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Th17 cell-Mediated Responses in Type 1 Diabetes Pathogenesis

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease characterized by a selective destruction of insulin-producing pancreatic beta cells. In general, Th1 cytokines are involved in the development of autoimmune diseases, whereas Th2 and regulatory cytokines result in disease prevention. More recently, a new population of IL-17-producing CD4+ T cells has been proposed to represent a distinct T helper cell lineage, named Th17 cells. Th17 cells are critically involved in the development of many autoimmune diseases; however, the exact role of this T cell subset in T1D pathogenesis remains controversial. Here, we review the conflicting evidences about a possible participation of Th17 cells in T1D development and progression, both in diabetic patients and experimental diabetes models.

Keywords: Autoimmune response; Type 1 diabetes; Th17 cells; IL-17

Abbreviations: T1D: Type 1 Diabetes; NOD mice: Non-Obese Diabetic Mice; STZ: Streptozotocin; G-CSF: Granulocyte Colony-Stimulating Factor; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; SCID: Severe Combined Immunodeficiency; Treg cells: Regulatory T cells; TCV: T-cell Vaccination; PLNs: Pancreatic Lymph Nodes; iNOS: Nitric Oxide Synthase; ATRA: All-Trans Retinoic Acid; RIP-LCMV: Rat Insulin Promoter-Lymphocytic Choriomeningitis Virus; EAE: Experimental Autoimmune Encephalomyelitis; OVA: Ovalbumin; BB rats: Biobreeding Rats; LjN6.2: *Lactobacillus johnsonii* Strain N6.2; CFA: Complete Freund´s Adjuvant

Type 1 Diabetes and Th17 cell Subset

Type 1 diabetes (T1D) is an autoimmune disease characterized by a selective destruction of insulin-producing beta cells in the pancreas. In susceptible individuals, the expansion and activation of autoreactive CD4+ and CD8+ T lymphocytes as consequence of a breakdown in immune regulation lead to an inflammatory response within the islets as well as to a humoral response induction with production of autoantibodies, resulting in insulin-producing beta cell damage [1]. The onset of autoimmune response is mediated by dendritic cells and macrophages that take up and process antigens released from pancreatic beta cells and migrate to the draining lymph nodes carrying these autoantigens. The processed antigens are presented to autoreactive T lymphocytes, resulting in T cell activation, clonal expansion and migration to pancreas. Once in the islets, they are further activated by local antigen-presenting cells to produce cytokines and chemokines that attract and activate other T cells and macrophages [2]. Proinflammatory cytokines produced not only by islet-infiltrating CD4+ and CD8+ T lymphocytes but also by NK T cells, dendritic cells and macrophages, induce the destruction of insulin-producing beta cells in pancreatic islets [3]. In general, IL-1β, TNF-α, IFN-γ, IFN-α, IL-2, IL-6, IL-12, IL-18, IL-21 and IL-17 promote the disease onset, whereas IL-4, IL-10, and TGF-β are known to confer protection to T1D [4]. The central role of T cells in the pathogenesis of T1D is well established [5]. Early studies using antibodies to deplete CD3+ T cells indicated that T1D in the NOD (*non-obese diabetic*) mouse is a T cell-mediated disease [6]. The importance of CD4+ T cells in the development and progression of T1D was demonstrated by several reports using pharmacological tool (anti-CD4 antibodies) or genetic deficiency (CD4-deficient mice) [7,8]. The killing performed by CD8+ T lymphocytes is likely to be a major mechanism of pancreatic beta cell destruction. These lymphocytes found in insulitic lesions of NOD mice

and human are able to promote beta cell cytotoxicity upon activation via MHC class I expressed on target cells. This cytotoxicity mechanism involves the perforin and granzyme pathway or the Fas and Fas ligand-dependent interactions [4].

Initially, the different subsets of CD4+ T helper (Th) cells were thought to be limited to Th1 and Th2 cells. Th1 cells are characterized by the expression of the transcription factor T-bet and the production of interferon-γ (IFN-γ), protecting the host against intracellular infections. However, Th2 cells express GATA-3 and secrete IL-4, IL-5 and IL-13, mediating the host defense against helminthic infections [9-11]. In general, Th1 cytokines (IL-2, IFN-γ) have been shown to be involved in the development of the autoimmune diseases, while Th2 and regulatory cytokines (IL-4, IL-10, TGF-β) have been involved in the disease prevention [12]. More recently, a new population of CD4+ T cells that selectively produce IL-17 has been proposed to represent a distinct T helper cell lineage, named Th17 cells [13]. Th17 cells are characterized by the expression of the transcriptional factor *Rorc* (human) or ROR-γt (mouse) [14] and production of IL-17A (also referred as IL-17), IL-I7F, IL-21 and IL-22 [15]. In addition to CD4+ Th17 cells, IL-17 is also produced by other cell types, including CD8+ T lymphocyte, δγ T lymphocyte and NK cells [16,17].

