Type 1 Diabetes in STAT Protein Family Mutations: Regulating the Th17/Treg Equilibrium and Beyond

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Improvements in the immunological, molecular, and genetic technologies such as next-generation sequencing have led to an exponential increase in the number of monogenic immune dysregulatory syndromes diagnosed, where type 1 diabetes (T1D) forms part of the autoimmune manifestations. Here, we reviewed the mutations in the signal transducer and activator of transcription (STAT) protein family, namely gain-of-function (GOF) mutations in *STAT1* and *STAT3* as well as *STAT5b* deficiency, that show strong association to T1D susceptibility. The equilibrium of T-helper 17 (Th17) and regulatory T cells (Tregs) is often found altered in patients affected by STAT GOF mutations. While the increased number of Th17 cells and the concomitant decrease in Treg cells may explain T1D in STAT3 GOF patients, the reduced number of Th17 cells found in those carrying STAT1 GOF mutations added a new level of complexity on the exact role of Th17 in the pathogenesis of T1D. Here, we describe the possible mechanisms through which STAT3 and STAT1 GOF mutations may perturb the fate and function of Th17 and Tregs and explore how this may lead to the development of T1D. We propose that the study of monogenic diseases, and in particular STAT mutations, may not only improve our understanding of the function of the human immune system but also shed light onto the pathogenic mechanisms of T1D and the genetic variants that confer predisposition to the disease.

Type 1 diabetes (T1D) is a multifactorial disease caused by the autoimmune destruction of pancreatic β-cells. In recent years, genome-wide association studies have identified single nucleotide polymorphisms (SNPs) in multiple susceptibility loci including *INS*, *PTPN22*, *CTLA4*, and *IL2RA* (1–3) that underlie the polygenic nature of T1D. These SNPs together with specific human leukocyte antigen (HLA) alleles that are strongly associated to T1D have given rise to a genetic risk score (GRS) that is used to predict T1D in susceptible individuals (4). Autoantibodies (AAbs) against islet antigens such as GAD, insulin, and IA-2 are used today as markers of diagnosis and disease risk (5). Since their role in the pathogenesis of T1D has been questioned, the majority of research efforts are concentrated on understanding the T-cell–mediated β-cell recognition and killing.

Autoreactive $CD4^+$ and $CD8^+$ T cells are considered the primary drivers of β-cell loss (6). Within $CD4^+$ T cells, T-helper 1 (Th1) and Th17 cells are considered the primary effector subsets involved in the disease pathogenesis (7,8). Th17 cells release proinflammatory cytokines (predominantly interleukin-17 [IL-17]) that directly compromise β-cell function. Additionally, they recruit and activate monocytes that also contribute to β-cell loss (9,10). Numerous data from animal studies have shown that inhibition of Th17 cells in NOD mice (IL-17 deficient) results in a significantly suppressed development of diabetes, a delayed onset of pathology, and a concomitant reduced insulitis (11–13). The effector function of Th17 cells is counterregulated by that of $FOXP3^+$ regulatory T cells (Tregs). Tregs are the master regulators of immune tolerance and are capable of halting the induction, proliferation, and effector function of Th17 cells, restoring the immune balance. Alterations in Treg number or function have been associated with the development of T1D and other autoimmune manifestations (14,15).

The development of Th17 and Treg cells is regulated by the signal transducer and activator of transcription (STAT) family of signal transducers. Interestingly, monogenic

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mutations in STAT family members give rise to primary immunodeficiencies and other immune dysregulatory syndromes, which are characterized by the co-occurrence of autoimmunity and often T1D. STAT proteins play an active role in immune-cell genesis and regulation. They are known to be fundamental for the development and maintenance of human memory T cells, and, as we will further discuss, are crucial in determining Th17 skewing and accumulation as well as regulating Treg fate (16). Interestingly, patients with mutations in STATs have a low T1D GRS, suggesting that a single genetic "hit" is able to significantly increase susceptibility to T1D (17). Here, we discuss how the STAT genes perturb the Th17:Treg equilibrium and how their mutation might be the root of the increased susceptibility of these patients to T1D.

