets. Interferon (IFN)-α and IFN-β were strongly expressed in islet cells. Major histocompatibility complex (MHC)-class I, IFN-γ, interleukin-18, and CXC motif ligand 10 were expressed and colocalized in affected islets. CD11c+ MHC-class II+ dendritic cells and macrophage subsets infiltrated most islets and showed remarkable features of phagocytosis of islet cell debris. CD4+ forkhead box P3+ regulatory T cells were not observed in and around the affected islets. Mononuclear cells expressed the Fas ligand and infiltrated most Fas-expressing islets. Retinoic acid–receptor responder 3 and activated caspases 8, 9, and 3 were preferentially expressed in β-cells. Serum levels of IFN-γ were markedly increased in patients with fulminant type 1 diabetes.

CONCLUSIONS—These findings demonstrate the presence of specific innate immune responses to enterovirus infection connected with enhanced adaptive immune pathways responsible for aggressive β-cell toxicity in fulminant type 1 diabetes.

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RESULTS

MDA5, RIG-I, and enterovirus-capsid protein expression. MDA5 was strongly expressed in β-cells, α-cells, and other types of islet cells of fulminant type 1 diabetic pancreata (Fig. 1A–D). In nondiabetic control and type 1 diabetic control subjects, weak MDA5 expression was observed in a few α-cells (Supplementary Fig. 1). Significant expression of RIG-I was observed preferentially in β-cells in all three patients with fulminant type 1 diabetes (Fig. 1E–H), yet it was not expressed in nondiabetic and type 1 diabetic control subjects (Supplementary Fig. 1). Enterovirus-capsid protein (VP1) was detected in β- and non-β-cells of fulminant type 1 diabetic pancreata confirming our previous report (Supplementary Fig. 2) (4) but not type 1 diabetic control and nondiabetic control subjects.

TLR3 and TLR4 expression. Both TLR3 and TLR4 were expressed in MNCs that had infiltrated islets of fulminant type 1 diabetic pancreata but not nondiabetic and type 1 diabetic control subjects (Table 1).

IFN-α, IFN-β, interferon regulatory factor-7, and major histocompatibility complex class I expression. In all three pancreata of the patients with fulminant type 1 diabetes, IFN-α and -β were strongly expressed (Fig. 2A–F). Some MNCs that had infiltrated around or in islets and pancreatic acinar and ductal cells also expressed theses cytokines in fulminant type 1 diabetes (Fig. 2A and B) but not in either control subject. The numbers of pancreatic acinar cells surrounded by CD8+ T cells were 11/mm², 24/mm², and 22/mm², respectively, in the pancreatic sections of cases 1, 2, and 3. Most IFN-α-expressing cells were β-cells, α-cells, and islet non-β- and non-α-cells (Fig. 2C–F). Interferon regulatory factor (IRF)-7 (7) was strongly expressed in β- and α-cells (Fig. 2G–I) and mostly stained around and in the nucleus of the islet cells (Fig. 2G). Major histocompatibility complex class I (MHC-I) was hyperexpressed in all islet cell subsets of fulminant type 1 diabetic pancreata (Fig. 2J). Nondiabetic control and type 1 diabetic control subjects did not show expression of IFN-α, IFN-β, or IRF-7 and hyperexpression of MHC-I in their islets.

CD11c+ cells in islets. Remarkable CD11c+ cells migration to the islets was observed in most islets of fulminant type 1 diabetic pancreata (Table 1). Intraislet CD11c+ cells expressed MHC class-II molecules (Fig. 2K–N). Confocal microscopy showed that some CD11c+ cells contained β-cell debris positive for insulin (Fig. 2O). Such findings were not observed in islets of nondiabetic or type 1 diabetic control subjects. Most CD11c+ cells were also positive for CD1a and some for CD68 (Fig. 2P), likely representing DCs and macrophage subsets. CD56+ or CD57+ NK cells and Tregs (CD4+ Foxp3+ cells) were not detected in or around islets of fulminant type 1 diabetic pancreata and either control. Tregs, CD4+ Foxp3+ cells, were not detected in or around the islets or in exocrine regions of the pancreas in fulminant type 1 diabetic, nondiabetic control, or type 1 diabetic control subjects (Table 1).

IL-18, IFN-γ, and CXCL10 expression. IL-18 was expressed in islet cells in all three fulminant type 1 diabetes (Fig. 3A and E). Most residual β-cells expressed both IFN-γ and IL-18 (Fig. 3G and H). IL-18, IFN-γ, and CXCL10 colocalized in most β- and islet non-β-cells (Fig. 3A–H). IL-12 was not expressed in any cells in affected pancreata. A few islets of type 1 diabetic control subjects (mean [range]: 2.8% [0–5.2]) expressed IL-18 and IFN-γ but not CXCL10. Nondiabetic control subjects did not express IL-18, IFN-γ, and CXCL10.

