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A human mutation in STAT3 promotes type 1 diabetes through a defect in CD8+ T cell tolerance

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Abstract:

Naturally occurring cases of monogenic type 1 diabetes (T1D) provide rare opportunities to establish direct mechanisms that cause this complex autoimmune disease. A recently identified de novo germline gain-of-function (GOF) mutation in the transcriptional regulator signal transducer and activator of transcription 3 (STAT3) was shown to cause neonatal T1D at birth. To investigate the role of STAT3 hyperactivity in T1D, we engineered a novel knock-in (KI) mouse incorporating this highly diabetogenic human mutation (K392R) in the STAT3 gene. These mice developed accelerated diabetes with severe insulinitis and insulin autoantibodies, thereby recapitulating the human autoimmune diabetes phenotype. Paired T cell receptor (TCR) and transcriptome (RNA) sequencing in single cells revealed that STAT3-GOF drives the proliferation and clonal expansion of highly cytotoxic effector CD8⁺ T cells that are resistant to terminal exhaustion. Single-cell ATAC-seq showed that these effector T cells are epigenetically distinct and revealed differential chromatin architecture induced by STAT3-GOF. Analysis of islet TCR clonotypes revealed an effector CD8⁺ T cell reacting against the known antigen IGRP, and STAT3-GOF in an IGRP-reactive TCR transgenic model demonstrated that STAT3-GOF intrinsic to CD8⁺ T cells is sufficient to accelerate diabetes onset. Taken together, these findings reveal a diabetogenic CD8⁺ T cell response that is restrained in the presence of normal STAT3 activity and drives diabetes pathogenesis.

Introduction

Type 1 diabetes (T1D) is caused by a breakdown of immune self-tolerance that leads to the T cell mediated destruction of pancreatic β cells and results in absolute insulin deficiency and hyperglycemia. Hallmarks of this autoimmune disease include the presence of insulinitis and islet autoantibodies against insulin, islet cell antigen (ICA), zinc transporter 8, and glutamic acid decarboxylase-65^{1, 2}. Genome-wide association studies (GWAS) show T1D is a polygenic disease with the majority of genetic risk attributable to human leukocyte antigen (HLA) alleles within the major histocompatibility complex (MHC) region³. Numerous other susceptibility alleles (e.g., *INS*, *CTLA4*, *STAT3*, *IL6R*)^{4, 5, 6, 7} have been identified from GWAS studies and help estimate individual risk, but ultimately additional functional studies are required to elucidate the cellular and molecular basis of these associations with disease. Naturally occurring cases of monogenic T1D, although rare (i.e., loss-of-function mutations in *Autoimmune Regulator* (*AIRE*) and *FOXP3*), have been most informative for understanding how central and peripheral mechanisms of immune tolerance break down and for suggesting new opportunities for therapeutic interventions⁸.

Recent large scale sequencing efforts in genetics have identified naturally occurring gain-of-function (GOF) mutations in the gene *signal transducer and activator of transcription 3* (*STAT3*) as a novel cause of monogenic diabetes^{9, 10}. However, it remains unclear how excessive STAT3 signaling leads to T1D. One challenge in studying STAT3 stems from its ubiquitous expression and important functions in diverse cell types. Within the immune system, the Janus kinase (JAK)-STAT signaling pathway regulates transcription in response to extracellular cues from cytokines. STAT3 is a transcription factor that regulates the expression of genes associated with cell survival, proliferation, activation, and differentiation¹¹. Outside of the immune system and directly within pancreatic cells, STAT3 plays a role in islet development as well as insulin secretion^{10, 12, 13}. Previous work has proposed multiple conflicting hypotheses regarding the relevant cell types involved in the development of diabetes caused by STAT3-GOF, such as an islet-intrinsic effect or perturbing the Treg / Th17 balance within the CD4⁺ T cell compartment^{12, 14, 15}. Observational studies have nominated a role for STAT3 in even more cell types; profiling peripheral blood in subjects with T1D has demonstrated changes in STAT3-dependent pathways including increased Th17 and TFh cells in new-onset T1D^{16, 17}, and IL-6 induced phosphorylation of STAT3 is significantly increased in CD4⁺ and CD8⁺ T cells of patients with T1D¹⁸. Additional studies of human diabetogenic STAT3-GOF mutations in a physiological context are

needed to resolve these results and establish the molecular and cellular link between STAT3 GOF mutations and T1D pathogenesis *in vivo*.

In this report, we generated STAT3 knock-in (KI) mice (STAT3^{+/K392R}) carrying a single copy of the highly activating and diabetogenic missense mutation resulting in a substitution of arginine for lysine at amino acid 392 in the STAT3 DNA binding domain (K392R), which was associated with neonatal T1D in an infant at birth^{9, 15}. Using this novel Mendelian mouse model, we performed a battery of phenotypic, biochemical, and functional analyses of the human STAT3-GOF mutation. Adoptive cell transfers and mouse genetic studies demonstrated a role for the mutation within the hematopoietic system, and specifically within CD8⁺ T cells. We found that the mutation induces an unbridled diabetogenic CD8⁺ T cell response, which we characterize using single cell transcriptomic, epigenetic, and T cell repertoire profiling. We determined that while CD8⁺ T cell function within the islets is typically restrained by T cell exhaustion, CD8⁺ STAT3^{+/K392R} T cells are resistant to terminal exhaustion and are maintained in a highly cytotoxic state. We show that STAT3-GOF only within CD8⁺ T cells is sufficient to accelerate T1D *in vivo*, thus demonstrating a cell-intrinsic role for the K392R mutation in CD8⁺ T cells and establishing a defect in CD8⁺ T cell exhaustion as a contributor to T1D development.

Results

Generation of a murine model of the human STAT3 K392R mutation

To investigate the role of STAT3 hyperactivity in T1D, we took advantage of CRISPR/Cas9 editing to engineer a novel knock-in (KI) mouse on the non-obese diabetic (NOD) background incorporating the highly diabetogenic GOF human mutation (K392R) in the STAT3 gene (**Fig. 1a**). To confirm that the STAT3-activating behavior of this mutation is conserved in mice, naïve CD4⁺ T cells were isolated from lymphoid organs and differentiated *in vitro* into Th17 and Treg subsets. The STAT3^{+/K392R} T cells exhibited increased differentiation of Th17 cells relative to the WT T cells while differentiation into Treg cells was reduced (**Fig. 1b**), which is consistent with gain-of-function behavior as previously described in humans^{19, 20}.

Heterozygous K392R mice recapitulate the human autoimmune diabetes phenotype

We then assessed whether STAT3^{+/K392R} mice exhibited the severe diabetes phenotype observed in humans. Both male and female STAT3^{+/K392R} mice developed diabetes more rapidly and with higher incidence than their WT siblings in both sexes (**Fig. 1c**). To control for potential off target effects of gene editing, we used two independent founder lines and confirmed this diabetic phenotype in the other founder line as well (data not shown); therefore, we selected one founder line to do subsequent experiments. Young, non-diabetic mice showed normal beta cell function during glucose tolerance testing (**Extended Fig. 1a**), and immunofluorescence of STAT3^{+/K392R} islets showed a normal distribution of alpha and beta cells within the islets (**Extended Fig. 1b**) suggesting the K392R mutation did not interfere with beta cell development or function as previously suggested¹². Rather, diabetes onset coincided with the presence of insulin autoantibodies (**Extended Fig. 1c**) and insulinitis (**Fig. 1d** and **Extended Fig. 1d**) with a rapid infiltration of B and T cells at disease-onset (**Fig. 1e**), supporting this mouse model's recapitulation of the autoimmune diabetes phenotype observed in human patients^{9, 15}.

Effector T cells drive STAT3-GOF autoimmune diabetes

To understand the cellular compartment driving the observed autoimmunity, immunophenotyping, genetic crosses and adoptive transfer experiments were performed. Given the time course of insulinitis development and diabetes incidence (**Fig. 1c, d**), we selected 6-10 weeks as the age for studying the onset of autoimmunity in peripheral lymphoid tissues and within the islets. We observed lymphoproliferation grossly by increased lymphoid organ size (**Extended Fig. 1e**) and absolute lymphocyte cell counts within these organs of mice 6-8 weeks of age (**Extended Fig. 1f**). Immunophenotyping of peripheral lymphocytes *ex vivo* by flow cytometry showed STAT3^{+/K392R} was associated with a small increase in Th17 cells but a more marked increase in Th1 cells and an increase in Tregs (**Fig. 2a**). In addition, STAT3^{+/K392R} mice had expansions of their CD8⁺ effector memory and CD4⁺ memory cellular compartments (**Fig. 2a**).

