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REVIEW

# Children diagnosed with both type 1 diabetes and celiac disease -an Immunological challenge

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**Type 1 diabetes (T1D) and celiac disease are both characterized by an autoimmune feature. As T1D and celiac disease share several common risk factors such as environment, genetics and immune dysregulation, patients have risk of developing the other disease subsequently. Patients with manifest T1D may have had a latent celiac disease, which is activated parallel to the anti-islet immune reactivity during the development of T1D. Contrary, a low prevalence of  $\beta$ -cell autoimmunity is found in young patients with celiac disease. The role of antigen-specific T cells and their relation to cytokines and chemokines is not well characterized in children with combination of T1D and celiac disease. Defective regulation and an impaired ability of responder T cells to be suppressed are suggested to contribute. We have previously shown that children suffering from these two immunological diseases in combination have a suppressed immune response to several antigens for example food antigens like gluten. Poor development of oral tolerance seen as immune aberrancies with low percentages of both early and late effector memory CD8<sup>+</sup> cells in the gut of children who are prone to T1D may predispose for development of celiac disease. This review highlights the immunological complexity in these two common pediatric immunological disorders that indicates that the combination of type 1 diabetes and celiac disease is an immunological challenge. It is obvious that we are far from understanding the immunological impact of these two autoimmune diseases in combination. This immunological challenge therefore needs to be elucidated to be able to predict and prevent these autoimmune diseases.**

**Keywords:** type 1 diabetes; celiac disease; children, immune system; immune response

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## The cell-mediated part of the immune system

### *T-helper and T-cytotoxic cells*

The obvious goal for the immune system is to protect us from viruses, bacteria and parasites invading our body. For this purpose the immune system keeps a number of different

sorts of immune cells for example T-helper (Th; CD4<sup>+</sup>), T-cytotoxic (Tc; CD8<sup>+</sup>) and T-regulatory (Treg; CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>CD127<sup>-</sup>) cells. T-helper as well as Tc cells differentiate from naïve cells to central memory and later on to effector memory cells. Memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells can be further divided into subpopulations by their expression of CD27 and CD28 and further differentiated by

expression of CD45RA and CCR7 into the subsets; naïve (CD27<sup>+</sup>CD28<sup>+</sup>CD45RA<sup>+</sup>CCR7<sup>+</sup>), effector memory (T<sub>EM</sub>) by early differentiation (CD27<sup>+</sup>CD28<sup>+</sup>CD45RA<sup>-</sup>CCR7<sup>-</sup>) or late differentiation (CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>-</sup>CCR7<sup>-</sup>), central memory (T<sub>CM</sub>, CD27<sup>+</sup>CD28<sup>+</sup>CD45RA<sup>-</sup>CCR7<sup>+</sup>), or as terminally differentiated (TEMRA) (CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+</sup>CCR7<sup>+</sup>) cells [1].

#### *Cytokine and chemokine production by T-helper and T-cytotoxic cells*

Naïve T cells can differentiate into for example T-helper cells, upon interaction with antigen-presenting cells in a specific cytokine milieu, focusing on helping other cells in the immune system. The original paradigm of two distinct subpopulations of Th cells; Th1 and Th2 cells have during the last decade been expanded to include also Th3, Th9, Th17 and Th22 cells based upon for example their lineage-specific production of cytokines and chemokines.

T-helper 1 together with Th2 cells were the first two subpopulations to be described by Mosmann *et al* in 1986 [2]. Naïve T cells, in a cytokine context of interleukin (IL)-12 and interferon (IFN)- $\gamma$ , presented for an antigen by dendritic cells will differentiate into Th1 cells. Activation of Th1 cells by signal transducer and activator of transcription (STAT)-4 and T-box expressed in T cells (T-bet) will cause cytokine secreting of especially IFN- $\gamma$ , IL-2 and tumor necrosis factor (TNF) [3, 4] and promote cell-mediated immunity for example cytotoxic and inflammatory responses mediated by macrophages, natural killer (NK) and T cells.

T-helper 2 cells require the transcription factors STAT6 and the zinc-finger GATA3 [5] as well as IL-4 and IL-33 for expansion and thereby produce a panel of different cytokines for example the interleukins; IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 [6]. These Th2 cells and their secreted cytokines are important players in humoral immunity for example antibody production by B-cells and enhanced eosinophil proliferation. Thus, human Th1 and Th2 cells produce certain patterns of cytokines, although the characterization of Th subpopulations are not as tightly restricted as found in animal models. Cytokines derived by Th1 and Th2 cells also exert their effects in collaboration with cytokines elaborated by other subsets of Th cells.

