Characterization of Diabetogenic CD8+ T Cells

IMMUNE THERAPY WITH METABOLIC BLOCKADE*

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Type 1 diabetes mellitus is caused by the killing of insulin-producing β cells by CD8+ T cells. The disease progression, which is chronic, does not follow a course like responses to conventional antigens such as viruses, but accelerates as glucose tolerance deteriorates. To identify the unique features of the autoimmune effectors that may explain this behavior, we analyzed diabetogenic CD8+ T cells that recognize a peptide from the diabetes antigen IGRP (NRP-V7-reactive) in prediabetic NOD mice and compared them to others that shared their phenotype (CD44+CD62LloPD-1+CXCR3+) but negative for diabetes antigen tetramers and to LCMV (lymphocytic choriomeningitis)-reactive CD8+ T cells. There was an increase in the frequency of the NRP-V7-reactive cells coinciding with the time of glucose intolerance. The T cells persisted in hyperglycemic NOD mice maintained with an insulin pellet despite destruction of β cells. We compared gene expression in the three groups of cells compared with the other two subsets of cells, and the NRP-V7-reactive cells exhibited gene expression of memory precursor effector cells. They had reduced cellular proliferation and were less dependent on oxidative phosphorylation. When prediabetic NOD mice were treated with 2-deoxyglucose to block aerobic glycolysis, there was a reduction in the diabetes antigen versus other cells of similar phenotype and loss of lymphoid cells infiltrating the islets. In addition, treatment of NOD mice with 2-deoxyglucose resulted in improved β cell granularity. These findings identify a link between metabolic disturbances and autoreactive T cells that promotes development of autoimmune diabetes.

Type 1 diabetes (T1D) in humans is an autoimmune disease involving the destruction of insulin-producing β cells in the islets of Langerhans by CD8+ T cells (1–6). In humans, the disease develops over a period of years after the first appearance of autoantibodies that identify individuals who are at risk. The kinetics of progression to T1D differs from those of other cell-mediated immune responses. In responding to foreign pathogens, there is rapid expansion of antigen-specific effector T cells followed by contraction and the appearance of memory T cells. Immune targets are destroyed within weeks. Primarily based on serologic data from studies of discordant triplets and relatives at risk for the disease, β cell destruction in T1D is thought to occur over a period of years, although we and others have postulated that there was waxing and waning of the process. More recent data has suggested that the progression is not uniform. The time required for metabolic changes in glucose tolerance was thought to reflect the need to destroy a large β cell mass before glucose tolerance deteriorates, but our studies of β cell killing in NOD mice and humans suggests instead that the progression of β cell killing may be rapid in the peridiagnosis period. In NOD mice, which first develop insulin at 5–6 weeks of age, the rate of β cell killing does not show a significant increase until after 11 weeks of age coincident with the appearance of glucose intolerance. In our studies of β cell killing in relatives of patients with T1D, only 25% of the 49 measurements that were obtained approximately every 6 months for up to 4.14 years before diagnosis showed increased levels, indicating that the destruction of β cells is an uncommon event. However, in at-risk subjects who were closer to diagnosis and manifest dysglycemia, 81% of the measurements in 27 subjects showed elevated levels of β cell death. These data showed that β cell killing is significantly greater when glucose intolerance is present.

These observations led us to consider whether there were interactions between the pathogenic immune cells and host factors that may affect the progression of disease. To understand these interactions, we focused our studies on CD8+ T cells that are reactive to an epitope of glucose-6-phosphatase catalytic subunit related protein (IGRP) and are known to cause β cell killing. These CD8+ T cells are the best characterized among pathogenic T cells (2–4, 7–15). In human autopsy studies, CD8+ T cells reactive to an epitope of IGRP have been found in pancreata from patients several years after the onset of disease (6). IGRP-reactive CD8+ T cells can be found in NOD mice by staining with MHC-I tetramer loaded with NRP-V7 peptide (5, 12, 16). They can cause diabetes when they receive help from CD4+ T cells (13). We analyzed gene expression among these cells to identify mechanisms that might account...
for the kinetics of disease and interactions between the host and the pathogenic immune response. We found that compared with CD8+ T cells that share the phenotype but not the antigen specificity of the IGRP-reactive T cells as well as viral antigen-reactive CD8+ T cells, the expansion and effector function of the NRP-V7-reactive T cells are dependent on glucose availability. We show that blocking glucose metabolism may selectively reduce the frequency of these cells and others that infiltrate the pancreatic islets. Our work suggests that elevated glucose may drive a feed-forward mechanism in which modest hyperglycemia provides fuel to effector T cells and leads to the maintenance of pathogenic T cells and accelerated β cell destruction.