THE STAT PROTEIN FAMILY OF SIGNAL TRANSDUCERS

The STAT family comprises seven proteins (STAT1, −2, −3, −4, −5a, −5b, and −6), which are all tightly cross-regulated (18). Cytokine receptor binding initiates the JAK-STAT cascade with Janus kinase (JAK) proteins leading to STAT phosphorylation and dimerization. STAT dimers translocate to the nucleus, where they bind to DNA and activate the transcription of many target genes (19). Mutations in genes encoding STAT1, −2, −3, and −5b impair their function and have been linked to immune dysregulatory syndromes both in the form of autoimmunity and immunodeficiency in past years (20). Gain-of-function (GOF) mutations in *STAT1* and *STAT3* as well as *STAT5b* deficiency have been specifically linked to T1D. For the latter, a correlation with T1D has been so far documented solely in mouse models (21).

T1D and STAT3 Mutations

STAT3 is a cytosolic signal transducer involved in many biological processes including cell growth, apoptosis, organogenesis, inflammation, infection, and oncogenesis (22,23). IL-2, IL-6, IL-10, and IL-21 together with transforming growth factor-β (TGF-β) are among the most important cytokines that activate the JAK-STAT3 cascade (24). There are 127 known genetic variants in the STAT3 gene, the majority of which are single-nucleotide substitutions (source: Human Gene Mutation Database, www.hgmd.cf.ac.uk). STAT3 protein deficiency due to loss-of-function (LOF) mutations has been documented mainly in hyperimmunoglobulin E syndrome (HIES) and other immune dysregulation syndromes (25,26). Patients with HIES are characterized by reduced production of IL-17 by $CD4^+$ T cells and, consequently, an increased susceptibility to recurrent debilitating infections by *Staphylococcus aureus* and fungi (27). Defects in Th17 responses and especially in IL-21R signaling might be responsible for the highly elevated IgE levels in this disorder (28).

On the other hand, *STAT3* GOF mutations, inherited as autosomal dominant traits, lead to a broad spectrum of clinical phenotypes. These include early-onset T1D, autoimmune enteropathy, autoimmune cytopenia, juvenile-onset arthritis, and autoimmune interstitial lung disease (29,30). A total of six patients with STAT3 GOF and T1D have been described so far (Table 1), and five additional patients have recently been identified in Exeter, U.K. (A. Hattersley and M. Johnson, personal communication). According to these reports, the prevalence of T1D in STAT3 GOF mutation–positive population is ~30% (30–32).

Some patients with STAT3 GOF mutations showed an increased Th17 frequency and a diminished number of Tregs, which could explain their autoimmune manifestations (30,32). The polyautoimmune clinical phenotype that is common in these patients could be attributed to the impaired function of Tregs. Haapaniemi et al. (31) performed quantitative and qualitative analyses in Tregs and described a decreased number of circulating $FOXP3^+$ Treg cells with impaired functional suppressive capacity compared with STAT3 wild-type (WT) control subjects. This further supports the key role of Treg and Th17 cells in the pathogenesis of T1D in this monogenic syndrome. Several studies have addressed the HLA haplotype in patients with STAT3 GOF–presenting T1D. While two patients possessed the high-risk HLA allele, two other patients developed T1D in the context of low-risk HLA, and one of them was also AAb negative (Table 1). The STAT3-mutated patients recently identified in Exeter showed similar clinical phenotypes and no association with high-risk HLA (A. Hattersley and M. Johnson, personal communication).

In an effort to understand the development of autoimmunity and T1D in these patients, below we summarize all the cellular alterations that may be introduced by *STAT3* GOF (Fig. 1). IL-6, IL-21, and TGF-β instruct naive T cells toward a Th17 phenotype. In conditions of hyperactive STAT3, IL-21 can induce a marked increase in the expression of IRF4 promoting IL-21–mediated Th17 differentiation (33,34). Similarly, IL-6 together with TGF-β induces the Th17 phenotype via STAT3, bypassing IRF4 (35,36). In addition, Th17 cells release IL-21, further amplifying their Th17 skewing in an autocrine manner (37).

To explain the decrease in Tregs in patients with GOF mutations in STAT3, we provide two models, a direct and an indirect one (Fig. 1). According to the first direct hypothetical model, STAT3 directly binds a silencer element within the *FOXP3* locus resulting in reduced binding by SMAD3, a known transcription factor required for Treg development (38,39). The second, indirect model is corroborated by data from Milner et al. (32), who showed that STAT3 GOF promotes the expression of SOCS3. Elevated levels of SOCS3 in turn inhibit STAT5 phosphorylation in response to IL-2, providing a plausible explanation for the decrease in Treg numbers observed in these patients (40–42).