Apparently, there are species-specific differences regarding how Th17 cells are differentiated from naïve T cells in mice and humans [18]. The combination of TGF-β plus IL-6 efficiently differentiates naïve CD4+ T lymphocytes into IL-17-producing T lymphocytes (Th17 cells) *in vitro* [19]. However, the presence of TGF-β plus IL-6 induces a population of Th17 cells with low pathogenic potential, and a subsequent exposure of Th17 cells to IL-23 seems to be required for the acquisition of pathogenic profile [18,20,21].

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Th17 cells play an important role in host defense against extracellular bacteria and fungi [22]. Both IL-17A and IL-17F are considered potent proinflammatory cytokines that promote the recruitment of neutrophils and monocytes in tissues and induce the production of antimicrobial peptides (mucins, β-defensins and S100 proteins) by epithelial barrier cells [23]. Moreover, these cytokines promote the induction of IL-6 synthesis, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), chemokines (CXCL1, CXCL5, IL-8, CCL2, and CCL7) and matrix metalloproteinases from fibroblasts, endothelial cells, and epithelial cells [24,25]. Furthermore, the association of IL-17 with other inflammatory cytokines such as TNF-α, IFN-γ, IL-1β amplifies the proinflammatory responses from various target cells [25,26]. Although IL-17 plays a protective role in host defense against extracellular pathogens, excessive activation of this pathway contributes to autoimmunity process [25]. Th17 cells and IL-17 levels are elevated in both human and mouse models of autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis [25,27-30]. However, the exact role of Th17 cells in the development of type 1 diabetes remains controversial [31,32]. On one hand there are some reports in the literature showing that Th17 cells play a pathogenic role in diabetes onset and progression. On the other hand, other articles report a dispensable role of Th17 cells in T1D pathogenesis. Surprisingly, there are also evidences showing that Th17 cells can be protective impairing disease development. This review aims to summarize evidences that correlated or not Th17 cell-mediated immune response with T1D pathogenesis, both in diabetic patients and experimental models.

Evidences of Th17 Immunity in T1D Patients

A possible contribution of Th17 cells in human T1D immunopathology has been provided by some reports in the literature (Table 1). Although moderate, a significant increase in the frequency of IL-17-secreting T cells from long-term patients with T1D when compared with healthy controls was observed by Bradshaw et al. However, they did not observe alterations in Th17 cells in recent-onset diabetic patients. Moreover, monocytes isolated from patients with T1D induced more IL-17-secreting T cells compared with monocytes from healthy control subjects, suggesting that the innate immune system in T1D may drive the adaptive immune system by expanding the Th17-effector cell population [33]. In addition, increased IL-17-secreting T cells was also observed in children with new-onset type 1 diabetes. The new-onset T1D subjects presented both CD4+ and CD8+ peripheral T cell populations biased toward IL-17 secretion [34]. Additional supporting evidence came from the study conducted by Honkanen et al. that also investigated Th17 immunity in children with T1D. Peripheral T cell from diabetic children exhibited enhanced IL-17 secretion and IL-17A transcripts after stimulation with anti-CD3/CD28 antibodies when compared with healthy children. Additionally, the authors showed that IL-17 had detrimental effects on human islet cells increasing inflammatory and proapoptotic responses in vitro, emphasizing the important role of IL-17 in human beta cells destruction [35]. In addition, Han et al. studied the immune profile of peripheral blood mononuclear cells isolated from at-risk (presence of 3 or more autoantibodies), new-onset and long-term diabetic patients by gene expression analysis. The authors reported that IL-17 mRNA levels were significantly higher in new-onset patients when compared with at-risk, long-term and healthy individuals. However, not all new-onset patients had detectable levels of IL-17 mRNA. The elevated IL-17 gene expression in new-onset patients suggests that IL-17 may be actively involved in T1D development [36]. In this context, Arif et al. addressed the question of the mechanisms by which IL-17 promotes beta cell death in patients with new-onset diabetes. Peripheral IL-17-producing T cells from diabetic patients exhibited increased response to beta cell antigens, observed by an augmented IL-17 production. In addition, IL-17A gene was six fold higher expressed in purified islet from diabetic patients when compared with control samples. However, IL-17 expression was not detected in the long-standing patients, suggesting that IL-17-producing cells are more involved in the early phases of T1D development. Interestingly, IL-17 alone had no apoptotic effect in cultured human islets but in combination with IL-1β, IFN-γ and TNF-α exacerbated islets apoptosis by increasing nitrite release. Treatment of human islets with IL-1β and IFN-γ promoted upregulation of IL-17A receptor by STAT1 and NF-κB transcriptional pathways, becoming more susceptible to the destructive action of IL-17. Looking at these results, Th17 immune response seems to act in synergism with Th1 cells in human T1D pathogenesis [37].