**Experimental Procedures**

**Mice and Reagents**—NOD and NOD/scid mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in our facility under specific pathogen free conditions. All animal use protocols were approved by the Yale University Institutional Animal Care and Use Committee.

**Infection with LCMV**—NOD mice 8 weeks of age were infected with 2–3 × 10⁶ pfu of V-Armstrong (intraperitoneal), 2–3 × 10⁶ pfu of LCMV clone 13 (intravenous), and 23,104 cfu of recombinant *Listeria monocytogenes* (intravenous) expressing the Gp33 and Gp276 epitopes (rLM33, a gift from Dr. H. Shen) (17).

**Identification of Antigen-specific T Cells**—H-2-K¹/peptide monomers were prepared by FPLC as previously described, and heavy and light chain proteins were refolded in a solution containing a high concentration of NRP-V7 (KYKANVFL) mimotope, negative control peptide TUM (KYQAVTTTL), or in mice infected with LCMV, Gp33, and Gp276 peptides (Genscript, Piscataway, NJ) as described previously (3, 4, 18). Single cell suspensions were prepared from the pancreatic, mesenteric, axillary/brachial, and inguinal lymph nodes, spleen, liver, and pancreas. Islets of Langerhans were hand-picked from 12-week-old NOD mice, and single cell suspensions were analyzed after dissociation with EDTA.

**Flow Analysis**—Flow cytometry acquisition was performed on a FACS LSRII cytometer and analyzed with FlowJo v7 software (Asland OR); non-viable cells were excluded. The cells were stained with antibodies to CD8α, CD44, CD127, PD-1, CD62L, CXCR3 and after fixation and permeabilization with Cytofix/Cytoperm (BD Biosciences) antibodies to T-bet, CD62L, CXCR3 and after fixation and permeabilization with Cytofix/Cytoperm (BD Biosciences) antibodies to T-bet, Eomes, and KLRG1.

**Gene Expression by Microarray**—Single cell suspensions of splenocytes were prepared from pools of 4–6, 10–18-week-old NOD mice or NOD mice 9 days after infection with LCMV virus. The cells were labeled with live/dead fixable stain, tetramer, and antibodies to CD44, CD62L, PD-1, and CXCR3. These antibodies also used sort tetramer + CD8+ T cells and tetramer − CD8+ T cells of the same phenotype (phenotype +). Cell pellets were prepared in buffer RLT (Qiagen, Valencia, CA) and stored at -80 °C. They were pooled, and RNA was isolated using RNeasy kit. cRNA was prepared and analyzed using Gene Chip Murine Genome U74Av2 Array (Affymetrix, Santa Clara, CA).

**Gene Array Analysis**—Raw microarray expression data were transformed (variance-stabilizing transformation) and normalized (quantile method) using the Lumi package from R and Bioconductor (19, 20) (The R Project for Statistical Computing). After quality control there were 11 samples including replicates; 5 for the NRP-V7-reactive cells, 3 for the phenotype+, and 3 for LCMV-reactive cells. The expression data were further filtered by removing transcripts with a detection *p* value cutoff >0.01. Genes with multiple probe sets were collapsed by choosing the probe set with the highest average expression across samples. Differentially expressed genes were identified by an absolute-fold change (>2) and by a statistically significant change in expression (<0.05), as determined by LIMMA using a Benjamini-Hochberg false discovery rate (FDR) (19). Biological and technical replicates were accounted for using the correlation parameter in LIMMA.

**Gene Set Enrichment Analysis**—Gene set enrichment analysis (GSEA) (21) obtained from the Broad Institute website was used to identify active pathways. Gene sets were first filtered based on size (min = 15, max = 500), leaving 781 pathway gene sets used in the analysis. Probes were collapsed to genes based on the gene symbol, choosing the probe with the maximum average value if multiple probes existed, resulting in 13,340 total genes. Gene set enrichment analysis was run on the gene list ranked by the signal-to-noise metric comparing NRP-V7-reactive and phenotype+ cells. The normalized enrichment score was calculated for each gene set, and statistical significance was determined using 5000 permutations of the gene set phenotype labels. Both the *p* value and FDR were calculated for each set. Gene sets with FDR < 0.05 were considered differentially expressed.