Given the expansion of the T cell compartment, we asked whether the adaptive immune compartment was necessary to induce diabetes by crossing STAT3^{+/K392R} mice to immunodeficient NOD.Rag1^{-/-} mice, which lack mature B and T cells. STAT3^{+/K392R} RAG1^{-/-} mice were completely protected from diabetes (**Fig. 2b**) and had no insulinitis, which confirmed the dependence on T and/or B cells in driving this disease. To further refine which compartment was essential in diabetes development, we investigated the role of the MHC in the STAT3^{+/K392R} diabetes. Similar to the

HLA haplotype DR3/4 which confers the majority of T1D risk in humans, wildtype NOD mice have the high-risk MHC haplotype H2^{g7}, which is essential for diabetes development. Thus, to determine whether STAT3^{+/K392R} diabetes was MHC-dependent, STAT3^{+/K392R} mice were crossed to the NOD.H-2^b strain, which carries an alternate MHC haplotype known to be protective from diabetes. NOD.H-2^b STAT3^{+/K392R} mice were completely protected from diabetes (**Fig. 2b**) and had no insulinitis upon histologic examination (data not shown) showing diabetes in STAT3^{+/K392R} mice was an MHC-dependent disease.

The MHC-dependence suggested that diabetes in STAT3^{+/K392R} mice was mediated through changes either in the MHC-dependent antigen expression in target pancreatic tissue or in the MHC-mediated activation of T cells involved in beta-cell destruction. To establish whether the key driver of T1D was in the T cell compartment or the tissue itself, bone marrow chimeras were generated by adoptively transferring WT or STAT3^{+/K392R} bone marrow into lethally irradiated WT mice. STAT3^{+/K392R} bone marrow resulted in accelerated diabetes compared to WT bone marrow (**Fig. 2c**). This result combined with the MHC-dependence showed STAT3^{+/K392R} induced autoimmune diabetes via a T cell mediated mechanism.

Previous work in human subjects with STAT3-GOF disease has suggested dysfunctional Treg cells as a potential cause of autoimmunity¹⁵. Therefore, we next assessed the suppressive activity of STAT3^{+/K392R} Treg cells *in vivo*. Adoptive transfer of islet-antigen-specific Tregs from BDC2.5 mice, which carry a CD4-specific TCR transgene that targets a pancreatic antigen derived from chromogranin A, are effective at preventing T1D in the NOD mouse model^{21, 22, 23}, and we utilized this model of Treg suppression by crossing BDC2.5 mice to the STAT3^{+/K392R} line. To specifically examine the role of the STAT3 mutation in Tregs, we performed adoptive transfer of naïve WT BDC2.5+ T cells into immunodeficient Rag1^{-/-} mice. This resulted in rapid development of diabetes that was ameliorated by both WT BDC2.5+ and STAT3^{+/K392R} BDC2.5+ Treg cells (**Extended Fig. 1g**). Therefore, STAT3^{+/K392R} did not significantly impair Treg function, which implicated Teff cells as key drivers of diabetes development due to the STAT3^{+/K392R} mutation.

T cell expansion is not due to priming by STAT3^{+/K392R} APCs

We next investigated whether the observed Teff expansion may be cell-intrinsic or due to improved T cell priming by STAT3^{+/K392R} in APCs and/or the local islet environment. We performed *ex vivo* immunophenotyping by flow cytometry of 8 week old nondiabetic STAT3^{+/K392R} APCs within the spleen and found STAT3^{+/K392R} did not significantly alter population frequencies of monocytes, pre-DCs or DCs (**Fig. 3a**). We next immunophenotyped APCs directly within the islets using mass cytometry, and observed the expression of classical molecules needed for antigen presentation to T cells, MHC class II and CD86, also appeared undisturbed in 8-10 week old nondiabetic mice (**Fig. 3b**). We then performed transcriptional profiling of the infiltrating immune cells using scRNA-seq of CD45⁺ lymphocytes isolated from islets of 8-10 week old nondiabetic mice. We obtained high quality scRNA profiles for 20,361 high quality single cells from STAT3^{+/K392R} and WT mice. Cells were clustered and then visualized using Seurat (**Extended Fig. 2a**), followed by a reclustering of only the CD11c⁺ APCs (**Fig. 3c**) for further analysis. Expression of canonical immune cell markers were computed and used to uniquely identify the six cell populations represented by each cluster, which included pDCs, cDCs, macrophages and monocytes (**Extended Fig. 2b**). Overall, cluster population frequencies were similar between STAT3^{+/K392R} and WT cells (**Fig. 3d**), consistent with our flow cytometry data (**Fig. 3a**). Corroborating our mass cytometry data (**Fig. 3b**), expression of antigen presenting genes *Cd86* and MHC-II (*H2-Ab1*) appeared comparable (**Fig. 3e**) among the different APC populations. Altogether, these results supported phenotypic similarity between STAT3^{+/K392R} and WT APCs.

Finally, we performed a functional experiment to test whether STAT3^{+/K392R} in APCs and/or the local islet environment was priming T cells and driving their increased proliferation. Naïve CD4⁺ BDC2.5Tg⁺ cells were isolated by FACS from non-diabetic BDC2.5Tg⁺ donors, labeled with CTV to track cellular activation and proliferation, and adoptively transferred into STAT3^{+/K392R} or WT hosts. Proliferation was evaluated 3.5 days after cell transfer by FACS analysis of the CTV dye dilution in the pLNs and iLNs (control) of recipient mice, and there was no evidence to support the local islet environment as a driver of T cell priming and activation (**Fig. 3f**). Overall, the lack of evidence to support an effect of STAT3^{+/K392R} on the APC compartment suggested the diabetogenic effect of STAT3^{+/K392R} was intrinsic to the Teff cells rather than their surrounding environment.

STAT3-GOF upregulates chemotactic and cytotoxic gene expression in CD8⁺ T cells

To better understand the molecular mechanisms of STAT3^{+/K392R} within Teff cells, we performed paired transcriptome and T cell receptor sequencing in single cells (scRNA / TCR-seq) from CD45⁺ lymphocytes isolated from islets of the 8-10 week old nondiabetic mice used in the earlier scRNA-seq experiment (**Extended Fig. 2a**). A total of 8,725 CD3⁺ T cells were reclustered and used for further analysis (**Fig. 4a**). Differentially expressed marker genes for each cluster were computed (**Extended Fig. 2c**) along with canonical marker gene expression (**Extended Fig. 2d, e**) and used to uniquely identify the cell populations represented by each T cell cluster. Comparisons of the STAT3^{+/K392R} vs WT clusters showed a marked expansion of effector CD8⁺ T cells and a corresponding decrease in naïve CD8⁺ T cells (**Fig. 4a**). Notably, no increase in Treg or Th17 cell populations in these islet infiltrates was observed. Differential gene expression comparing the STAT3^{+/K392R} vs WT CD8⁺ T cells showed STAT3^{+/K392R} drove a highly cytotoxic CD8⁺ T cell phenotype with upregulation of genes involved in chemotaxis (e.g., *Ccl4* and *Ccl5*) and cytotoxicity (e.g., *Gzma*, *Gzmb*, *Gzmk*) (**Fig. 4b**). To confirm these findings at the protein level, we performed flow cytometry and found that STAT3^{+/K392R} CD8⁺ T cells isolated from the pancreatic lymph node showed increased Ccl5 expression relative to WT (**Fig. 4d**), and CD8⁺ T cells from the spleen showed increased Granzyme A and Granzyme B protein expression (**Extended Fig. 2f**).

To provide deeper characterization of the expanded STAT3^{+/K392R} CD8⁺ T cells, a total of 2,034 CD8⁺ T cells were reclustered for further analysis (**Fig. 4d**). Clustered cell populations were identified by their expression of canonical CD8 markers (**Fig. 4e**), in addition to using module scores to distinguish terminally exhausted T cells (*Cd101*, *Cd200r2*, *Cd7*, *Cd200r1*, *Il10*) and transitory T cells (*Cx3cr1*, *Klrg1*, *Il2ra*, *Il18rap*, *Slpr5*) (**Fig. 4f**), as previously described²⁴. Focusing on the expanded non-naïve cell populations, STAT3^{+/K392R} cells exhibited a bias towards the transitory (vs terminally) exhausted CD8⁺ T cell phenotype (**Fig. 4g**) with STAT3^{+/K392R} transitory:terminally exhausted ratio 2.5 times that of WT. As the gene signatures of the effector and transitory clusters (**Fig. 4h**) closely matched that of the differential gene expression seen across CD8 T cells in aggregate (**Fig. 4b**), it suggested the phenotypic difference in CD8 T cells caused by STAT3^{+/K392R} was driven by an increased proportion of transitory cells with a cytotoxic gene expression profile, while WT cells preferentially exhibited a terminally exhausted phenotype.