In order to obtain insight into the possible effect of the known missense GOF mutations in STAT3, we mapped the amino acid substitutions in the crystal structures of

Table 1—Cases of T1D in patients with STAT3 GOF mutations currently described in the literature

Patient	STAT3 mutation	TID HLA type	Age at diagnosis (weeks)	Concomitant manifestations	Islet cell–specific AAbs	Th17/Treg alteration	Reference
Patient 1	p.Thr716Met	DRB1*03-DQB1*02/DRB1*03-DQB1*02 (high risk)	2	Celiac disease; primary hypothyroidism	Positive	Not described	Flanagan et al. (30) (patient 2 also presented by Haapaniemi et al. [31])
Patient 2	p.Lys392Arg	DRB1*04-DQB1*0302 (high risk)	0	Celiac disease; desquamative interstitial pneumonitis; T cell large granular lymphocyte leukemia	Positive	Yes	
Patient 3	p.Asn646Lys	DQA1*01-B1*05/DQA1*01 (low risk)	3	Eczema	Negative	Not described	
Patient 4	p.Asn646Lys	DQA1*03-B1*03:02/A1*01-B1*06:02 (low risk)	43	Eczema; juvenile arthritis	Positive	Not described	
Patient 5	p.Arg152Trp	Not described	Not described	Lymphoproliferation; recurrent herpes zoster; lung nodules	Not described	Yes	Milner et al. (32)
Patient 6	p.Glu415Lys	Not described	Not described	Enteropathy; achalasia; atopic dermatitis; lymphoproliferation; lung nodules; short stature; recurrent urinary tract infections	Not described	Yes	

The four patients described by Flanagan et al. (30) presented with early-onset T1D with positive antibody detection in patients 1, 2, and 4. Patients 3 and 4 developed diabetes in a context of low-risk HLA predisposition, where patient 3 was also negative in autoantibody screening. In light of these findings, we can hypothesize a fundamental role of cellular-mediated immunity in T1D pathogenesis, at least in the context of STAT3 GOF mutations.

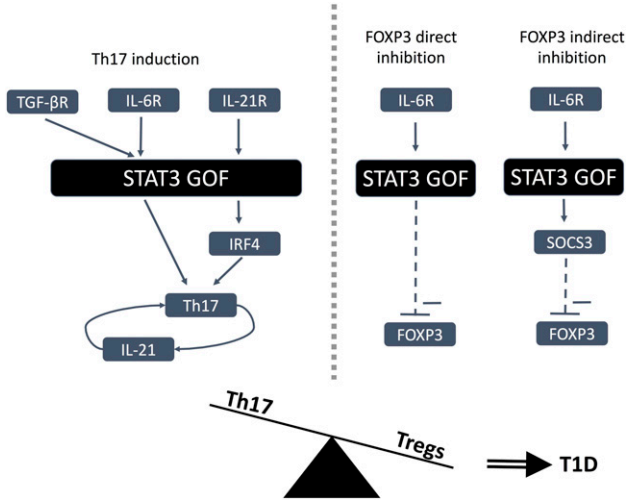

Figure 1—The altered balance between Th17 and Tregs in patients with STAT3 GOF and the potential mechanisms underlying it. In the context of STAT3 GOF, an increase in IRF4 expression induction is able to drive the skewing of Th17. At the same time, TGF-β and IL-6 together with IL-21 are able to induce a similar amplification of the induction of this cell type. There are two possible ways through which STAT3 GOF could instead affect Tregs development: directly via STAT3 inhibition of FOXP3 or indirectly via SOCS3-dependent inhibition of FOXP3. R, receptor.