The concept of Th1 and Th2 immunity can also be detailed by their different sets of chemokine receptors and chemokine production allowing them to migrate to different tissues. Chemokines can be broadly divided into two categories: inflammatory, which are induced or strongly upregulated in peripheral tissue by inflammation; and constitutive, which fulfil housekeeping functions and may be

involved in constitutive leukocyte traffic [7]. Different sets of chemokine receptors are expressed on Th cells. The chemokines; C-C motif ligand (CCL) 2 (monocyte chemoattractant protein (MCP)-1) [8], CCL3 (macrophage inflammatory protein (MIP)-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ) and CCL5 (regulated on activation, normal T cell expressed and secreted (RANTES)) are often discussed in relation to Th1 immunity. However, CCL5 binds to the promiscuous receptor C-C motif receptor (CCR5) found to be expressed on Th1 cells but also found on Th2 cells [9]. Also CCL3 and CCL4 bind to the receptor CCR5 [9] which illustrate the complexity of chemokines and their receptors.

Suppression of proliferation of Th1 and Th2 cells can be conducted for example by T-helper 3 cells, a subgroup of T-regulatory cells, through administration of TGF- $\beta$ . Interleukin-10 with the concurrent inhibition of IL-12 may also augment the expansion of Th3 cells by decreasing the development and maturation of Th1 cells, which in contrary can inhibit Th3 cell expansion [10].

Naïve T cells can turn into T-helper 9 cells by activation of the cytokines transforming growth factor (TGF)- $\beta$  and IL-4 [11, 12] together with the promotor region of PU.1 from the ETS-family and the transcription factors STAT6, GATA3 and interferon response factor (IRF) 4 [13]. These Th9 cells are identified by especially their production of IL-9 [12] but also production of IL-10, IL-17, IL-21 and IL-22 even though their role for function of Th9 cells is still undiscovered [14, 15]. Interleukin-9 has been associated with both allergic and autoimmune diseases [16]. Contrary, recent studies in animal models suggests a protective function of IL-9 in cancer for example by inhibiting tumor growth in a mast cell-dependent manner [17].

Interleukin-9, together with TGF- $\beta$ , have shown able to in vitro differentiate naïve CD4<sup>+</sup> T cells into T-helper 17 cells. In a so-called positive autocrine loop the production of IL-9 by Th17 cells can themselves amplify further Th17 cells [18]. Differentiation of naïve T cells into Th17 cells also require TGF- $\beta$  and IL-6 and the nuclear receptor RAR-related orphan receptor (ROR) -C for its initial Th17 polarization. Also the interleukins IL-1 $\beta$ , IL-6, IL-21 and IL-23 are shown to be involved in the differentiation of Th17 cells. T-helper 17 cells themselves produce especially IL-17A, IL-17F, IL-21, IL-22 and granulocyte macrophage colony-stimulating factor (GM-CSF) [19, 20], are identified as effector cells [21] and suggested to be involved in the pathogenesis of autoimmune diseases. However recent studies suggest that not all Th17 cells are pathogenic and the local cytokine milieu instead determine their faith [22].

T-helper 22 cells are characterized by their production of

IL-22<sup>[23]</sup>. Interleukin-22 functions in synergy with for example IL-17 but also with TNF- $\alpha$  and IFN- $\gamma$ <sup>[24]</sup>. T-helper 22 cells differentiate in response to TNF- $\alpha$  and IL-6<sup>[23]</sup> and effects via the IL-22-IL22R complex<sup>[25]</sup>. These Th22 cells are suggested to play for example an important role in autoimmune diseases like graft versus host disease, rheumatoid arthritis, hepatitis and psoriasis<sup>[25]</sup> as well as in allergy and epidermal immunity and remodeling<sup>[26]</sup>.

T-cytotoxic cells are important effector cells of the immune system. By destroying cells that express foreign antigens on their surface through major histocompatibility complex (MHC) class I molecules they protect against viral infections and also against certain cancers. Overall, virus-specific CD8<sup>+</sup> T cells produce a similar range of cytokines for example IL-2, TNF- $\alpha$ , GM-CSF and IFN- $\gamma$  and chemokines for example CCL3, CCL4 and CCL5 as CD4<sup>+</sup> T-cells. This indicates that also cytotoxic T cells can be divided into subpopulations and can obtain cytokine secreting phenotypes that require transcription factors suchlike those of helper T cells<sup>[27]</sup>. Thus, Tc1 cells secrete for example IFN- $\gamma$ <sup>[28]</sup> and naïve CD8<sup>+</sup> T cells can be differentiated into Tc17 cells by the same conditions as for Th17 polarizing<sup>[29]</sup>. Furthermore, IL-21 and IL-23 can stimulate activation of Tc22 cells producing IL-22<sup>[24]</sup>.