STAT3-GOF epigenetically regulates chemotactic and cytotoxic genes in effector CD8⁺ T cells

In parallel to our single cell transcriptomic approach, we also used single cell epigenetics to examine the chromatin state in infiltrating immune cells in the islets. We performed scATAC-seq on CD45⁺ infiltrating islet cells of 8-10 week old nondiabetic mice similar to those used in the scRNA-seq studies and obtained high-quality ATAC-seq profiles from 17,466 single cells with a median number of 5,610 fragments per cell and a median enrichment of Tn5 insertions in transcription start sites for 17.11 (**Extended Fig. 3**). To identify cell-types and determine a correspondence between the scRNA-seq clusters and the scATAC-seq clusters we used gene scores, which are computed by aggregating the ATAC-seq signal across the gene body and promoter in each cell (**Fig. 5a**). By analyzing the corresponding clusters, we were able to link our gene expression findings with epigenetic changes in the same cell types. There were 197 peaks with significantly increased chromatin accessibility whose nearest gene was one of the 129 significantly upregulated genes ($\log FC \geq 0.25$ and $\text{padj} \leq 0.05$, **Fig. 5b, c**). Key molecules characteristic of cytotoxic CD8⁺ T cells, including *Ccl5*, *Klrg1*, and *Gzma*, had multiple nearby significant peaks (**Fig. 5b**), indicating epigenetic regulation of the effector T cell gene expression program induced in STAT3^{+/K392R} compared to WT mice. We next sought to determine which transcription factors might be responsible for the observed epigenetic remodeling in the CD8⁺ T cell compartment. Comparing transcription factor motif accessibility between STAT3^{+/K392R} and WT CD8⁺ T cells using chromvar²⁵ identified 406 motifs with significantly different activity ($\text{FDR} \leq 0.1$, $|\text{mean difference}| \geq 0.01$). Eomes and Tbx21 were among the significantly more accessible transcription factors and have well established roles in effector T cell development and function²⁶ (**Fig. 5d, e**). Transcription factors involved in programming tissue residency in CD8⁺ T cells were among the most significantly different, in particular the increased accessibility of Runx3²⁷ and decreased accessibility of Klf2²⁸ (**Fig. 5d, e**). In sum, STAT3^{+/K392R} leads to increased activity of TFs involved in tissue infiltration and cytotoxic gene expression programs in CD8⁺ effector T cells.

STAT3-GOF in diabetogenic CD8⁺ T cells are sufficient to accelerate T1D

Finally, we sought to establish whether these STAT3^{+/K392R} effector CD8⁺ T cells were directly responsible for the autoimmune diabetic phenotype. We analyzed the TCR repertoire data obtained from the scRNA / TCR-seq experiment to characterize the specificity of the infiltrating CD8⁺ T cells and determine whether they were indeed directly diabetogenic. We recovered high-confidence TCR sequences for 91% of T cells (**Extended Fig. 4a**). We used the Gini index to quantify the extent of TCR clonal expansion within each cluster and found the STAT3^{+/K392R}

effector CD8⁺ T cell cluster exhibited increased clonal expansion relative to WT (**Extended Fig. 4b**) suggesting that these cells were recognizing and proliferating in response to islet antigens. We then analyzed the top 20 most abundant CD8⁺ TCR clones and identified Clone 6158, which was present in both STAT3^{+/K392R} and WT mice (**Fig. 6a**). This specific clone contained CDR3 sequences nearly identical to those found in the TCR-8.3 (**Fig. 6b**), a CD8-restricted TCR specific for the islet-specific glucose-6 phosphatase-related protein [IGRP] antigen that is known to be diabetogenic in NOD mice (8.3Tg⁺)²⁹. Since this clone was shared between the two genotypes, we crossed the 8.3Tg⁺ mouse with the STAT3^{+/K392R} line and assessed diabetes incidence to isolate whether the underlying transcriptional and epigenetic changes were key drivers underlying the STAT3^{+/K392R} diabetogenic effect. Consistent with a STAT3-GOF intrinsic effect on CD8⁺ T cells, STAT3^{+/K392R} 8.3Tg⁺ mice became rapidly diabetic and with a much higher diabetes incidence than WT 8.3Tg⁺ (**Fig. 6c**).

To then test whether STAT3-GOF in diabetogenic CD8⁺ T cells was sufficient to accelerate T1D, we used an established adoptive transfer model in which polyclonal CD4⁺ T cells are transferred with naïve 8.3-TCR transgenic CD8⁺ T cells and start inducing diabetes within a month of transfer into NOD.SCID recipients^{30, 31, 32}. NOD.SCID mice adoptively transferred with polyclonal WT CD4⁺ T cells and naïve CD8⁺ T cells from STAT3^{+/K392R} 8.3Tg⁺ mice experienced significantly accelerated diabetes onset compared to mice who instead received their naïve CD8⁺ T cells from WT 8.3Tg⁺ mice (**Fig. 6d**). As no other immune populations were present in the recipient NOD.SCID mice, this experiment established STAT3-GOF in diabetogenic CD8⁺ T cells is sufficient to drive the autoimmune diabetic phenotype. We also performed adoptive transfer experiments to understand whether CD4⁺ cells might also be playing a key role in STAT3-GOF diabetes and did not find evidence to support this. First, naïve CD8⁺ cells from WT 8.3Tg⁺ mice transferred with polyclonal CD4⁺ cells from either STAT3^{+/K392R} or WT mice into NOD.SCID mice did not cause a noticeable difference in diabetes incidence, which suggested the diabetogenic effect of STAT3^{+/K392R} was likely intrinsic to its role in CD8⁺ T cells rather than an indirect result of CD4 help to the CD8⁺ population (**Extended Fig. 4c**). Second, the adoptive transfer of either WT BDC2.5⁺ or STAT3^{+/K392R} BDC2.5⁺ CD4⁺ Teff cells into NOD.Rag1^{-/-} mice did not result in differing diabetes incidences, suggesting CD4⁺ cells alone were insufficient drivers of the accelerated diabetic phenotype (**Extended Fig. 4d**). Altogether, these findings showed effector CD8⁺ cells were the key drivers of STAT3-GOF diabetes, and in combination with our

molecular data, established a direct mechanism used by STAT3 to promote T1D through a defect in CD8+ T cell tolerance.

Discussion

In sum, we generated a novel Mendelian model of monogenic autoimmune diabetes based on a human STAT3-GOF mutation, identified how it caused an unbridled autoreactive effector CD8+ T cell response at the epigenetic and transcriptomic levels, and confirmed that these findings are sufficient to accelerate T1D *in vivo*, thus establishing a novel direct pathway involved in T1D pathogenesis. Our findings are surprising given prior assumptions from *ex vivo* and *in vitro* studies where STAT3-GOF caused T1D via Th17/Treg imbalance^{14, 15} or an islet intrinsic defect¹², and highlights the value of *in vivo* study of human mutations to uncover mechanisms underlying diseases.

However, our findings are also consistent with STAT3's known role in driving formation of memory CD8+ T cells³³, which have a highly proliferative potential and long-term survival, consistent with the CD8+ T cell expansions we observed in our STAT3-GOF mouse model in the secondary lymphoid organs and islets. STAT3 is required to sustain the expression of EOMES³⁴, another key transcription factor involved in the effector to memory cell transition and which we observed had more motif activity in our ATAC-seq data. Because the mutation we studied is in the DNA binding domain, we speculate STAT3-GOF is inducing an opening of chromatin regions within activated CD8+ T cells that are associated with chemotaxis (e.g., *Ccl4* and *Ccl5*), cytotoxicity (e.g., *Gzma*, *Gzmb*, *Gzmk*), and memory homeostasis (e.g., *Tbx21* and *Eomes*), which leads to a more diabetogenic CD8+ T cell population.

Additionally, our findings suggest STAT3^{+K392R} causes excessive CD8 T cell effector activity by impeding the development of terminal exhaustion and instead retaining CD8 effector cells in a highly cytotoxic (and typically transitory) phenotype. It has been shown CD8 exhaustion follows a spectrum³⁵, with different exhausted CD8 T cell subsets retaining different levels of effector functions^{36, 37, 38}. The transitory CD8 population, similar to Tex^{int} previously described³⁵, carries more proliferative capacity and effector-like function (e.g., upregulation of genes such as *Tbx21* and *Gzmb*) compared to terminally exhausted CD8 cells that are less functional²⁴. This aligns with our findings of an expanded CD8 T cell compartment containing a similar cytotoxic profile, and which subsequently induces acceleration of diabetes onset. The transitory population also expands in response to PD-1 pathway

blockade and is thought to play a critical role in therapeutic response to checkpoint blockade used in cancer immunotherapy^{24, 35}. Separately, it has been shown that T cell exhaustion is important in determining autoimmune disease outcomes³⁹ and PD-1 deficient NOD mice develop accelerated diabetes^{40, 41}. Our findings help bridge these important sets of knowledge by expanding the role of transitory exhausted T cells to autoimmune disease and T1D. This is consistent with recent observations from studying T1D progression in humans showing individuals with a higher activated transitional memory CD8 phenotype tended to progress more rapidly to T1D, while individuals with a more terminally exhausted CD8 phenotype had a slower rate of T1D progression⁴². Our results also align with the positive associations between exhausted CD8+ T cells and T1D immunotherapy treatment response during landmark T1D prevention and reversal trials using the anti-CD3 monoclonal antibody teplizumab^{43, 44}. This underscores how using a mouse model rooted in a human mutation can provide cellular and molecular insights into the determinants of response in human T1D clinical trials. In addition, this work may help further our understanding of how unintended autoimmune toxicities, such as T1D, result from cancer immunotherapies and how we might develop strategies to prevent them⁴⁵. Altogether, our new model has allowed us to determine how this transitory state may be a key tolerance pathway that keeps T1D in check.