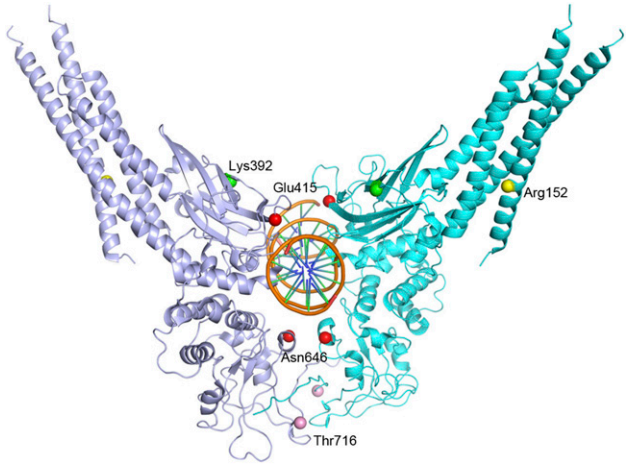

Figure 2—Location of the pathogenic mutations of STAT3b. The crystal structure of STAT3b bound to double-stranded DNA (PDB code 4E68) was visualized using the software Pymol (www.pymol.org). The STAT3b chains in the homodimer are shown as light blue and cyan cartoon, the double-stranded DNA is colored orange. The location of the mutations in the structure is shown with red (Glu415Lys and Asn646Lys, affecting DNA binding), yellow (Arg152Trp, affecting the helical region), green (Lys392Arg, whose effect is at the present unclear), and pink (Thr716Met, modifying the dimerization surface).

homodimeric, phosphorylated (PDB code 1BG1), and non-phosphorylated murine STAT3b core protein bound to double-stranded DNA (PDB code 4E68) (Fig. 2) (43,44). Arginine 152 is located in the N-terminal four-helix bundle of STAT3; its substitution with a tryptophan introduces a hydrophobic patch that could modify the local structure of the protein, although it is unclear how it could enhance its function. Amino acids Glu415 and Asn646 are both in close proximity with the DNA, and their substitution with positively charged lysines likely enhances the DNA binding affinity of STAT3 and may even modify its binding specificity. The amino acid Thr716 corresponds to Phe716 in the murine STAT3b, thus the effect of the mutation cannot be directly evaluated. However, the residue is part of an extended peptide that contributes to the dimerization interface of the protein. Thus, the substitution of a hydrophilic threonine with a hydrophobic methionine at the interface may affect the quaternary structure of STAT3b, both by destabilizing the Stat3b homodimerization and perhaps enhancing the formation of heterodimers with selected partners. The effect of the conservative Lys392Arg substitution is unclear, and further studies will be required to clarify its effect on the function of STAT3.

T1D and STAT1 Mutations

STAT1 is a cytosolic protein involved in signal transduction from type I interferon (IFN) (IFN-α and IFN-β) and IFN-γ. Other known STAT1 activators are IL-6, IL-10, IL-27, and IL-35 (24). Patients with autosomal dominant STAT1 LOF mutations are more prone to mycobacterial and other bacterial infections (45,46). Patients with autosomal recessive STAT1 LOF mutations are primarily susceptible to mycobacterial and viral infections as reported in some case reports (47,48). Both possibly reflect the failure of IFN-γ–mediated immunity. On the other hand, STAT1 autosomal dominant GOF mutations have been primarily associated with chronic mucocutaneous candidiasis and immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX)-like syndromes (49,50). Patients with STAT1 GOF–associated chronic mucocutaneous candidiasis are characterized by a predisposition to chronic noninvasive infections of the skin, nails, and mucous membranes as well as autoimmune manifestations. Concomitant T1D has been documented only in a minority of cases, with a prevalence of 4%, according to a recent review (51,52).

IPEX-like syndromes are characterized by multiorgan immune dysregulation including T1D, thyroiditis, and other autoimmune phenomena. In a recent study, a cohort of five patients with IPEX-like features but WT FOXP3 were genetically screened for underlying STAT1 GOF mutations. Three of these patients had GOF defects in STAT1 and presented with concomitant T1D (50). A total of 12 patients with coexisting STAT1 GOF mutations and T1D have been reported. Interestingly, only a minority of these patients were positive for islet-specific AAbs (50–52). This is in line with the current idea that in some patients, T1D can evolve in the absence of AAbs. In some of these patients, Th17 and Tregs were investigated as potential contributors to their immunological dysregulation. Paradoxically, Th17 reduction was a common finding in STAT1 GOF patients, but it