**Cell Culture Studies**—Splenocytes harvested from 11 weeks or older NOD mice were labeled with cell tracer violet then stimulated with 0.01 μg/ml anti-CD3 in culture in media supplemented with glucose and 50 units/ml IL-2 but in the presence of 4.1 mM, 1.37 mM, or 0.15 mM oligomycin. After 24 h, cells were washed to remove anti-CD3 then re-cultured overnight in glucose and IL-2 supplemented culture medium at the concentrations discussed above then harvested and analyzed for cellular proliferation by flow cytometry. Uptake of the glucose analog, 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) (Sigma) was measured by flow cytometry in splenocytes from NOD mice that were cultured in 20 mM glucose for 48 h.

**Treatment with 2-Deoxyglucose (2DG)**—To determine the role of metabolic pathways, 11–13-week-old NOD mice were treated with 2DG (500 mg/kg, intraperitoneally) for 5 days, and the frequency of tetramer+ and phenotype+ T cells in the spleen and lymph nodes was analyzed by flow cytometry. The relative proportion of tetramer+ versus phenotype+ CD8+ T cells was compared in the treated and untreated mice.

**Analysis of Islet Cells**—The granularity of β cells and islet-infiltrating T cells was analyzed by flow cytometry. Islets from prediabetic NOD mice were hand-picked after collagenase digestion of the pancreas and dispersed into single cells using described methods (22). Before flow analysis the cells were stained with Fluoro-Zn and TMRE. Electronic gates were first placed around endocrine cells based on forward and side scatter.
Zn+TMRE+ cells. The granularity of the β cells were distinguished using side scatter (23) Infiltrating lymphocytes could be identified by forward and side scatter characteristics and comparison to islet cells from NOD/scid mice.

Statistical Analysis—Unless indicated data are presented as the mean ± S.E. For comparisons of two groups, Student’s t tests or Mann Whitney tests were performed as indicated using GraphPad Prism (version 6.03). We analyzed changes over time with a two-way ANOVA (GraphPad, v6). Comparisons of secondary lymphoid structures and changes over time were done with a mixed linear model using SAS (v9.3, Cary, NC). p values < 0.05 were considered statistically significant. Designations throughout the manuscript are: *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Results

Appearance of Diabetogenic CD8+ T Cells during Progression of Diabetes—We measured the frequency of the IGRP-reactive, NRP-V7-reactive T cells in the secondary lymphoid structures (spleen, pancreatic, mesenteric, axillary, and brachial lymph nodes) in NOD mice at different ages by tetramer staining. The cells were first detected in some mice in the pancreatic lymph nodes at 6 weeks of age (Fig. 1, p = 0.009) (ANOVA, n = 3–5/group at each time point). Compared with the first measurement at 6 weeks, there was an increase in the frequency of tetramer + cells in the mesenteric (*, p = 0.03), pancreatic (**, p < 0.001) lymph nodes, and spleen (***, p < 0.001) over 14 weeks.

Compared with the tetramer – CD8+ T cells in the spleen, pancreatic lymph nodes, and in the islets, the NRP-V7-reactive cells showed higher levels of expression of CD44, PD-1, and CXCR3 (all p < 0.0001) (Fig. 2c) (it was not possible to identify tetramer + cells within the islets most likely because of modulation of the T cell receptor (TCR)). The phenotype of the tetramer + cells was similar in the spleen, pancreatic, and mesenteric lymph nodes. The tetramer + cells also expressed T-bet and Eomes (Fig. 2d) and produced IFNγ, CD107a, and TNF when activated with PMA and calcium ionophores (not shown). There were significant changes in cell surface markers on the NRP-V7-reactive T cells from the mesenteric and pancreatic lymph nodes over time (Fig. 2e). CD44 increased from 10 weeks until the onset of diabetes (p < 0.01), and CD69 also increased after diabetes onset (p < 0.01), suggesting cell activation. CXCR3 was at the highest levels just before diabetes onset but declined afterward. Other markers such as CD27, KLRG1, CD62L, PD-1, and CD127 did not show significant changes.