Historically, T1D development has been studied in the context of central tolerance mechanisms (via the study of human *AIRE* mutations) and peripheral suppressor mechanisms (via the study of human *FOXP3* mutations). Therefore, our findings fill an important void necessary for understanding this complex autoimmune disease by establishing that CD8+ T cell tolerance also plays a key role in T1D immunopathogenesis^{46, 47, 48}. Our studies support an important protective role for CD8 T cell exhaustion in T1D and suggest modulating CD8 T cell function may be a therapeutic opportunity for the prevention and treatment of human T1D.

Figure 1. STAT3^{+K392R} mutant mice recapitulate the human type 1 diabetes phenotype | **a**, STAT3^{K392R} mutation located in the DNA binding domain was inserted into WT (i.e., NOD) mice using CRISPR/Cas9 and confirmed by Sanger sequencing **b**, *In vitro* CD4 differentiation of naïve CD4⁺ T cells into Th17 and Treg subsets. **c**, Diabetes incidence curves (Females: WT, n=93; STAT3^{+K392R}, n=125; Males: WT, n=19; STAT3^{+K392R}, n=68) **d**, Time course of insulinitis in WT and STAT3^{+K392R} mice with and without diabetes (n=3-11 mice per gender per time point) **e**, viSNE plots of islets using mass cytometry compare immune and endocrine cellular compositions between a WT mouse without diabetes and littermate STAT3^{+K392R} mouse with recently-diagnosed diabetes to highlight the presence of immune infiltration with STAT3^{+K392R} diabetes. Results representative of viSNE plots from 8-14 week old non-diabetic WT (n=6) and diabetic STAT3^{+K392R} (n=4) mice. Error bars indicate mean $\pm$ SD. ns = non-significant (P>0.05), * P $\leq$ 0.05, ** P $\leq$ 0.01, *** P $\leq$ 0.001.

Figure 2. STAT3^{+/K392R} effector T cell compartment **a**, Immunophenotyping of splenocytes *ex vivo* in 8 week old male mice (n=4 per group) **b**, Diabetes incidence in STAT3^{+/K392R} RAG1^{-/-} mice (B- and T-cell dependence) and STAT3^{+/K392R} H2^{b/b} mice (MHC-dependence) (STAT3^{+/K392R} females, n=132; STAT3^{+/K392R} males, n=70; STAT3^{+/K392R} RAG1^{-/-}, n= 9; STAT3^{+/K392R} H2^{b/b}, n= 20) **c**, Experimental design and subsequent diabetes incidence in bone marrow chimeras – lethally irradiated WT recipients adoptively transferred with bone marrow from WT (n=18) or STAT3^{+/K392R} (n=17) mice. Error bars indicate mean $\pm$ SD. ns = non-significant (P>0.05), * P $\leq$ 0.05, ** P $\leq$ 0.01, *** P $\leq$ 0.001.

Figure 3. STAT3^{+/K392R} APC Phenotype and T-cell priming by the local islet environment is unchanged | a, Flow cytometry gating (values shown are frequency relative to parent gate) and population frequencies of APC subpopulations from splenocytes isolated *ex vivo* in non-diabetic 8 week old male mice (n=4 per group) **b**, Representative histograms for MHCII and CD86 protein expression on conventional DCs (CD11c+MHC II+) and classical monocytes (CD11b+Ly6c+) in the islets of non-diabetic 8-10 week old mice using mass cytometry. Performed in 3-4 mice per group **c**, UMAP projection of reclustered antigen-presenting myeloid cells (CD11c+ cells) from islet immune infiltrate used in scRNA-seq experiment (see **Extended Fig. 2a**) **d**, UMAP projection showing distribution of STAT3^{+/K392R} and WT cell transcriptomes **e**, Violin plots showing gene expression profile of *MHCII* and *CD86* in APC myeloid clusters **f**, CTV profile among BDC2.5 CD4+ T cells in pancreatic and inguinal (control) lymph nodes (pLN and iLN, respectively) 3.5 days after adoptive transfer into STAT3^{+/K392R} and WT recipients. Cell proliferation was evaluated by FACS analysis of the CTV dilution in the pLNs and iLNs of recipient mice and results are expressed as the percentage of proliferating cells within the recovered CTV-labeled BDC2.5 CD4+ T cells. Representative of three sets of STAT3^{+/K392R} / WT recipient mice. Error bars indicate mean +/- SD. ns = non-significant (P>0.05).

Figure 4. STAT3^{+/K392R} drives expansion of the CD8⁺ effector compartment with upregulation of cytokine and chemokine genes | **a**, UMAP projection of reclustered T cells (CD3⁺ cells) from islet immune infiltrate used in scRNA-seq experiment (see **Extended Fig. 2a**) with associated cell counts per cluster and comparison of cluster frequencies between the two genotypes (STAT3^{+/K392R} vs WT) **b**, Volcano plot showing STAT3^{+/K392R} vs WT differential gene expression in CD8⁺ clusters. Log₂ fold cutoff 0.25 used. Genes of interest are labeled. **c**, Flow cytometry of Ccl5 protein expression in CD8⁺ memory T cells from the pancreatic lymph node in nondiabetic males at 14 weeks (n=3 per group). Error bars indicate mean $\pm$ SD, ns = non-significant (P>0.05), * P $\leq$ 0.05. **d**, UMAP projection of reclustered CD8⁺ T cells **e-f**, Marker gene or module score expression in CD8⁺ T cell clusters. In **f**, genes used to calculate module scores for terminally exhausted T cells were *Cd101*, *Cd200r2*, *Cd7*, *Cd200r1*, *Il10* and for transitory T cells were *Cx3cr1*, *Klrg1*, *Il2ra*, *Il18rap*, *Slpr5*, as previously described²⁴ **g**, Relative population frequencies among non-naïve CD8⁺ cell clusters **h**, Volcano plot showing differential gene expression in CD8⁺ effector and transitory cell clusters relative to all CD8⁺ cells.

Figure 5. STAT3^{+/K392R} increases chromatin accessibility within cytotoxic CD8⁺ loci resulting an epigenetically distinct state | **a**, UMAP projection of scATAC-seq profiles of T cells (CD3⁺) subclustered from CD45⁺ islet infiltrates of 8-10 week old male non-diabetic mice, WT (n=3, pooled) vs STAT3^{+/K392R} (n=3, pooled) | **b**, Normalized pseudobulk ATAC-seq tracks of CD8⁺ T cell clusters split by genotype around genes identified as upregulated in corresponding scRNA-seq CD8⁺ T cell clusters. Peaks significantly upregulated (from c) are shown in red | **c**, Volcano plot showing differential peaks whose nearest gene is present in the significantly upregulated genes (**Fig. 4b**) between CD8⁺ T cell clusters (STAT3^{+/K392R} vs WT) | **d**, Volcano plot showing differential transcription factor motifs between CD8⁺ T cell clusters (STAT3^{+/K392R} vs WT) | **e**, Transcription factor motif activity for CD8⁺ T cell clusters split by genotype. Motif sequence shown above each violin plot.

Figure 6. STAT3^{+/K392R} drives clonal expansion of diabetogenic CD8⁺ T cells | **a**, Top 20 clones by count in each sample **b**, Top 10 specific clones with corresponded CDR3 sequences displayed in table. The CDR3 sequence for Clone 6158 in red is nearly identical to that of the CD8-restricted TCR specific for islet specific antigen IGRP (TCR-8.3, sequence shown below table). The only difference between Clone 6158 and TCR-8.3 is a serine, denoted in blue, replaces an alanine of the TCR beta chain **c**, Diabetes incidence of 8.3Tg⁺ (IGRP-specific TCR) mice with (n=7) and without (n=11) STAT3^{+/K392R} confirms increased diabetogenicity **d**, Diabetes incidence after adoptive transfer of polyclonal WT CD4⁺ T cells with naïve CD8⁺ T cells from 8.3Tg⁺ mice with (n=5) and without STAT3^{+/K392R} (n=10) into NOD.SCID mice. Results are pooled from 2 independent cohorts.

Extended Data Figure 1. Phenotyping of STAT3^{+/K392R} mouse model. | **a**, Intraperitoneal glucose tolerance testing of young (<8 weeks of age) WT (n=11) vs STAT3^{+/K392R} (n=8) mice. **b**, Immunofluorescence of islet in STAT3^{+/K392R} Rag1^{-/-} showing normal distribution of alpha cells (glucagon+) and beta cells (insulin+). Scale bar, 100µm. **c**, Prevalence of insulin autoantibodies (IAA) in nondiabetic mice 4-8 weeks of age (Females: WT, n=20; STAT3^{+/K392R}, n=20; Males: WT, n=17; STAT3^{+/K392R}, n=23). **d**, H&E of pancreas from diabetic STAT3^{+/K392R} mouse at 13 weeks showing severe insulinitis. Scale bar, 100µm. Yellow arrows indicate dense lymphocytic infiltrate into the islet. **e**, Lymphoproliferation in non-diabetic female littermates at 6 weeks - spleens and pancreatic lymph nodes as representative examples **f**, Absolute cell counts in pancreatic lymph node of WT (n=6) vs STAT3^{+/K392R} (n=6) non-diabetic mice at 7-8 weeks, p=0.006. **g**, *In vivo* Treg suppression assay showing diabetes incidence after adoptive transfer of WT CD4⁺ BDC2.5⁺ Teff cells without (n=6) and with Tregs from WT (n=5) or STAT3^{+/K392R} (n=3) BDC2.5⁺ mice into NOD.Rag1^{-/-} mice. Results pooled from 2 independent cohorts. Error bars indicate mean +/- SD. ns = non-significant (P>0.05), * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

Extended Data Figure 2. scRNA-seq of infiltrating islets additional cluster characterization. | **a**, UMAP projection of scRNA-seq profiles of cells isolated from CD45+ islet infiltrates of 8-10 week old male non-diabetic mice, WT (n=3, pooled) vs STAT3^{+/K392R} (n=3, pooled) **b**, Summary of marker genes used to identify APC clusters. **c**, Additional marker gene characterization for the scRNA-seq T cell clusters **d**, Expression of selected genes visualized for each T cell **e**, Summary of marker genes used to identify T cell clusters. **f**, Additional flow cytometry data on GzmA and GzmB protein expression for both genotypes.