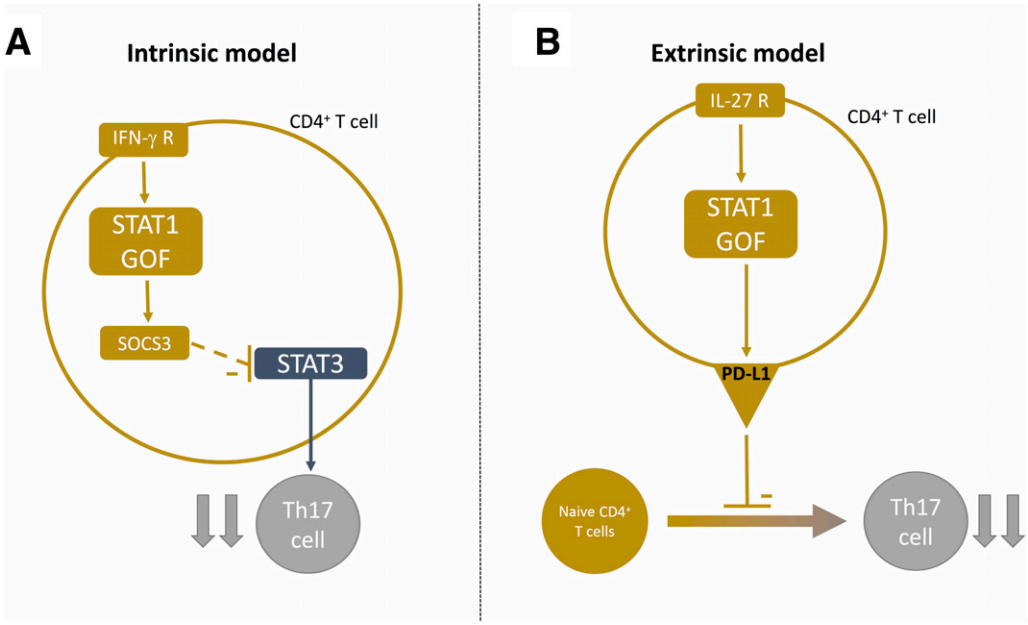

Figure 3—Models linking STAT1 GOF to Th17 deficiency. In the intrinsic model (*A*), STAT1 GOF inhibits STAT3 activity via SOCS3 and consequently impairs Th17 development. In the T-cell extrinsic model (*B*), inhibition of Th17 occurs in *trans*, via PD-L1 overexpression in neighboring cells. It is currently unknown how a reduction in Th17 cells may increase susceptibility to T1D. R, receptor.

remains unclear how STAT1 GOF affects Th17 development. We hypothesize that STAT1 affects Th17 development via a T-cell intrinsic and a T-cell extrinsic mechanism. In the T-cell intrinsic mechanism (Fig. 3*A*), STAT1 induces SOCS3 upon IFN-γ stimulation. SOCS3 in turn inhibits STAT3 activity and consequently suppresses Th17 development (53). In the T-cell extrinsic mechanism (Fig. 3*B*), STAT1 induces programmed death-ligand 1 (PD-L1), which prevents Th17 development in *trans* (54,55).

Although a deficiency in Th17 explains the increased susceptibility to *Candida albicans* in STAT1 GOF patients, it remains puzzling how a reduction in Th17 cells can lead to autoimmunity. With the intent to understand the susceptibility to autoimmunity in these patients, a quantitative and qualitative evaluation of Tregs was undertaken by Uzel et al. (50). No differences in Tregs were observed between STAT1-mutated and WT controls (50). In a recent study conducted in natural killer cells, STAT1 GOF mutants impaired STAT5b phosphorylation and reduced natural killer cell proliferation after stimulation with IL-2 and IL-15, suggesting that STAT1 may also control how Tregs respond to IL-2R signaling (56). More work is necessary for elucidating the role of Tregs, Th17, and perhaps other cellular pathways in the immune dysregulation and susceptibility to T1D that characterize patients with STAT1 GOF mutations.

T1D and STAT5b Deficiency

STAT5b is a cytosolic signal transducer involved in multiple cell functions. STAT5b deficiency is very rare, and only a handful of patients with the deficiency have been described; however, its real prevalence may be underestimated in endocrine clinics. Growth defects, Treg-related primary immune deficiencies, and endocrine abnormalities are all possible manifestations described in STAT5b-deficient patients (57). The cytokines IL-2, IL-7, IL-15, and IL-21 have a central role in the initiation of the JAK-STAT5b pathway (24). In particular, IL-2 induces phosphorylation of STAT5 (both STAT5a and STAT5b), which is a necessary step for the induction of $FOXP3^+$ Tregs (58). Given its pivotal role in the generation of Treg cells, STAT5 has become an interesting topic of research in recent years, particularly for understanding the pathogenesis of T1D. Despite there being no cases in the literature describing patients with a diagnosis of T1D and a concomitant defect in STAT5b, STAT5b is known to be fundamental in the accumulation of $CD4^+CD25^+$ Treg cells (59). The importance of the STAT5b pathway was highlighted by studies on NOD mice, where an overexpression of STAT5b was found to be protective against T1D (21). According to Jin et al. (21), the upregulation of $CD4^+CD25^+$ Treg cells resulting from the hyperactivity of STAT5b might explain the decreased incidence of T1D in NOD mice (60). Therefore, the role of STAT5b in human T1D pathogenesis cannot be excluded, even though more research is needed to clarify the function of STAT5b in Treg regulation and its interplay with other STAT family proteins.