Compared with immune responses to conventional (e.g. viral) antigens, the immune response leading to T1D is more prolonged and less robust. To understand the differences in the viral antigen and diabetes (auto)antigen-specific responses we also compared the NRP-V7-reactive cells to LCMV-reactive cells 9 days after infection of NOD mice with LCMV. We identified viral antigen-reactive CD8+ T cells with Class I MHC tetramers with the gp33 peptide 9 days after infection with the virus. The LCMV-reactive cells showed similar expression of CD44, CD27, CD62L, PD-1, and CXCR3 compared with the NRP-V7 reactive cells but expressed higher levels of CD25, CD69, and KLRG1 (p < 0.01, p < 0.0001, and p < 0.0001, respectively), consistent with the acute changes in effector T cells to viral infection (Fig. 2f).

Gene Expression in NRP-V7-reactive T Cells—To distinguish the features of the autoreactive, viral antigen-reactive CD8+ T cells from other cells in NOD mice that share the phenotype of these cells, we sorted CD8+ T cells that showed high expression of CD44, PD-1, and CXCR3 and low expression of CD62L and compared gene expression by microarray to the gp33 and NRP-V7 tetramer + CD8+ T cells, sorted from the spleens mesenteric and pancreatic lymph nodes (data may be accessed at www.ncbi.nlm.nih.gov).

Fig. 3a displays the number of genes with significant differences between the NRP-V7-reactive cells and the polyclonal phenotype+ or LCMV-reactive cells (FDR < 0.05 and -fold change >1.8), and genes that show the same directional changes in comparison to both LCMV and phenotype+ cells. As expected from our phenotype analysis, gene expression in NRP-V7-reactive cells clustered more closely with phenotype+ than gp-33 reactive cells (Fig. 3b). We focused on expression of the 54 common genes that showed significant differences
between the NRP-V7-reactive and phenotype + cells to identify features associated with the pathogenicity of the NRP-V7-reactive cells and differentiated them from these other populations. There were several genes associated with differentiation pathways that differed between the NRP-V7-reactive and phenotype + population. Among these, neuropilin 1 (NRP1), a marker expressed on tolerant CD8 + T cells and Tregs, was reduced as well as ICOS, which is associated with resolution of CD8-mediated lung injury in a transplant model (26–28). Consistent with our findings by flow cytometry (the mRNA expression levels of other transcription factors), Tbet was similar between NRP-V7-reactive, LCMV-reactive, and phenotype + T cells, but Eomes was lower on the LCMV-reactive cells (not shown).

To identify the functional phenotype of these cells, we compared the enrichment of the most differentially expressed (FDR < 0.05) NRP-V7 and phenotype + signature genes to the ranked gene list of memory cells (MPECs) or short-lived effector cells (SLECs) (29). The NRP-V7 tetramer + cells showed a significant enrichment of MPEC signature ($p < 10^{-7}$) when compared with phenotype + cells, whereas the phenotype + cells showed a significant enrichment of SLEC signature ($p = 2.9 \times 10^{-10}$) (Fig. 3c). Similarly, when we compared the enrichment of the most differentially expressed NRP-V7 and LCMV signature genes, NRP-V7- and LCMV-reactive cells showed significant enrichment of MPEC ($p = 2.4 \times 10^{-7}$) and SLEC ($p = 1 \times 10^{-14}$) signatures, respectively (Fig. 3c).

**Differences in the Expression of Genes from Proliferative and Metabolic Pathways in NRP-V7-reactive Cells and Other CD8 + T Cells That Share Their Phenotype**—Using Gene set enrichment analysis, we identified 106 pathways that were significantly altered in the NRP-V7-reactive versus phenotype + CD8 T cells (FDR < 0.05) (Fig. 4) and then examined these pathways in the LCMV-reactive cells. There were three pathways...
enhanced in the NRP-V7 versus phenotype+ cells. Cellular proliferation and DNA replication pathways were increased in the phenotype+ cells and gp-33 reactive cells versus NRP-V7-reactive cells (Table 1). Ex vivo, we confirmed these findings and found greater proliferation of the phenotype+ versus NRP-V7-reactive T cells from mice at 10 weeks of age as identified by a lower percentage of Ki67+ tetramer+ versus phenotype+ T cells from the same mice (Fig. 5) \( (p < 0.01) \).