Serum IFN-γ levels in patients with fulminant type 1 diabetes. Serum levels of IFN-γ in patients with fulminant type 1 diabetes were approximately three times higher than those in nondiabetic and type 1 diabetic control subjects (Fig. 3J).
TABLE 1
Frequency of islets with MNCs that express TLR3, TLR4, CD11c, CD4+Foxp3, and FasL in three fulminant type 1 diabetic cases, and nondiabetic and type 1 diabetic control subjects

|                  | Frequency of TLR3+ MNCs (%) | Frequency of TLR4+ MNCs (%) | Frequency of CD11c+ cells (%) | Frequency of CD4+Foxp3+ cells (%) | Frequency of FasL+ MNCs (%) |
|------------------|-----------------------------|----------------------------|-------------------------------|----------------------------------|----------------------------|
| Case 1           | 11.3 (7/62)                 | 1.6 (1/62)                 | 95.9 (70/73)                  | 0 (0/75)                        | 82.6 (38/46)               |
| Case 2           | 5.0 (2/40)                  | 1.8 (1/56)                 | 100 (64/64)                   | 0 (0/63)                        | 90.0 (36/40)               |
| Case 3           | 18.8 (6/32)                 | 9.4 (5/53)                 | 91.2 (52/57)                  | 0 (0/70)                        | 95.2 (40/42)               |
| Mean             |                             |                            | 11.7 (6.9 (15/134)           | 4.3 (4.4 (186/194)              | 89.3 (6.3 (114/128)         |
| PT1D (n = 3)     |                             |                            |                               |                                  |                            |
| Nondiabetic control subjects (n = 15) | 0 (0/652)                 | 0.3 ± 0.6 (0.17) (3/742) | 0.2 ± 0.5 (0.1-1.4) (2/636) | 0 (0/692)                        | 0.3 ± 0.7 (0.1-1.9) (3/763) |
| Type 1 diabetic control subjects (n = 4) | 0 (0/228)                 | 2.4 ± 2.1 (0.9-8.9) (10/392) | 0 (0/351)                        | 18.2 ± 16.9 (3.8-46.6) (81/417) |

P value
- PT1D vs. non-diabetes <0.0001
- PT1D vs. type 1 diabetes <0.002
- Nondiabetics vs. type 1 diabetes NS

Values are means ± SD unless otherwise indicated. PT1D, fulminant type 1 diabetes.

DISCUSSION

Both RIG-1 and MDA5 were strongly expressed in β-cells of both diabetic and MDA5 were strongly expressed in β-cells of allergic type 1 diabetic pancreata. MDA5 was also hyperexpressed in β-cells of diabetic β-cells of patients with fulminant type 1 diabetes. Hyperexpression of RIG-I and MDA5 with subsequent IFN-γ production and DC and macrophage activation (6), were strongly expressed in both diabetic β-cells and diabetic β-cells. These results indicate that all islet cells are in an activated state of innate immunity in response to enterovirus infection (6). In islets of autopsied pancreatic duct cells, e.g., in type 1 diabetic control subjects, RARRES3, cleaved caspases 8, 9, and -3 were expressed weakly in some β-cells (Supplementary Fig. 5). In islets of autopsied pancreatic duct cells, e.g., in type 1 diabetic control subjects, RARRES3, cleaved caspases 8, 9, and -3 were expressed weakly in some β-cells (Supplementary Fig. 5).
CD4+ Foxp3+ cells, which represent a pivotal subset of Tregs, were not observed in or around the islets of fulminant type 1 diabetic pancreata, suggesting that the extremely polarized local condition to predominance for Th1 in response to enteroviral infection suppresses Treg differentiation from naive T CD4+ precursors (17). In turn, the Treg-depleted islet condition enhances Th1 cytokine (i.e., IFN-γ) generation.

Notably, IL-18, an IFN-γ-inducing factor, was extensively expressed in islet cells of infected fulminant type 1 diabetic pancreata. In response to viral infection, IL-18 is promptly secreted from virus-activated macrophages, DCs, and T cells (18), stimulating production of IFN-γ synergistically with IFN-α and -β through a unique pathway that sometimes occurs independently of IL-12 or NK cells (19). Conversely, IL-18 can be induced by IFN-γ alone or in combination with other cytokines in islet β-cells (20). Thus, for fulminant type 1 diabetes, enterovirus itself or enterovirus-activated T cells and macrophages most likely infiltrate islets to induce IL-18 production in these cells. In addition, IFN-α and -β, produced in islet cells and islet-infiltrating MNCs, can enhance IL-18-mediated signaling (21). Subsequently, islet-secreted IL-18 may induce IFN-γ production via receptors on the islet cells or islet stromal cells in an autocrine/paracrine manner. Once this positive autocrine/paracrine circuit for production of IL-18, IFN-γ, and CXCL10 is established in islet cells, destructive mechanisms involving CXCR3+ T cells and macrophages might persist until complete destruction of the β-cells (4).

We found that Fas was highly expressed in affected islet β-cells and islet-infiltrating FasL+ cells. Taken together with the finding that MHC-I and IFN-α, -β, and -γ were
strongly expressed in affected islet cells, effector mechanisms for β-cell apoptosis in fulminant type 1 diabetes are likely mediated in part by MHC-I and by the Fas-FasL pathway (22). Inflammation-induced Fas-FasL expression in β-cells was reported to lead to rapid and massive β-cell destruction (23). Other apoptotic mechanisms through the IFN-γ–dependent JAK/STAT pathway (24) and innate immune pathway (25) will also exert β-cell destruction.

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K.A. and Y.N. conducted immunohistochemical staining, RT-PCR data analysis, and discussed, reviewed, and edited the article. S.T. contributed to planning and discussion and edited the article. T.M. and A.S. sampled autopsied pancreas and participated in discussion. T.A., M.S., and H.S. contributed to discussion and reviewed and edited the article. S.T., T.M., M.I., D.A., and T.A. recruited serum samples, measured serum levels of IFN-γ, and participated in discussion. F.F., A.K., and M.K. contributed to analysis of immunostained sample data. J.I., H.F., and T.E. contributed to analysis of nondiabetic pancreas and Treg+ lymph nodes. T.K. analyzed data and wrote and edited the article.

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