Extended Data Figure 3. scATAC-seq of infiltrating islets additional cluster characterization | **a**, aggregated scATAC-seq fragments exhibit the nucleosome periodicity characteristic of high-quality ATAC-seq libraries **b**, Summary of cell calling and library quality control.

a

b

Extended Data Figure 4. Additional characterization of single-cell TCR data from infiltrating islets and functional experiments | **a**, TCR expression in UMAP clusters from subset of CD3⁺ T cells identified in scRNA-seq analysis **b**, Gini index showing clonal expansion in STAT3^{+/K392R} broken down by cluster and genotype **c**, Diabetes incidence after adoptive transfer of naïve CD8⁺ T cells from WT 8.3Tg⁺ mice with polyclonal CD4⁺ T cells from mice without (n=10) and with STAT3^{+/K392R} (n=5) into NOD.SCID mice **d**, Diabetes incidence after adoptive transfer of CD4⁺ BDC2.5⁺ Teff cells from mice without (n=6) and with STAT3^{+/K392R} (n=9) into NOD.Rag1^{-/-} mice. Results pooled from 2 independent cohorts.

Methods:

Mice

The single nucleotide variant 1454 A>G of NM_011486.3 identified in a STAT3 gain-of-function patient was inserted using CRISPR/Cas9 genome editing by The Jackson Laboratory in a custom request⁴⁶. Two founder lines were used for experiments to minimize the risk of off-target effects. Additionally, The Jackson Laboratory provided other mice used in these experiments: NOD (Jax #001976), NOD.RAG1^{-/-} (Jax #003729), NOD.SCID (Jax #001303), NOD.H2^{b/b} (Jax #002591), NOD.BDC2.5+ (Jax #004460), NOD.8.3TCR+ (Jax # 005868)⁴⁶, unless otherwise specified in methods.

Mice were maintained in the University of California San Francisco (UCSF) specific pathogen-free animal facility in accordance with the guidelines established by the Institutional Animal Care and Use Committee (IACUC) and Laboratory Animal Resource Center and all experimental procedures were approved by the Laboratory Animal Resource Center at UCSF. Mice aged 4–8 weeks were used for all experiments unless otherwise specified in the text or figure legends. Mice were age-matched in figures displaying a single representative experiment and in pooled data.

Lymphocyte Isolation, Cell Sorting, and Flow cytometry

LN and spleen were isolated by dissection from mice and then mashed through a 70-µm filter. Spleen cells were lysed in ammonium-chloride-potassium lysis buffer to remove red blood cells. Cells isolated from spleen and LNs were counted, and $1-5 \times 10^6$ cells were first stained in PBS and Ghost Live/Dead (Tonbo), followed by blocking in 2.4G2 before staining with the appropriate antibodies for flow cytometry. For transcription factor staining, cells were fixed overnight in the eBioscience Foxp3/Transcription Factor/Fixation-Concentrate kit (Thermo Fisher Scientific). After fixation, cells were permeabilized and stained with the appropriate antibodies. For ICS, cells were stimulated for 4 h in Brefeldin A (eBioscience) and eBioscience Cell Stimulation Cocktail (500×). Cells were then fixed and permeabilized using the BD cytofix/cytoperm kit before staining with the appropriate antibodies. Islets were purified following standard collagenase protocols as described²² and dissociated by incubating with a non-enzymatic solution (Sigma, St, Lois, MO) followed by trituration per the manufacturer's instructions.

Antibodies used for flow-cytometry were as follows: PE-Cy7-conjugated CD4 (clone RM4-5; Tonbo), PE-Cy7-conjugated B220 (clone RA3-6B2; BioLegend), PE-Cy7-conjugated CD62L (clone MEL-14; Tonbo), PE-Cy7-conjugated CD45 (clone 30-F11; Invitrogen), PE-conjugated IL-17A (clone eBio17B7; Invitrogen), PE-conjugated CD62L (clone MEL-14; Tonbo), PE-conjugated CD44 (clone IM7; BioLegend), PE-conjugated CD8 (clone 53-6.7; Tonbo), PE-conjugated ROR γ (Q31-378; BD Biosciences), PE-conjugated IL-13 (clone eBio13A; Invitrogen), FITC-conjugated IFN γ (clone xMG1.2; Invitrogen), FITC-conjugated Foxp3 (clone FJK-16s; Invitrogen), FITC-conjugated CD44 (clone IM7; BioLegend), FITC-conjugated CD8 (clone 53-6.7; Tonbo), APC-conjugated CD25 (clone PC61.5; Tonbo), APC-conjugated CD4 (clone GK1.5; BioLegend), APC-conjugated IL-2 (clone JES6-5H4; Invitrogen), APC-conjugated CD62L (clone MEL-14; Tonbo), PerCP-conjugated CD45 (clone 30-F11; Invitrogen), eFluor 450-conjugated TCR β (clone H57-597; Tonbo), eFluor 450-conjugated CD8 (clone 53-6.7; Tonbo), PerCP-Cy5.5-conjugated CD4 (clone GK1.5; BioLegend), Ghost UV 450 (Tonbo), and Ghost 510 (Tonbo).

Cytokine Production

Lymphocyte cells were activated at 10^6 cells/ml with 0.5 mM ionomycin, 10 ng/ml PMA and 3 mg/ml of Brefeldin A at 37°C for 3–4 hour before labeling with LIVE/DEAD fixable dead stain, and staining for CD4, CD8, IL-17 and INF γ using Cytotfix/Cytoperm.

T cell differentiation

T cells were enriched from spleen and LNs using the MagniSort CD4 negative selection kit (Thermo Fisher Scientific). Naive CD4⁺ T cells were isolated by flow cytometry based on the markers CD4⁺CD62L⁺CD44[–]CD25[–] or using the Easysep mouse naive T cell isolation kit. 10^5 naive T cells were cultured for 4 d (Th1, Th17, iTreg cell) in a 96-well flat-bottom plate coated with 2 μ g/ml anti-CD3 (clone 2C11; Tonbo) and 2 μ g/ml anti-CD28 (clone 37.51; Tonbo) with the relevant cytokines and blocking antibodies: classical Th17 (20 ng/ml IL-6, 2 ng/ml TGF β , 10 μ g/ml anti-IL4 [clone 11B11; Tonbo], and 10 μ g/ml anti-INF γ [clone XMG1.2; Tonbo]), pathogenic Th17 (20 ng/ml IL-6, 20 ng/ml IL-1 β , 20 ng/ml IL-23, 10 μ g/ml anti-IL4, and 10 μ g/ml anti-INF γ), Th1 (20 ng/ml IL-12, 100 U/ml IL-2, and 10 μ g/ml anti-IL4), iTreg cell (20 ng/ml TGF β and 100 U/ml IL-2), or Th0 (100 U/ml IL-2). Th17 cultures were performed in Iscove's medium, Th1/iTreg cell cultures were performed in RPMI. All media were supplemented with 10% FBS, penicillin/streptomycin, glucose, pyruvate, β -mercaptoethanol, and Hepes. Cytokines

were purchased from R&D Systems (murine IL-4, murine IL-6, and human IL-2), Miltenyi (murine IL-12, murine IL-1 β , and murine IL-23), or HumanKine (human TGF β).

Mass Cytometry of Islets

Islets were purified following standard collagenase protocols as described²² and dissociated by incubating with a non-enzymatic solution (Sigma, St. Lois, MO) followed by trituration per the manufacturer's instructions. Single cell suspensions were fixed for 10 min at RT using 1.6% PFA (Fisher Scientific, Hampton, New Hampshire). Mass-tag cellular barcoding was performed as previously described⁴⁷, followed by sample pooling. Cells were then stained as previously reported⁴⁸. Primary conjugates of mass cytometry antibodies were prepared using the MaxPAR antibody conjugation kit (Fluidigm, South San Francisco, CA) according to the manufacturer's recommended protocol and each antibody clone and lot was titrated to optimal staining concentrations using primary murine samples. Prior to sample acquisition, cells were stained with 125 nM Ir191/193 DNA intercalator (Cell-ID Intercalator-Ir, Fluidigm) for 20min, washed in Di water, filtered through a 35 μ m nylon mesh and resuspended to 0.5×10^6 cells/ml with 0.1% EQ four element calibration beads (Fluidigm). Data acquisition was done with a CyTOF 2 mass cytometer (Fluidigm) at an event rate of 300–500 cells/s. After data acquisition, .fcs files were concatenated, normalized using mass bead signal⁴⁹ and debarcoded using a single-cell debarcoding algorithm⁴⁷. Manual gating and viSNE analysis of .fcs files was performed using Cytobank⁵⁰.