There was also increased expression of genes associated with oxidative phosphorylation pathways in the phenotype+ cells (Table 1). To confirm the functional differences, we cultured phenotype+ and NRP-V7-reactive T cells with oligomycin,
which blocks ATP synthase used in oxidative phosphorylation but not aerobic glycolysis (30). The level of cellular proliferation was greater at the start of the experiments in the phenotype+ cells without oligomycin versus tetramer+ cells (Fig. 6a), as predicted from the gene array analysis and similar to the data in Fig. 5. Oligomycin reduced the frequency of proliferating phenotype+ cells but had little effect on proliferation of the NRP-V7-reactive cells (Fig. 6, a and b, p < 0.05). In addition, we found that the uptake of 2-NBDG was significantly greater in the NRP-V7-reactive versus phenotype+ (p < 0.05) or cells without the same phenotype (Fig. 6c, p < 0.001).

Treatment with DG Reduces NRP-V7-reactive T Cells and Improves β Cell Granularity—To determine whether aerobic metabolism is crucial for survival and pathogenicity of NRP-V7-reactive cells, we treated NOD mice with 2DG for 6 days. We compared the frequency of tetramer+ and phenotype+ cells in the spleen and pancreatic lymph nodes from litter mates treated with saline for the same time period by flow cytometry. There was a reduction in the frequency of tetramer+ cells and a decrease in the ratio of tetramer+ : phenotype+ T cells in the mice treated with 2DG in the lymph nodes (p = 0.009, Mann-Whitney) (Fig. 7a).

To test whether this intervention affected β cells and islet infiltrates, we studied the effects of the 2DG treatment on the granularity of β cells by flow cytometry and islet-infiltrating lymphocytes. We previously showed that there are degranulated β cells in the islets of NOD mice with new onset disease that can recover with successful immune therapy (24). β cells from dissociated hand-picked islets were identified by staining with FluroZn and TMRE, and the granularity of the cells was assessed by side scatter. Degranulated β cells were found in the islets of 10–12-week-old NOD (Fig. 8a and Ref. 24). Treatment with 2DG reduced the islet infiltrating lymphocytes (red arrows; Fig. 8a) and improved the granularity of the β cells compared with the control mice (Fig. 8, a, blue and green arrows, b, p = 0.004, Mann-Whitney test). The effects of the 2DG were not due to direct effects of the drug on β cells because we did not find changes in β cells in NOD/scidγc−/− mice that
do not have immune cells (not shown). These results indicate that the 2DG-reduced cellular infiltrates into the islets and improved β cell function, consistent with its effects on decreasing the survival of diabetogenic T cells.

**Discussion**

Studies of the past two decades have identified diabetes antigen-specific T cells that can cause diabetes in NOD mice, and T cells in human studies have similar specificities (3, 6, 12–15). Most investigations of regulation of these cells have focused on immunologic ligands that initiate and regulate their activation, but little is known about the characteristics of the cells or the factors that lead to their pathogenicity. To address this, we studied diabetes antigen-specific CD8+ T cells that have previously been shown to mediate autoimmune diabetes in NOD mice (13). Our studies show that the diabetogenic cells can be identified at 10 weeks in the secondary lymphoid structures; their appearance corresponds to deterioration in glucose tolerance without overt hyperglycemia (24). The expression of activation markers (CD44 and CD69) and Tbet and Eomes is consistent with a memory Tc1 phenotype. CD8 T cells reactive with another diabetes antigen (insulin L9 peptide) showed a similar phenotype to the NRP-V7-reactive cells. The NRP-V7-reactive cells also shared some phenotypic features with LCMV-reactive cells including high expression of PD1, which with FOXO1, is thought to create a positive feedback pathway.
to desensitize cells to antigen but support their survival (18). A gene array analysis showed lower expression of genes associated with pathways of cellular proliferation and oxidative phosphorylation in the NRP-V7-reactive T cells compared with CD8+ T cells that were not diabetes antigen-specific or LCMV-reactive CD8+ T cells. Moreover, when we blocked glucose aerobic glucose metabolism in vivo with 2DG, there was reduced frequency of the NRP-V7-reactive cells and increased β cell granularity in vivo. They suggest an unrecognized relationship between nutrient availability and selection and expansion of diabetogenic T cells and suggest how elevated glucose may contribute to accelerated β cell killing.