An imbalance of regulatory T cells (Treg)/Th17 cells in diabetic patients can also be related with T1D pathogenesis, since Ferraro et al. observed that the Treg/Th17 cells ratio was about five times lower in

Authors [Ref]	T1D Subjects	Observations
Bradshaw et al. [33]	Recent and long-term patients	Increased frequency of IL-17 secreting cells in long-term diabetic patients, increased production of IL-1β and IFN-γ by monocytes driving Th17 profile.
Marwaha et al. [34]	New-onset patients	Increased proportion of both CD4+ and CD8+ T cells that produce IL-17.
Honkanen et al. [35]	Diabetic children	Peripheral T cells: Increased IL-17 secretion; increased levels of IL-17A, RORC2, IL-22 transcripts; IL-17 increased inflammatory and apoptotic responses in human islets cells.
Han et al. [36]	At-risk, new-onset and long-term patients	Increased IL-17 mRNA levels in new-onset diabetic patients when compared with at-risk, long-term and healthy individuals.
Arif et al. [37]	New-onset patients	Presence of circulating beta cells-specific Th17 cells, increased IL-17A expression in pancreatic tissue, IL-17 plus IL-1β, IFN-γ and TNF-α promoted beta cell apoptosis.
Ferraro et al. [38]	Long-term patients	Increased numbers of Th17 cells in pancreatic lymph nodes, increased IL-17 production after islet-antigen stimulation, decreased numbers and function of Treg cells, Treg/Th17 cell ratio was 4-5 times lower than health group.

Table 1: Investigations about the role of Th17 immune response in patients with T1D.

pancreatic lymph nodes (PLNs) from patients with T1D. The diabetic group exhibited increased frequency of IL-17-producing cells in PLNs, but not in peripheral blood, when compared with health individuals. Moreover, the frequency of Treg cells were decreased in PLNs from T1D group and their suppressive capacity was defective. However, despite these observations, it is still unknown whether the Treg/Th17 imbalance is a cause or a consequence of T1D development [38].

Taking together, the studies mentioned above strongly correlate Th17 immunity with human T1D pathogenesis. Th17 cells seem to act as pathogenic effectors cells in T1D setting and the increased levels of IL-17 in diabetic patients confirm the participation of this cell subset during disease development and progression. Contrary to the data provided from studies with diabetic patients, the role of Th17 cells during the development and progression of T1D in animal models is very controversial. Next we summarize the growing body of controversial data present in the literature about the role of Th17 immunity in experimental models of T1D (Table 2).

Central versus Accessory Role of Th17 cells in Experimental T1D Pathogenesis

Since the discovery of Th17 cells as a new subset of T helper cells, there is growing body of evidences suggesting a central role of Th17 cells and IL-17 in experimental T1D development. Vukkadapu et al. aimed to indentify key changes in BDC2.5TCR transgenic mice (TCR specific to islet antigens) during the course of T1D development using microarray analysis. The authors showed that plasma IL-17 levels were elevated during the onset of severe insulits (10 days old) and *Il17* gene was upregulated in the pancreas during late-stage of insulitis (3 weeks old), suggesting a possible role of this cytokine in disease pathogenesis [39]. Moreover, Miljkovic et al. observed increased concentrations of serum IL-17 in streptozotocin-induced diabetes model (rat and mouse) 10-15 days after the last dose of streptozotocin. The authors also showed the ability of IL-17 to upregulate the expression of nitric oxide synthase (iNOS), causing toxicity in cultures of the MIN6 beta cell line and in mouse pancreatic islets [40]. Using the same experimental model of

Authors [Ref]	Animal model	Intervention	Th1/Th17	Details
Vukkadapu et al. [39]	BDC2.5/ NOD mice	-	↑ IL-17	Upregulation of *Il17* gene in pancreas and increased IL-17 plasma levels during disease onset.
Miljkovic et al. [40]	STZ induced diabetes	-	↑ IL-17	Increased IL-17 level in diabetic animals, IL-17 promoted upregulation of iNOS and increased beta cell toxicity.
Mensah-Brown et al. [42]	STZ induced diabetes	IL-23 administration	↑ IFN-γ ↑ IL-17	Enhanced diabetogenic process, increased pancreatic inflammation and beta cell loss.
Jain et al. [43]	NOD mice	Ig-GAD2	↑ IFN-γ ↓ IL-17	Delayed T1D onset at insulitis stage, increased production of IFN-γ and inhibition of IL-17 secretion: reduced islet inflammation, restored normoglycemia at prediabetic stage.
Emamaullee et al. [44]	NOD mice	Anti-IL-17 antibody or Recombinant IL-25	↓ IL-17	Inhibition of Th17 cells on effector phase: Prevented progression of T1D, reduced islet inflammation and autoantibody formation. IL-25 treatment: restored euglycemia, reduced frequency of Th2 and Th17 cells, increased Treg population.
Liu et al. [45]	NOD mice and NOD.*Idd3* mice	-	↑ IL-17	Cells from NOD mice: increased production of IL-21, increased Th17 differentiation, increased production of pro-Th17 mediators by APCs.
Zhang et al. [46]	NOD mice	IL-12 administration	↑ IFN-γ ↓ IL-17	Prevention of T1D development, decreased insulitis, increased healthy islets, decreased Th-17 cytokines levels.
Spolski et al. [47]	IL-21 knockout NOD mice	-	↓ IL-17	Resistance of T1D development, reduced numbers of Th17 cells and IL-17 levels.
Zhao et al. [49]	NOD mice	Bone marrow stromal cells	↓ IL-17 = IFN-γ	Decreased blood glucose levels, decreased insulitis, increased Treg cells and decreased Th17 cells.
Wang et al. [50]	STZ induced diabetes	T-cell vaccination	↓ IFN-γ ↓ IL-17	Decreased blood glucose levels, decreased Th1 and Th17 cytokines, increased Th2 cytokines. Transference of Th17 cells accelerated disease development.
Yaochite et al. [51]	STZ induced diabetes (IL-17 receptor deficient mice)	-	↓ IL-17	Absence of IL-17 signaling: impaired diabetes development, reduced peri-insulits, increased beta cell mass preservation.
Shi et al. [53]	NOD mice	-	↑ IL-17	NOD x Balb/c mice: Decreased Treg cells, increased Th17 cells. Diabetic NOD x Nondiabetic NOD: higher Th17/Treg ratio.
Bending et al. [56]	Adoptive transfer system (Th17 cells from BDC2.5 mice to NOD/SCID mice)	-	Th17 →Th1	Tranfer of Th17 cells induced diabetes in NOD/SCID recipient after conversion into Th1 cells. Neutralizing IFN-γ antibody prevented disease development, anti-IL-17 antibody had no effect.
Martin-Orozco et al. [57]	Adoptive transfer system (Th17 cells *in vitro*-differentiated from BDC2.5 mice to NOD/SCID mice)	-	Th17 →Th1	Transfer of Th17 cells induced diabetes in NOD/SCID recipient after conversion into Th1 cells.
Wan et al. [54]	Adoptive transfer system (Th17 cells *in vitro*-differentiated from BDC2.5 mice to NOD/SCID and NOD mice)	-	Th17→Th1 (NOD/SCID mice) Stable Th17 (NOD mice)	NOD/SCID recipient: Transferred Th17 cells converted into Th1 cells to promote disease. NOD recipient: Absence of conversion, promoted pancreatic inflammation without clinical diabetes.
Van et al. [71]	Adoptive transfer system (splenocytes from NOD mice to NOD/SCID mice)	All-trans retinoic acid	↓ IFN-γ = IL-17	Inhibition of T1D development, decreased islet inflammation, suppression of Th1 but not Th17 cells, Treg expansion.