Bone Marrow Chimeras

Bone marrow was removed from 4-8 week nondiabetic, CD45.2 congenically labelled donor mice, filtered through a 70-mm filter, centrifuged, and resuspended in sterile PBS to a concentration of 10^7 live cells per 200 μ l. CD45.1 congenically labelled female NOD recipient mice were lethally irradiated (1200 rad) 8-16 h before bone marrow transplant. A single-cell suspension of CD45.2 bone marrow in sterile PBS (10^7 live cells per recipient mouse) was transplanted to each recipient mouse by IV tail injection. Reconstitution was confirmed by flow cytometry of the peripheral blood at 4-8 weeks post transplantation. Nonfasting blood glucose levels in recipient mice were monitored weekly by using an Accu-Check glucometer (Roche Diagnostic Corp). Diabetes onset was considered to have occurred when nonfasting blood glucose concentration exceeded 250mg/dl for 2 consecutive days.

In vivo Treg Suppression Assay

NOD.BDC2.5+ and NOD.BDC2.5+STAT3^{+/K392R} lymphocyte single cell suspensions were made from axillary, inguinal, mesenteric and para-aortic LNs, as discussed above. CD4+CD25+CD62L+ Tregs and CD4+CD25-CD62L+ Teffs were isolated by cell sorting using a FACS Aria sorter, prior to being incubated in ex-vivo cultures at 37°C for ten days. 25-50 x10³ purified cells were plated on 96-well U-bottom plate, stimulated with mouse anti-CD3/CD28 dynabeads (3:1 bead to cell ratio; Thermo Fisher Scientific, Inc.) and supplemented with complete medium consisting of DMEM containing 10% heat-inactivated fetal bovine serum (Biosource International), 5μM HEPES, nonessential amino acids, 0.5mM sodium pyruvate, 1mM glutaMax I (all from Invitrogen), penicillin-streptomycin, recombinant human IL-2 (2000IU/mL for Treg and 200IU/mL for Teff cultures; Proleukin) and 55μM 2-ME (Sigma-Aldrich). On day ten of ex-vivo culture, 5 x10⁶ expanded NOD.BDC2.5+ or NOD.BDC2.5+STAT3^{+/K392R} Tregs along with 5x 10⁶ NOD.BDC2.5+ Teffs were harvested, washed twice, rested in complete media without any IL-2 supplementation for 6 hours at 37°C, and resuspended in 100μL of PBS before the individual cell suspensions were injected intravenously via the retroorbital vein into anesthetized NOD.Rag2^{-/-} mice. Successful intravenous infusion was confirmed by direct visualization and palpation of the periorbital area. Nonfasting blood glucose levels in recipient mice were monitored daily by using an Accu-Check glucometer (Roche Diagnostic Corp). Diabetes onset was considered to have occurred when nonfasting blood glucose concentration exceeded 250mg/dl for 3 consecutive days.

Adoptive Transfer of Diabetes using 8.3Tg+ CD8+ T cells

NOD, NOD.8.3Tg+ and NOD.8.3Tg+STAT3^{+/K392R} lymphocyte single cell suspensions were made from nonpancreatic lymph nodes and spleen from nondiabetic donors, as discussed above. NOD cells were CD4-enriched using MACS EasySep CD4-negative selection kits (StemCell), and 8.3Tg+ cells were naïve CD8-enriched using MACS EasySep naïve CD8-negative selection kits (StemCell) . Aliquots at each step were analyzed to confirm purity. Purified cells were pooled in a 1:2 ratio of naïve CD8+8.3Tg+ T cells to polyclonal CD4+ T cells, and retroorbitally injected at 1.5 x 10⁶ cells per recipient NOD.SCID mouse. Nonfasting blood glucose levels in recipient mice were monitored three times per week by using an Accu-Check glucometer (Roche Diagnostic Corp) from days 7-30 post-transfer. Diabetes onset was considered to have occurred when nonfasting blood glucose concentration exceeded 250mg/dl for 2 consecutive days.

CTV-Labeling and Adoptive Transfer of T cells

NOD.BDC2.5+.Foxp3^{RFP} nondiabetic mice donated from the Tang Lab at UCSF were used to generate lymphocyte single cell suspensions from nonpancreatic lymph nodes and spleen, as discussed above. CD4+CD62L+Vβ4+Foxp3- cells were isolated by cell sorting using a FACS Aria sorter, labeled using the Invitrogen CellTrace Violet Proliferation Kit, and 1.0×10^6 CTV-labeled cells were injected i.v. into WT or STAT3^{+/K392R} nondiabetic recipients. 3.5 days post-adoptive transfer, pancreatic and inguinal lymph nodes were removed and analyzed for the presence of CTV-labeled cells to assess cell proliferation.

Single cell RNA-seq library preparation and sequencing

Islets were purified following standard collagenase protocols as described²² and dissociated by incubating with a non-enzymatic solution (Sigma, St, Lois, MO) followed by trituration per the manufacturer's instructions. After live CD45+ cells were isolated by cell sorting using a FACS Aria sorter, cells were spun down and resuspended in PBS. Prior to loading on the 10x Chromium instrument, cells were counted using a haemocytometer and the concentration of cells adjusted to $\sim 1 \times 10^3$ cells μL^{-1} . A viability of at least 90% for all samples were confirmed by trypan blue staining. Samples were handled on ice where possible. Cells were then processed by the UCSF Immunology Core facility using the Chromium Single Cell 5' Library and the Gel Bead Kit following the manufacturer's user guide (10x Genomics, Pleasanton, CA, CG000086_SingleCellVDJRagentKitsUserGuide_RevB). Single-cell libraries were sequenced on a Novaseq S4 Flowcell PE 2x150.

Single cell RNA-seq analysis

Reads were processed and aligned to the mm10 reference genome assembly using the 10X cellranger count pipeline. Doublets were assessed using the R implementation of scrublet⁵¹, 'scrublet'. Seurat objects for each sample were created and merged. Barcodes with a doublet score greater than 0.15, mitochondrial reads greater than 5%, or less than 200 features were removed.

Pre-processing, clustering, and dimensionality reduction were performed using Seurat⁵². Clusters were identified using marker genes including *Cd3e*, *Cd19*, and *Itgax*. T cell clusters 1, 2, and 3 were selected for re-clustering and

were subsequently re-processed using the same procedure. A small number of additional contaminants (doublets and non-T cells) were identified and removed. Differential genes were determined with Seurat's 'FindAllMarkers' and 'FindMarkers' functions and represented as heatmaps (plotted with 'pheatmap') or volcano plots (plotted with 'ggplot2'). For clarity, ribosomal genes, mitochondrial genes, genes starting with "Gm", and genes ending with "Rik", are not shown in the volcano plots.

Single cell TCR analysis

TCR sequences were aligned to the mm10 reference genome using the 10X cellranger vdj pipeline. High confidence clonotypes for each sample were merged to obtain a single clone id by matching identical CDR3 amino acid sequences between the samples. These merged clones were added to the Seurat object metadata for downstream analysis

Single cell ATAC-seq library preparation and sequencing

Cells were processed using the 10X single cell ATAC reagents and protocols as described previously⁵³. Briefly, bulk cells were transposed, single cells were loaded into droplets for barcoding using the 10X chromium platform, and then DNA was amplified and prepared for sequencing. Libraries were sequenced at the Stanford Functional Genomics Facility.

Single cell ATAC analysis

Reads were processed and aligned to the mm10 reference genome assembly using the 10X cellranger atac-count pipeline. Fragments files were loaded into ArchR for all downstream analysis⁵⁴. ArchR default settings were used for doublet calling, quality filtering (based on transcription start site enrichment and number of fragments per cell) and computing the cell by tile matrix.

The tile matrix was used for dimensionality reduction and clustering of the cells. ArchR gene scores were used to determine cluster identities and T cells were selected for re-clustering, analogous to the scRNA workflow. ArchR was then used to create a cell by peak matrix and cell by motif deviation matrix. Markers for each matrix were determined using the 'getMarkerFeatures' utility and then displayed via heatmap or volcano plot. Accessibility for

selected genomic regions were visualized using the ArchR browser where each track for a particular region is shown on the same fixed scale and normalized by reads in transcription start sites genome-wide.

Histology

Pancreata were removed and fixed in 10% neutral-buffered formalin (Sigma-Aldrich). Paraffin-embedded step-sections were stained with hematoxylin and eosin (H&E) and insulinitis was scored as described previously⁵⁵.