Our analysis of autoantigen-specific CD8+ T cells is similar to previous reports by Trudeau et al. (25) and Chee et al. (31). Those investigators described the phenotype of the NRP-V7-reactive cells as effector memory T cells. In our analysis we have included the phenotype of insulin-reactive cells, which like the findings of Trudeau et al. (25), we find at a much lower frequency than NRP-V7-reactive cells. We have also differentiated the autoantigen-reactive T cells from those reactive with viral antigen (gp33) as well as the phenotype+ CD8+ T cells. The phenotype that we identified has differences from those described for human autoantigen-reactive cells. Skowera et al. (32) found that IGRP-reactive T cells in patients with T1D include naïve and effector cells. There was variability in CD45RO and CCR7 expression among these cells. In view of our recent observations that the time of maximal effector T cell function may be before the onset of hyperglycemia, the phenotype of the diabetes antigen-specific cells may change after diagnosis (33). Although it appears that there are differences in the phenotypes of IGRP-reactive T cells in humans and NOD mice, a prospective study of the phenotype of human diabetogenic T cells has not been reported.

The transcriptional features of murine or human autoantigen-reactive T cells have not previously been described and were not reported by Chee et al. (31). Our analysis showed that gene expression in the diabetogenic CD8+ cells is more consistent with MPEC than the LCMV-reactive or phenotype+ cells that were a mixture of cells but more closely terminally differentiated SLEC. MPEC have a less terminally differentiated phenotype, may self-renew, or may remain resting memory cells for long periods of time, which may account for the durability of the NRP-V7-reactive cells (34–37). Although the factors that lead to the differentiation of the NRP-V7-reactive cells into MPECs are not known, this observation may account for the prolonged kinetics of disease progression in contrast to the rapid occurrence of viral antigen responses in which the antigen specific cells show a SLEC phenotype.

The differences between the populations of CD8+ T cells have identified features that may be important in designing therapies. The differential (lower) expression of genes associated with oxidative phosphorylation distinguish the NRP-V7 cells from conventional MPECs. To carry out their function, effector T cells use metabolic pathways that are distinct from memory cells (30, 38–40). Memory T cells generally rely on mitochondrial oxidative phosphorylation involving fatty acid oxidation for their longevity. The processes that cause the switch in cellular metabolism are not clear, but the microenvironment and nutrient availability may be important factors (38). Changes in metabolic substrates (i.e. glucose) in diabetes presents a unique situation as it changes as a result of the effector function. In this way, there may be a reciprocal relationship between effector activity and the consequences of that activity that modulate the immune response, the differentiation of effector cells, and use of metabolic pathways. The utilization and dependence of the
diabetogenic T cells on aerobic glycolysis is also consistent with their ability to produce IFNγ and other cytolytic cytokines (38). In keeping with the array data we found an increase in the expression on Myc (1.35-fold, \( p = 0.06 \)) and a decreased expression of malic enzyme 2 (Me2) (0.75-fold, \( p = 0.03 \)) compared with phenotype+ cells. The transcription factor Myc has been found to control metabolic reprogramming upon T cell activation (41). The differences that we found in cell cycle gene expression were unexpected as effector T cells utilizing aerobic glycolysis would be expected to show higher rates of proliferation (38–40). Rather than an exclusive dependence on a single metabolic pathway, it is most likely that the NRP-V7-reactive and phenotype+ cells use both oxidative phosphorylation and aerobic glycolysis but to varying degrees and perhaps with differences between individual mice. Consistent with this notion is the variability in the time to diabetes within these inbred mice housed under identical conditions. Other metabolic pathways may also be utilized (38).

A curious finding from our studies is the dramatic and continued increase in the frequency of the NRP-V7-specific T cells beginning at 10 weeks with only modest changes after diagnosis of diabetes and elimination of β cell antigens. This time period
The percentage of degranulated $\beta$ cells was reduced by treatment with 2DG.

In summary, we have shown differences in gene expression of diabetogenic T cells compared with other cells that share their phenotype or are viral antigen-reactive. Our studies suggest mechanisms that may be used to prevent the expansion or even eliminate these pathologic cells.

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