Joseph et al. [72]	IL-17 knockdown NOD mice	-	Absence of IL-17	No effect on frequency or disease onset. Dispensable role of IL-17 in T1D pathogenesis.
Van Belle et al. [55]	RIP-LCMV mice	-	Absence of IL-17	T1D development in absence of detectable IL-17A production. Dispensable role of IL-17A in T1D pathogenesis.
Ankathatti Munegowda et al. [52]	Adoptive transfer system (OVA-specific Th17 cells to RIP-OVA mice)	-	-	Transferred Th17 cells stimulated CD8+ T cells. Activated CD8+ T cells promoted islets destruction via perforin-pathway. Indirect role of Th17 cells in T1D pathogenesis.
Lau et al. [73]	Bio-breeding diabetes prone rats (BBDP rats)	*Lactobacilus johnsonii* (LjN6.2)	↑ IL-17	LjN6.2 feeding: Diabetes resistance, increased IL-17, IL-23 and IL-6 levels. Protective role of Th17 immune response.
Nikoopour et al. [77]	NOD mice	Mycobacterial adjuvants	↑ IL-17	Prevention of T1D onset, increased levels of IL-17 in spleen, lymph nodes and pancreas. Transference of polarized Th17 cells from CFA-treated NOD mice into NOD/SCID recipient delayed disease onset: important role of IL-17 in this protection.
Tse et al. [78]	NAPDH oxidase deficient NOD mice	-	↓IFN-γ ↑ IL-17	NADPH oxidase deficiency promoted Th17 polarization: protection against T1D and decreased insulitis.

Abbreviations: T1D: Type 1 Diabetes; NOD mice: Non-Obese Diabetic Mice; STZ: Streptozotocin; SCID: Severe Combined Immunodeficiency; Treg cells: Regulatory T cells; RIP-LCMV: Rat Insulin Promoter-Lymphocytic Choriomeningitis Virus; OVA: Ovalbumin; CFA: Complete Freund´s Adjuvant.

Table 2: Investigations about the role of Th17 immune response in animal models of T1D.

T1D, Mensah-Brown et al. demonstrated that administration of IL-23, a cytokine that positively regulates the IL-17 production and promotes the expansion of Th17 cells [41], enhanced the diabetogenic process after the injections of subdiabetogenic concentrations of streptozotocin [42]. The prolonged IL-23 treatment promoted an increase in islet infiltrating cells, loss of beta cell mass, increased levels of TNF-α, IFN-γ and IL-17 in the islets and expression of iNOS in islet-infiltrating cells. In this study, the authors correlated the IL-17 expression with the intensity of mononuclear cell infiltrate and beta cell damage [42]. The important role of IL-17 in T1D pathogenesis was also observed by Jain et al. in which the production of IFN-γ induced by adjuvant-free antigen restored normoglycemia in NOD mice through the inhibition of IL-17 production. In this study, the administration of peptide GAD2 incorporated into an immunoglobulin molecule (Ig-GAD2) resulted in IFN-γ production that diminished splenic and pancreatic IL-17 causing reversal of T1D [43]. Taking together, these studies revealed that T1D progression is related to Th17 cells activity.