Immunofluorescence

For immunofluorescence, pancreata were fixed in 2% paraformaldehyde (Pierce) in PBS for 2 hrs at 4 °C followed by overnight incubation in 30% (w/v) sucrose (Sigma Aldrich) in PBS. Tissues were embedded in Optimal Cutting Temperature Compound (Tissue-Tek) and stored at –80 °C before sectioning (50-200 µm) on a cryostat (Leica). Thin sections were dried on Superfrost Plus (Fisher Scientific) slides and semi-thick (200 µm) sections were moved directly to 0.3% Triton X-100 (Sigma-Aldrich), 0.2% BSA (Sigma-Aldrich), 0.1% sodium azide (Sigma-Aldrich) in PBS (Immunomix). Slides were stained in a humidified chamber and semi-thick sections were stained in 24-well plates with one section per well. Slides were briefly rehydrated in PBS before permeabilization in Immunomix for 1hr at RT followed by blocking with BlockAid (ThermoFisher), primary antibody staining at RT, and, when necessary, secondary antibody staining at RT for 1hr. Semi-thick sections were permeabilized in Immunomix with shaking at RT overnight followed by blocking with BlockAid at RT for 2hr, primary antibody staining at RT for 2hr, and, when necessary, secondary antibody staining at RT for 2hr. Semi-thick sections were then moved to Superfrost Plus slides and all sections were mounted with ProLong Diamond Antifade Mountant (ThermoFisher). Images were acquired on a Leica SP5 (Leica) laser scanning confocal microscope.

Statistical analysis

All experiments were performed using randomly assigned mice without investigator blinding. No data were excluded. Statistical significance between two groups was calculated using an unpaired, parametric, 2-tailed Student's t test. Experimental groups included a minimum of three biological replicates. Intragroup variation was not assessed. All statistical analysis was performed using Prism 7 (GraphPad Software). Figures display

means $\pm$ standard deviation. A P value of less than 0.05 was considered statistically significant. No statistical methods were used to predetermine sample size.

References:

1. Gepts, W. & De Mey, J. Islet cell survival determined by morphology. An immunocytochemical study of the islets of Langerhans in juvenile diabetes mellitus. *Diabetes* **27 Suppl 1**, 251-261 (1978).
2. Gepts, W. Pathologic Anatomy of the Pancreas in Juvenile Diabetes Mellitus. *Diabetes* **14**, 619-633 (1965).
3. Erlich, H. *et al.* HLA DR-DQ Haplotypes and Genotypes and Type 1 Diabetes Risk. *Diabetes* **57** (2008).
4. Fung, E.Y.M.G. *et al.* Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. *Genes And Immunity* **10**, 188 (2008).
5. Ferreira, R.C. *et al.* Functional IL6R 358Ala allele impairs classical IL-6 receptor signaling and influences risk of diverse inflammatory diseases. *PLoS Genet* **9**, e1003444 (2013).
6. Wang, J. *et al.* Common variants on cytotoxic T lymphocyte antigen-4 polymorphisms contributes to type 1 diabetes susceptibility: evidence based on 58 studies. *PloS one* **9**, e85982 (2014).
7. Sharma, A. *et al.* Identification of non-HLA genes associated with development of islet autoimmunity and type 1 diabetes in the prospective TEDDY cohort. *Journal of autoimmunity* **89**, 90-100 (2018).
8. Warshauer, J.T., Bluestone, J.A. & Anderson, M.S. New Frontiers in the Treatment of Type 1 Diabetes. *Cell metabolism* **31**, 46-61 (2020).
9. Flanagan, S.E. *et al.* Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. *Nature genetics* **46**, 812-814 (2014).
10. Velayos, T. *et al.* An Activating Mutation in STAT3 Results in Neonatal Diabetes Through Reduced Insulin Synthesis. *Diabetes* **66**, 1022-1029 (2017).
11. O'Shea, John J. & Plenge, R. JAK and STAT Signaling Molecules in Immunoregulation and Immune-Mediated Disease. *Immunity* **36**, 542-550 (2012).
12. Saarimäki-Vire, J. *et al.* An Activating STAT3 Mutation Causes Neonatal Diabetes through Premature Induction of Pancreatic Differentiation. *Cell Reports* **19**, 281-294 (2017).
13. Gorogawa, S. *et al.* Insulin secretory defects and impaired islet architecture in pancreatic beta-cell-specific STAT3 knockout mice. *Biochem Biophys Res Commun* **319**, 1159-1170 (2004).
14. Fabbri, M., Frixou, M., Degano, M. & Foustieri, G. Type 1 Diabetes in STAT Protein Family Mutations: Regulating the Th17/Treg Equilibrium and Beyond. *Diabetes* **68**, 258-265 (2019).
15. Milner, J.D. *et al.* Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood* **125**, 591-599 (2015).
16. Marwaha, A.K. *et al.* Cutting Edge: Increased IL-17–Secreting T Cells in Children with New-Onset Type 1 Diabetes. *The Journal of Immunology* **185**, 3814 (2010).

17. Viisanen, T. *et al.* Circulating CXCR5⁺PD-1⁺ICOS⁺ Follicular T Helper Cells Are Increased Close to the Diagnosis of Type 1 Diabetes in Children With Multiple Autoantibodies. *Diabetes* **66**, 437 (2017).
18. Hundhausen, C. *et al.* Enhanced T cell responses to IL-6 in type 1 diabetes are associated with early clinical disease and increased IL-6 receptor expression. *Science translational medicine* **8**, 356ra119 (2016).
19. Wienke, J. *et al.* A novel human STAT3 mutation presents with autoimmunity involving Th17 hyperactivation. *Oncotarget* **6** (2015).
20. Durant, L. *et al.* Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity* **32**, 605-615 (2010).
21. Stadinski, B.D. *et al.* Chromogranin A is an autoantigen in type 1 diabetes. *Nature immunology* **11**, 225-231 (2010).
22. Tang, Q. *et al.* In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *The Journal of experimental medicine* **199**, 1455-1465 (2004).
23. Tarbell, K.V., Yamazaki, S., Olson, K., Toy, P. & Steinman, R.M. CD25⁺ CD4⁺ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *The Journal of experimental medicine* **199**, 1467-1477 (2004).
24. Hudson, W.H. *et al.* Proliferating Transitory T Cells with an Effector-like Transcriptional Signature Emerge from PD-1⁺ Stem-like CD8⁺ T Cells during Chronic Infection. *Immunity* **51**, 1043-1058.e1044 (2019).
25. Schep, A.N., Wu, B., Buenrostro, J.D. & Greenleaf, W.J. chromVAR: inferring transcription-factor-associated accessibility from single-cell epigenomic data. *Nature Methods* **14**, 975-978 (2017).
26. Kaech, S.M. & Cui, W. Transcriptional control of effector and memory CD8⁺ T cell differentiation. *Nature Reviews Immunology* **12**, 749-761 (2012).
27. Milner, J.J. *et al.* Runx3 programs CD8⁺ T cell residency in non-lymphoid tissues and tumours. *Nature* **552**, 253-257 (2017).
28. Weinreich, M.A. *et al.* KLF2 transcription-factor deficiency in T cells results in unrestrained cytokine production and upregulation of bystander chemokine receptors. *Immunity* **31**, 122-130 (2009).
29. Verdaguer, J. *et al.* Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. *The Journal of experimental medicine* **186**, 1663-1676 (1997).
30. Jun, H.S., Santamaria, P., Lim, H.W., Zhang, M.L. & Yoon, J.W. Absolute requirement of macrophages for the development and activation of beta-cell cytotoxic CD8⁺ T-cells in T-cell receptor transgenic NOD mice. *Diabetes* **48**, 34-42 (1999).
31. De Leenheer, E. & Wong, F.S. Adoptive Transfer of Autoimmune Diabetes Using Immunodeficient Nonobese Diabetic (NOD) Mice. *Methods in molecular biology (Clifton, N.J.)* **1433**, 135-140 (2016).
32. Christianson, S.W., Shultz, L.D. & Leiter, E.H. Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4⁺ and CD8⁺ T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. *Diabetes* **42**, 44-55 (1993).
33. Cui, W., Liu, Y., Weinstein, J.S., Craft, J. & Kaech, S.M. An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8⁺ T cells. *Immunity* **35**, 792-805 (2011).