Screening for STAT Protein Family Mutations in Patients With T1D

According to recent findings, a number of patients might benefit from genetic screening for STAT. In our opinion, STAT-targeted sequencing (or whole-exome sequencing)

should be performed in certain patients with T1D, such as *1*) patients with T1D presenting with reduced C-peptide and neonatal T1D irrespective of their AAb status who test negative for the known genes of monogenic diabetes (alternatively, the screening gene panel for neonatal diabetes could be modified to accommodate *STAT1*, *STAT3*, *IL2R, FOXP3*, *CTLA4*, *LRBA*, and *AIRE*); *2*) all patients diagnosed with T1D under 5 years of age presenting with/without growth retardation concomitantly and with at least one additional autoimmunity (e.g., autoimmune enteropathy and autoimmune disorders, especially of the endocrine system) showing familiarity; and *3*) patients with T1D and a low T1D GRS (17).

Table 2—Summary showing the altered Th17/Tregs equilibrium described in STAT1 GOF and STAT3 GOF

STAT1 GOF	STAT3 GOF
↓ Th17	↑ Th17
= Treg	↓ Treg

Discerning the Role of STAT Protein Family Mutations in Islet Autoimmunity and Beyond

STAT proteins are not exclusively expressed by the immune cells. They are also expressed by β-cells, and, as it was recently shown, they play a key role in β-cell differentiation. Saarimäki-Vire et al. (61) showed that an activating mutation of STAT3 causes premature endocrine differentiation through direct induction of NEUROG3 expression, leading to pancreatic hypoplasia. This suggests that the some of the clinical features seen in STAT3 GOF patients, such as T1D and perhaps growth retardation, may be due to organ-specific effects of the mutation.

To dissect the role of STATs on the development and functions of the immune system and that of the β-cells, future research is necessary. Genetically modified mice, i.e., knock-in for the corresponding human mutations, could be generated with CRISPR/Cas9 technology in the NOD or autoimmune-resistant background. The impact of STAT mutations could be also addressed in human T and B cells by in vitro isogenic systems. In these systems, cells are manipulated by CRISPR/Cas9 to express the mutated form of STAT and probed for their activation, proliferation, and function.

CONCLUSIONS

Here, we reviewed how STAT monogenic mutations may predispose to T1D. Quantitative changes in Th17 and Treg cells were often present, with Tregs sometimes altered also from a qualitative point of view. While an increased number of Th17 cells with a concomitant decrease in Treg cells explain T1D susceptibility in STAT3 GOF patients, different mechanisms are likely responsible for the onset of T1D in patients with STAT1 GOF mutations who show a reduction in Th17 cells and no alterations in their Treg numbers (Table 2). Currently, many fundamental aspects of STAT biology, and therefore the mechanisms underlying T1D, remain unanswered. The paradoxical and complicated functions of STATs are challenging questions that are directly linked to our understanding of the immunological mechanisms that lead to T1D. We could use these experiments of nature to learn whether specific mutations in *STAT1*, *STAT3*, and *STAT5b* correlate with the immunological and clinical phenotypes of T1D and, furthermore, to broaden our understanding of the differences between polygenic T1D and that caused by mutations in single genes. We believe that determination of the HLA genotype, T1D GRS, AAb status, ethnicity, BMI, and HbA_{1c}, combined with a comprehensive immunological analysis, are necessary for the future reports as they could inform us on the genetic, molecular, and immunoregulatory barriers that define susceptibility to T1D. From a therapeutic clinical perspective, small molecules that interfere with STAT function may be of great potential as we learned by the therapeutic effect of STAT5b overexpression in NOD mice. Currently, some promising results have been obtained in the field of cancer treatment and other conditions with chronic inflammation, such as psoriasis (62). Thus, studying the role of STAT family of transcription factors in T1D pathogenesis could be the focus of future research as it may open novel therapeutic avenues.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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