In another study conducted by Emamaullee et al. the authors investigated the role of Th17 cells in NOD mouse model using two approaches to inhibit Th17 cells: administration of neutralizing anti-IL-17 antibody or recombinant IL-25. In the early stage (5 weeks of age), the treatment with anti-IL-17 antibody or recombinant IL-25 did not prevent diabetes development. However, both treatments conferred protection against T1D when the administration was initiated on effector phase of disease (10 weeks of age), reducing insulitis, decreasing autoantibody formation and increasing regulatory T cell population. Interestingly, IL-25 but not anti-IL-17 treatment was able to control established diabetes in hyperglycemic mice [44]. Liu et al. demonstrated that naïve T cells from NOD mice have a greater capacity to differentiate into Th17 cells rather than T cell from diabetes-resistant NOD.*Idd3* mice. Antigen presenting cells (APCs) from these two mouse strain displayed differences to support Th17 differentiation, especially relative to IL-21 production. Moreover, NOD exhibited the Treg/Th17 ratio shifted toward pathogenic cells, which the opposite is observed in the resistant mouse strain. The impairment in the generation of Th17 cells can be related with the disease resistance in NOD.*Idd3* strain [45]. Zhang et al. showed that T1D onset in NOD mice is mediated by Th17 cells and the intermittent IL-12 administration prevented insulitis and inhibited T1D development in this experimental model. The authors showed that the IFN-γ produced downstream of IL-12 signaling inhibited the development of Th17 cells. In addition, IL-12 indirectly inhibited the Th17 cells by suppressing the Th17-associated cytokines:

IL-1β, IL-6 and IL-23. The inhibition of Th17 cells and the balance of cytokines resulted in the prevention of T1D development, diminished islet inflammation and increased number of preserved islets [46]. Spolski et al. showed that IL-21-deficient NOD mice did not develop T1D. In addition, mice lacking IL-21 exhibited reduced numbers of IL-17 secreting cells in spleen and pancreatic lymph nodes, suggesting a possible participation of IL-17 in beta cell damage [47]. In addition, one study revealed that efficient migration of pancreatic dendritic cells to pancreatic lymph nodes is dependent on IL-21 receptor, which leads to infiltration of autoreactive CD8+ T cells to the pancreas during T1D [48]. The amelioration of the T1D as result of the Th17 cell modulation was also observed by Zhao et al. after bone marrow stromal cells transplantation. The bone marrow stromal cells are known to have many immunomodulatory properties and once infused in NOD mice promoted reversal of T1D and insulits by decreasing serum IL-17 levels and increasing IL-10 and TGF-β levels [49].

Using T-cell vaccination (TCV) approach, Wang et al. observed that TCV therapy resulted in protection against disease development and preservation of pancreatic islets in streptozotocin-induced diabetes model. This treatment was able to shift the response from Th1 pattern toward Th2 response and also decrease the levels of IL-17 and IL-23 produced by intrapancreatic infiltrating lymphocytes (Th17 cells) via Stat3-mediated ROR-γt inhibition. The transfer of Th17 cells (*in vitro*-differentiated) into prediabetic mice resulted in the development of sustained hyperglycemia whereas naïve T cells had no effect. The administration of neutralizing anti-IL-17 antibody, but not anti-IFN-γ, after the transfer of Th17 cells impaired the disease development, suggesting an important role of Th17 cell function in STZ-induced diabetes model [50]. In accordance with this, our research group reported recently the important role of Th17 cells during the early stages of diabetes development in STZ model using mice lacking the expression of IL-17 receptor. Six days after diabetes induction, IL-17 receptor deficient mice showed impairment in STZ-induced diabetes progression, reduced peri-insulitis and beta cells preservation when compared with wild-type mice, suggesting that IL-17 signaling contributes to initiation of diabetes development [51]. Collectively, these reports indicate that Th17 cells may be directly involved in inflammatory response against pancreatic islets and the suppression of Th17 cells may represent a possible target for the prevention or reversal of T1D.

On the other hand, an indirect participation of Th17 cells in T1D pathogenesis was proposed by Munegowda et al. CD4+ OVA-specific

Th17 cells were generated *in vitro* and then transferred into a RIP-OVA model depleted or not of CD8+ T cells by the administration of neutralizing anti-CD8 antibody. The authors showed that T1D was directly mediated by T CD8+ cells stimulated by Th17 cells and Th17 cells alone (in absence of CD8+ T cells) were not able to promote the disease. The CD8+ T cells activated by transferred CD4+ Th17 cells destroyed pancreatic islets via perforin-dependent pathway. On the contrary, Th17 cells by themselves played a major role in the pathogenesis of EAE by the production of proinflammatory Th17 cytokines [52]. Looking at these results, Th17 cells can exhibited an accessory role in T1D activating other pathogenic effector cells rather than participating directly in beta cell damage.