34. Ciucci, T., Vacchio, M.S. & Bosselut, R. A STAT3-dependent transcriptional circuitry inhibits cytotoxic gene expression in T cells. *Proceedings of the National Academy of Sciences of the United States of America* **114**, 13236-13241 (2017).
35. Beltra, J.C. *et al.* Developmental Relationships of Four Exhausted CD8(+) T Cell Subsets Reveals Underlying Transcriptional and Epigenetic Landscape Control Mechanisms. *Immunity* **52**, 825-841.e828 (2020).
36. Siddiqui, I. *et al.* Intratumoral Tcf1+PD-1+CD8+ T Cells with Stem-like Properties Promote Tumor Control in Response to Vaccination and Checkpoint Blockade Immunotherapy. *Immunity* **50**, 195-211.e110 (2019).
37. Im, S.J. *et al.* Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* **537**, 417-421 (2016).
38. Wu, T. *et al.* The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. *Science Immunology* **1**, eaai8593 (2016).
39. McKinney, E.F., Lee, J.C., Jayne, D.R., Lyons, P.A. & Smith, K.G. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature* **523**, 612-616 (2015).
40. Keir, M.E. *et al.* Tissue expression of PD-L1 mediates peripheral T cell tolerance. *Journal of Experimental Medicine* **203**, 883-895 (2006).
41. Ansari, M.J. *et al.* The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. *The Journal of experimental medicine* **198**, 63-69 (2003).
42. Wiedeman, A.E. *et al.* Autoreactive CD8+ T cell exhaustion distinguishes subjects with slow type 1 diabetes progression. *The Journal of clinical investigation* **130**, 480-490 (2020).
43. Long, S.A. *et al.* Partial exhaustion of CD8 T cells and clinical response to teplizumab in new-onset type 1 diabetes. *Sci Immunol* **1** (2016).
44. Herold, K.C. *et al.* An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. *New England Journal of Medicine* (2019).
45. June, C.H., Warshauer, J.T. & Bluestone, J.A. Is autoimmunity the Achilles' heel of cancer immunotherapy? *Nature medicine* **23**, 540-547 (2017).
46. Madisen, L. *et al.* A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* **13**, 133-140 (2010).
47. Zunder, E.R., Lujan, E., Goltsev, Y., Wernig, M. & Nolan, G.P. A continuous molecular roadmap to iPSC reprogramming through progression analysis of single-cell mass cytometry. *Cell stem cell* **16**, 323-337 (2015).
48. Levine, L. *et al.* *Single-cell metabolic analysis by mass cytometry reveals distinct transitional states of CD8 T cell differentiation*, 2020.
49. Finck, R. *et al.* Normalization of mass cytometry data with bead standards. *Cytometry A* **83**, 483-494 (2013).
50. Kotecha, N., Krutzik, P.O. & Irish, J.M. Web-based analysis and publication of flow cytometry experiments. *Curr Protoc Cytom* **Chapter 10**, Unit10.17 (2010).

51. Wolock, S.L., Lopez, R. & Klein, A.M. Scrublet: Computational Identification of Cell Doublets in Single-Cell Transcriptomic Data. *Cell Syst* **8**, 281-291.e289 (2019).
52. Stuart, T. *et al.* Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902.e1821 (2019).
53. Satpathy, A.T. *et al.* Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nature biotechnology* **37**, 925-936 (2019).
54. Granja, J.M. *et al.* ArchR: An integrative and scalable software package for single-cell chromatin accessibility analysis. *bioRxiv*, 2020.2004.2028.066498 (2020).
55. Katz, J.D., Wang, B., Haskins, K., Benoist, C. & Mathis, D. Following a diabetogenic T cell from genesis through pathogenesis. *Cell* **74**, 1089-1100 (1993).

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Author Contributions:

J.T.W. conceived the study, designed and performed experiments, analyzed data and wrote the manuscript. J.A.B. designed and performed biostatistical analysis of RNA, TCR, and ATAC sequencing data. A.C conceived and designed experiments with J.T.W, performed flow cytometry experiments and analyzed data. Q.S. performed genomic experiments. N.S and Y.P. designed, performed and analyzed data for the in vivo Treg suppression assay with Q.T. supervision and guidance. J.W and A.G. designed, performed and analyzed data for CD8 adoptive transfer experiments with J.G. supervision and guidance. J.P. designed and performed flow cytometry experiments. A.T.S. supervised genomic experiments and analysis. M.S.A directed the study and wrote the manuscript with J.T.W.

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Figures

Figure 1

STAT3^{K392R} mutant mice recapitulate the human type 1 diabetes phenotype | a, STAT3K392R mutation located in the DNA binding domain was inserted into WT (i.e., NOD) mice using CRISPR/Cas9 and confirmed by Sanger sequencing b, In vitro CD4 differentiation of naïve CD4⁺ T cells into Th17 and Treg subsets. c, Diabetes incidence curves (Females: WT, n=93; STAT3^{K392R}, n=125; Males: WT, n=19; STAT3^{K392R}, n=68) d, Time course of insulinitis in WT and STAT3^{K392R} mice with and without diabetes (n=3-11 mice per gender per time point) e, viSNE plots of islets using mass cytometry compare immune and endocrine cellular compositions between a WT mouse without diabetes and littermate STAT3^{K392R} mouse with recently-diagnosed diabetes to highlight the presence of immune infiltration with STAT3^{K392R} diabetes. Results representative of viSNE plots from 8-14 week old non-diabetic WT (n=6) and diabetic STAT3^{K392R} (n=4) mice. Error bars indicate mean $\pm$ SD. ns = nonsignificant (P>0.05), * P $\leq$ 0.05, ** P $\leq$ 0.01, *** P $\leq$ 0.001.

Figure 3

STAT3^{+/K392R} APC Phenotype and T-cell priming by the local islet environment 324 is unchanged |

a, Flow cytometry gating (values shown are frequency relative to parent gate) and population frequencies of APC subpopulations from splenocytes isolated ex vivo in non-diabetic 8 week old male mice (n=4 per group) b, Representative histograms for MHCII and CD86 protein expression on conventional DCs (CD11c⁺MHC II⁺) and classical monocytes (CD11b⁺Ly6c⁺) in the islets of non-diabetic 8-10 week old mice using mass cytometry. Performed in 3-4 mice per group c, UMAP projection of reclustered antigen-presenting myeloid cells (CD11c⁺ cells) from islet immune infiltrate used in scRNA-seq experiment (see Extended Fig. 2a) d, UMAP projection showing distribution of STAT3^{+/K392R} and WT cell transcriptomes e, Violin plots showing gene expression profile of MHCII and CD86 in APC myeloid clusters f, CTV profile

among BDC2.5 CD4+ T cells in pancreatic and inguinal (control) lymph nodes (pLN and iLN, respectively) 3.5 days after adoptive transfer into STAT3+/K392R and WT recipients. Cell proliferation was evaluated by FACS analysis of the CTV dilution in the pLNs and iLNs of recipient mice and results are expressed as the percentage of proliferating cells within the recovered CTV-labeled BDC2.5 CD4+ T cells. Representative of three sets of STAT3+/K392R / WT recipient mice. Error bars indicate mean +/- SD. ns = non-significant (P>0.05).

Figure 4

STAT3+/K392R drives expansion of the CD8+ effector compartment with upregulation of cytokine and chemokine genes | a, UMAP projection of reclustered T cells (CD3+ cells) from islet immune infiltrate used in scRNA-seq experiment (see Extended Fig. 2a) with associated cell counts per cluster and comparison of cluster frequencies between the two genotypes (STAT3+/K392R vs WT) b, Volcano plot showing STAT3+/K392R vs WT differential gene expression in CD8+ clusters. Log N fold cutoff 0.25 used. Genes of interest are labeled. c, Flow cytometry of Ccl5 protein expression in CD8+ memory T cells from the pancreatic lymph node in nondiabetic males at 14 weeks (n=3 per group). Error bars indicate mean +/- SD, ns = non-significant (P>0.05), * P ≤ 0.05. d, UMAP projection of reclustered CD8+ T cells e-f, Marker gene or module score expression in CD8+ T cell clusters. In f, genes used to calculate module scores for terminally exhausted T cells were Cd101, Cd200r2, Cd7, Cd200r1, Il10 and for transitory T cells were Cx3cr1, Klrg1, Il2ra, Il18rap, S1pr5, as previously described²⁴ g, Relative population frequencies among non-naïve CD8+ cell clusters h, Volcano plot showing differential gene expression in CD8+ effector and transitory cell clusters relative to all CD8+ cells.

Figure 5

STAT3+/K392R increases chromatin accessibility within cytotoxic 354 CD8+ loci resulting in an epigenetically distinct state | a, UMAP projection of scATAC-seq profiles of T cells (CD3+) subclustered from CD45+ islet infiltrates of 8-10 week old male non-diabetic mice, WT (n=3, pooled) vs STAT3+/K392R (n=3, pooled) b, Normalized pseudobulk ATAC-seq tracks of CD8+ T cell clusters split by genotype around

genes identified as upregulated in corresponding scRNA-seq CD8+ T cell clusters. Peaks significantly upregulated (from c) are shown in red c, Volcano plot showing differential peaks whose nearest gene is present in the significantly upregulated genes (Fig. 4b) between CD8+ T cell clusters (STAT3+/K392R vs WT) d, Volcano plot showing differential transcription factor motifs between CD8+ T cell clusters (STAT3+/K392R vs WT) e, Transcription factor motif activity for CD8+ T cell clusters split by genotype. Motif sequence shown above each violin plot.

Figure 6

STAT3+/K392R drives clonal expansion of diabetogenic CD8+ T cells | a, Top 20 clones by count in each sample b, Top 10 specific clones with corresponded CDR3 sequences displayed in table. The CDR3 sequence for Clone 6158 in red is nearly identical to that of the CD8-restricted TCR specific for islet specific antigen IGRP (TCR-8.3, sequence shown below table). The only difference between Clone 6158 and TCR-8.3 is a serine, denoted in blue, replaces an alanine of the TCR beta chain c, Diabetes incidence of 8.3Tg+ (IGRP-specific TCR) mice with (n=7) and without (n=11) STAT3+/K392R confirms increased diabetogenicity d, Diabetes incidence after adoptive transfer of polyclonal WT CD4+ T cells with naïve CD8+ T cells from 8.3Tg+ mice with (n=5) and without STAT3+/K392R (n=10) into NOD.SCID mice. Results are pooled from 2 independent cohorts.