An imbalance of Treg cells and Th17 cells may contribute to the onset of T1D in NOD mice. Comparing Balb/c mice with NOD mice, Shi et al. observed that NOD mice exhibited enhanced proportion of Th17 cells and decreased frequency of Treg cells in spleen and pancreatic lymph nodes compared with nondiabetic Balb/c mice. Moreover, diabetic NOD mice exhibited higher Th17/Treg ratio when compared with nondiabetic NOD mice, suggesting that the onset of T1D is associated with the imbalance of the Th17/Treg axis during disease progression. Additionally, the oral treatment of NOD mice with *Cordyceps sinensis*, a fungus with immunoregulatory properties, promoted a delay in T1D development, decreasing the ratio of Th17 cells to Treg cells [53]. A more recent report published by Wan et al. showed that Th17 cells were unable to undergo Th1 conversion upon transfer into NOD mice, requiring the Treg cell depletion to transfer the disease [54].

The Plasticity of Th17 cells During T1D Development

Although of the reports cited above supported an important role for Th17 cells/IL-17 in T1D pathogenesis, data from transfer models shed different light on this [55]. Two groups using a model of adoptive transfer showed the plasticity of Th17 cells in T1D setting, indicating that Th17 cell transfer induces the disease through conversion to Th1 cells *in vivo* [56,57]. The plasticity in CD4+ T cell subsets has been discussed, but the circumstances under this phenomenon are not well understood [32]. Bending et al. used the BDC2.5TCR transgenic mouse to investigate the role of Th17 cells in T1D. They found, using highly purified populations of cells, that although Th17 cells appeared to transfer disease to NOD/SCID recipients, this was in fact due to a conversion of these cells into Th1 cells. Moreover, the transferred Th17 cells lost the ability to promote the disease after the treatment with neutralizing IFN-γ antibody. On the other hand, the treatment with anti-IL-17 specific antibodies did not prevent disease development, suggesting a major role of Th1 cells in the induction of T1D in NOD mice [56]. At the same time, additional supporting evidence about Th17 plasticity and the central role of Th1 cells in T1D came from the study conducted by Martin-Orozco et al. The authors differentiated BDC2.5TCR CD4+ T cells into Th17 cells *in vitro*, transferred them to NOD/SCID recipients and observed that Th17 cells promoted disease development with extensive islet destruction. However, the donor BDC2.5TCR Th17 cells were converted into Th1 cell *in vivo*, indicating that Th17 cells exhibit plasticity in lymphopenic hosts and the T1D development in these animals is dependent on IFN-γ production [57]. Corroborating these two studies, Wan et al. showed that polarized BDC2.5TCR Th1 cells induced diabetes within 4 to 5 days after adoptive transfer into NOD/SCID mice, while BDC2.5TCR Th17 cells delayed the disease onset up to 9 days, time needed to complete the conversion into Th1 cells. Neutralization of IFN-γ, but not IL-17,

abrogated the diabetes development in NOD/SCID mice. On the other hand, the transfer of Th17 cells into immunocompetent mice, such as NOD mice, did not result in cell conversion and promoted pancreatic inflammation without hyperglycemia [54]. Contrary to these reports, Liu et al. showed that Th17 cells can induce T1D independently of IFN-γ. The authors generated islet-specific Th17 cells from IFN-γ-/- deficient BDC2.5TCR transgenic mouse and demonstrated that they were efficient in promoting the T1D upon adoptive transfer into recipient mice [45].

The plasticity of Th17 cells has also been recently demonstrated by several reports that described the existence of a subtype of Th17 cells able to produce both IL-17 and IFN-γ which is named IL-17/IFN-γ double positive T cells or Th17/Th1 cells [20,58-61]. It has been shown that Th17-polarizing treatments in the absence of TGF-β induced double-secreting Th17 cells which co-express IL-17 and IFN-γ whereas polarization in the presence of TGF-β induced IL-17 single-secreting Th17 cells [62,63]. Moreover, recent observations indicate that IL-17+/IFN-γ+ double producers arise from Th17 exposed to certain cytokine milieus and not from Th1 cells that retain their phenotype [64,65].

In addition to IL-17 and IFN-γ single-producing T cells, the number of IL-17/IFN-γ double-positive T cells is increased in both human and mouse inflamed tissues [20,65-68]. The presence of this double positive T cell population that shares features of both Th1 and Th17 was detected in the gut of patients with Crohn's disease, in the brain lesions and peripheral blood of multiple sclerosis patients and in the synovial fluid of established arthritis rheumatoid patients [58,69,70]. However, the role of this Th17/Th1 cell population in T1D setting is unclear. Honkanen et al. reported the existence of peripheral IL-17/IFN-γ double positive CD4 T lymphocytes in 4 out of 11 diabetic children tested. The concomitant presence of IL-17 and IFN-γ may result in additive beta cells damage due to the synergistic effects mediated by these two cytokines on the islets cells [35]. Bending et al. showed that transferred Th17 cells started to produce IFN-γ in NOD/SCID recipient mice. High frequency of IL-17/IFN-γ double positive cell population was observed in pancreatic lymph nodes 8 days after Th17 cells transference. NOD/SCID recipient mice developed T1D; however, the capacity of Th17/Th1 cells by themselves in promoting disease was not evaluated [56].

In general, little is known about the function of these Th17/Th1 cell subset and assessing the heterogeneity within each population is important for deciphering their origin and respective roles during inflammation, host defense and autoimmunity [66].

A Dispensable Role of Th17 cells/IL-17 in Experimental T1D Pathogenesis

A dispensable role of Th17 cells in T1D pathogenesis was described by Van et al. The authors demonstrated that the treatment with all-trans retinoic acid (ATRA) inhibited the development of T1D in an adoptive transfer animal model. Splenocytes from NOD mice were adoptively transferred to NOD/SCID mice and the recipients were treated or not with ATRA. The treated animals presented a delay in the diabetes onset, decreased numbers of CD8+ infiltrating T cells and expansion of Foxp3+ regulatory T cells. The treatment with ATRA suppressed IFN-γ but not IL-17 producing T CD4+ cells in pancreatic lymph nodes and islets, suggesting a central role of Th1 cells in the pathogenesis of T1D [71]. Using a lentiviral transgenesis to generate NOD mice in which IL-17 is silenced by RNA interference, it was recently demonstrated that the absence of IL-17 did not protect NOD

from T1D development. The loss of IL-17 had no effect on the incidence or disease onset on spontaneous or cyclophosphamide-induced diabetes, suggesting that IL-17 and consequently Th17 function may be dispensable in T1D pathogenesis [72]. Van Belle et al. observed the development of autoimmune diabetes in the absence of detectable IL-17A in a CD8 driven virally induced model. The authors developed a CD8-driven rat insulin promoter-lymphocytic choriomeningitis virus (RIP-LCMV) murine model that express viral antigens on pancreatic beta cells inducing a rapid CD8+ T cells response against viral peptides. The RIP-LCMV mice were crossed to IL-17A eGFP reporter mice to detect the production of IL-17A *in situ*. Using this model of diabetes, the authors did not observe the presence of IL-17 in spleen, PLNs and pancreas, neither when the cells were isolated and restimulated *in vitro*, in both diabetic and prediabetic mice. In addition, when autoantigen-specific CD4+ T cells were transferred into RIP-LCMV mice they did not convert into Th17 phenotype, producing preferentially IFN-γ but not IL-17A. Taking the results together, it is possible to suggest that IL-17 is unlike to be an essential molecule in autoimmune T1D [55].

A Protective Role of Th17 cells/IL-17 in Experimental T1D Development

A possible protective role of IL-17 and Th17 cells during T1D was proposed. The Bio-Breeding diabetes prone (BBDP) spontaneous develops T1D, and the Bio-Breeding diabetes resistant (BBDR) rat only develops the disease after environmental changes. Lau et al. correlated the T1D development in these models with resident gut flora and Th17 immune response pattern [73]. Previously, the same group showed that BBDP rats fed with *Lactobacillus johnsonii* strain N6.2 (LjN6.2) became resistant to T1D development, and the treatment with PBS or *Lactobacilus reuteri* (Lr) did not prevent disease onset [74]. After this study, the authors investigated the mechanisms involved in T1D resistance promoted by LjN6.2. Nondiabetic BBDP rats fed with LjN6.2 showed a significant Th17 bias represented by elevated levels of IL-17 and IL-23 in gut-associated mesenteric lymph nodes when compared with diabetic BBDP rats. In addition, LjN6.2 promoted an increase in the levels of IL-6 and IL-23, cytokines involved in induction and maintenance of Th17 cells. These current results suggest that Th17 cells generated by LjN6.2 feeding are protective during T1D, probably inhibiting the T cell conversion to a diabetogenic phenotype [73]. A protective role of IL-17 in preventing T1D development was also reported by Nikoopour et al. There are many evidences showing that immunization with mycobacterial preparations prevents the onset and progression of experimental T1D [75-77]. The injection of mycobacterial products in NOD mice promoted an increase of IL-17 production in spleen, draining lymph nodes and pancreatic tissue. Interestingly, adoptive transfer of IL-17 producing cells differentiated from Complete Freund´s Adjuvant-treated NOD mice into NOD/SCID recipient mice delayed the T1D development with maintenance of their Th17 phenotype after the adoptive transfer. In addition, the use of IL-17 neutralizing antibody reduced the disease protection, suggesting an important regulatory role of IL-17 in diabetes progression [77]. In parallel, using NADPH oxidase deficient NOD mice, Tse et al. showed that the lack of NADPH oxidase resulted in protection against T1D. The mechanism involved in this protection was the polarization of T cells to Th17 phenotype instead of Th1 pattern in NOD mice NADPH oxidase deficient, suggesting that Th1 cells are more pathogenic than Th17 cells in promoting T1D [78].

These studies implicate that retention of the Th17 differentiation state may inhibits T cell conversion to the diabetogenic phenotype,

thus preventing or significantly inhibiting the onset of T1D. Moreover, Th17 cells may not be intrinsically diabetogenic, but have the capacity to become diabetogenic in the absence of intervention [73]. However, we cannot discard the influence of pathogens in these studies above mentioned. The infection can induce the expansion of Treg cells resulting in inhibition of effector cells. The impairment of T1D development observed in these studies can be related with the increased frequency of Treg cells instead of the presence Th17 cells. Further investigations are needed to clarify this possible protective role of Th17 cells during T1D development in absence of pathogens.

It is important to note that the function of Th17 cells can possibly be modulated by different cytokines during the polarization process [20,21]. McGeachy et al. showed that myelin-reative T cells cultured with TGF-β plus IL-6 can produce IL-17 and IL-10, exhibiting a regulatory phenotype, whereas IL-23 induce a pathogenic profile in EAE setting [20]. In accordance, Singh et al. reported that BDC2.5 CD4+ T cells polarized to Th17 pattern by TGF-β plus IL-6 exhibited a regulatory phenotype, and failed in inducing the disease in NOD mice. Contrary, the preactivation of T cells with IL-23 and IL-6 induced diabetogenic Th17 cells that could lead to the T1D in NOD mice [3]. Thus, these studies suggest that the microenvironment during T cell polarization is essential to determine the profile of Th17 cells: regulatory Th17 profile or pathogenic Th17 profile.

Concluding Remarks

Even with the wide range of published reports about the possible role of Th17 immunity in T1D, it is still uncertain the exact function of Th17 cells during T1D development and progression. There is a body of data suggesting that Th17 cell-mediated immune response plays a central role in disease pathogenesis both in T1D patients and experimental models. On the other hand, some reports show a dispensable participation of Th17 immunity in experimental T1D development. A third line of evidence suggests a protective role of Th17 cells in preventing disease development. It is not clear whether Th17 cells are involved in the initiation of autoimmune response or if they contribute directly to the active phase of disease development. Emamaulle et al. proposed that Th17 cells can be involved in directing an immune response in the secondary lymphoid tissues rather than participating directly in beta cell destruction, since low frequency of Th17 cells within the insulitic lesions was observed in NOD mice [44]. Another possibility is that Th17 cells might recruit or activate macrophages to the islets promoting an uncontrolled immune response that leads to intense inflammatory cell infiltration inside the islet, facilitating T1D development. This activation of inflammatory cells in the islets can further sustain Th1 differentiation or IFN-γ production by macrophages and NK T cells that will promote islet beta cell death *in situ*. In this point of view, Th17 cells seem to exhibit an accessory role in T1D pathogenesis, amplifying the immune response against beta cells [57]. Confirming this indirect role of Th17 cells in T1D, it was demonstrated that Th17 cells have the ability to activate pathogenic CD8 T cells that promote beta cells destruction [52].

It is probable that there are multiple pathways through which beta cells are destroyed during the development of T1D. It is known that beta cells are very susceptible to the proapoptotic actions of IL-1β and IFN-γ or TNF-α and IFN-γ [79] and IL-17 can possible act exacerbating pancreatic cell destruction. Taking this into account, Th1 cells and Th17 cells could work in synergism to promote T1D. Arif et al. proposed in 2011 a possible mechanism by which Th1 and Th17 cells act to promote beta cell death. Th1 cells and macrophages

produce IFN-γ and IL-1β that bind to their receptors present in beta cell surface, resulting in STAT-1 and NF-κB activation and expression of IL-17 receptor. After this, Th17 cells produce IL-17 that binds to its receptor leading to increase of beta cell damage that was initiated by IFN-γ and IL-1β [37]. Thus, both Th1 and Th17 cells can be considered key participants in the autoreactive response against pancreatic beta cells, however, the factors involved in the initiation of the autoimmune response remain unclear [32].

The plasticity of Th17 cells was recently highlighted *in vivo* in mouse models of ocular inflammation, colitis, or diabetes showing that after adoptive transfer of Th17 cells, these cells converted rapidly into IFN-γ-producing cells that were critical for disease development [54,56,57,65,66]. Th17 cells only induced diabetes efficiently after conversion into IFN-γ-producing cells in lymphopenic hosts [53,55,56]. However, the transference of Th17 cells in immunocompetent animals should be done to evaluate the possible convertion of the transferred cells into Th1 phenotype and the capacity in inducing diabetes in the presence of an intact immune system. Whether Th1 and Th17 cells are mutually antagonistic remains unknown and the presence of T cells producing IL-17 and IFN-γ in inflammatory tissues complicates this relationship. Further studies should be performed to address whether such converted IFN-γ- producing cells belong purely to the Th1 phenotype or have more characteristics of the double positive Th17/Th1 subset [66]. Moreover, little is known about the role of IL-17/IFN-γ double positive cells during T1D development, and a deep investigation about the presence, plasticity and function of these cells during the course of the disease is necessary.

Most reports evaluated the role of Th17 immunity in T1D development focusing on the presence and production of IL-17, and we cannot discard that Th17 cells may be involved in T1D through different effector mechanisms than IL-17 production (such as IL-21 and IL-22 production), and further studies are necessary to test whether the complete absence of Th17 cells would indeed be protective [72].

In an attempt to elucidate these wide range of questions and possibilities, further studies are required to clarify the exact role of Th17 immune response in T1D pathogenesis. Furthermore, understanding how and when these cells act during the autoimmune response will enable the development of new drugs that target Th17 cells improving the treatment of T1D.

Acknowledgments

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