Notably, the same cytokine might suppress autoimmune and inflammatory processes resulting in a benign immunological process or alternatively, under different conditions, induce inflammation or autoimmunity.

### *T-regulatory cells*

T-regulatory (Treg) cells are suggested to maintain self-tolerance of the immune system and have immune suppressive functions for example by inducing protection against viral, bacterial, and parasite antigens in vivo<sup>[30, 31]</sup>. Among humans, approximately 1-2% of the CD4 T cells with high expressing of CD25 (CD4CD25<sup>hi</sup>) are found not to proliferate but instead inhibit proliferation and cytokine secretion by activated CD4CD25 responder T cells<sup>[32]</sup>. Thus, lymphocytes with regulatory function are important components of the peripheral tolerance.

Forkhead box P3 (FoxP3) is a member of the forkhead transcription factor family that plays a critical role in the maintenance and function of peripheral T cells<sup>[33]</sup>. Ectopic expression of the transcription factor FoxP3 can confer a suppressor phenotype to naïve CD4 T-cells<sup>[34]</sup>. Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) is also a useful marker of activated CD4CD25 Treg cells with immune regulatory functions. An abnormal CTLA-4 expression is suggested to result in fatal lymphoproliferative disease.

Interaction of CD80 and/or CD86 with CTLA-4 on activated T cells inhibit the down-regulatory effect of CTLA-4 on effector cells, thus raise the threshold that is required for CD4CD25 mediated suppression<sup>[30, 31]</sup>. The previous described Th3 cells secreting TGF- $\beta$  as well as Tr1 cells secreting IL-4 are other types of Treg cells<sup>[30, 31]</sup>. In contrast to CD4CD25, these cells are developed in the periphery and depend on cytokines for suppression.

The principal function of thymus-derived, natural CD4CD25 cells is to prevent autoimmunity. Besides their cytokine-independent but contact-dependent mechanisms of action, they can also induce other CD4 T cells to become suppressor cells. However, only few natural CD4CD25 cells circulate in human peripheral blood. Adoptive transfer of TGF- $\beta$  and IL-2 ex vivo generated CD4 T-reg cells is suggested to be a potential treatment of autoimmune diseases because these Treg cells have the functional and phenotypic properties including suppressive activity similar to natural occurring CD4CD25 cells<sup>[35]</sup>.

A CD4CD25<sup>hi</sup> population expressing the FoxP3 is commonly used for characterization of Treg cells<sup>[32, 36]</sup>. In addition, CD101, expressed on for example activated T cells<sup>[37]</sup>, has shown highly associated with both in vitro and in vivo suppressor activity within CD4<sup>+</sup>CD25<sup>+</sup> Treg cells<sup>[38]</sup>. Also the ectonucleotidase CD39, involved in suppression of inflammation, have shown to be expressed on FoxP3<sup>+</sup> Treg cells<sup>[39]</sup>, named CD39<sup>+</sup>FoxP3<sup>+</sup>Tregs<sup>[40]</sup>. The IL-9 receptor, CD129 expressed on T cells, together with secreted IL-9 are critical for the early stages of human intrathymic T cell development to increase the suppressive function of Tregs and is thereby probably of importance for regulation<sup>[41]</sup>. Contrary, as activated Treg cells express low levels of CD45RA and CD127 both these clusters of differentiation can be used to distinguish different types of Treg cells<sup>[42, 43]</sup>.

However, characterization of the true human Treg cell is still not conclusive. This far, CD4<sup>+</sup>CD25<sup>hi</sup> cells with high expression of CD39, CD101, CD129 and FoxP3 but low expression of CD45RA and CD127 may be the best way for characterization of human Treg cells.

### **Common autoimmune diseases in children**

In autoimmune disease, the adaptive immune system mistakenly begins attacking specific healthy cells and tissues in almost any part of the body, though often target connective tissues (skin, muscle and joints)—and fails to shut off the attack. Autoimmune diseases in children are generally rare, and when they occur they can be challenging to diagnose, due to for example nonspecific symptoms, and thereafter also difficult to treat. Two of the most common

diseases with autoimmune characteristic in children are type 1 diabetes (T1D) and celiac disease.

### Type 1 diabetes

Type 1 diabetes is a disease, in genetically predisposed individuals, known to be caused by destruction of the pancreatic  $\beta$  cells by an autoimmune origin [44]. The autoimmune process can exist for months or even up to years in a so-called preclinical phase [Castano, 1990 #244]. Classic manifestation of the disease includes ketosis and hyperglycemia that occurs due to loss of insulin-producing  $\beta$  cells [44].

The infiltrating cells in insulinitis lesions include both CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as macrophages. In non-obese diabetic (NOD) mice, diabetes has shown to be transferable by CD4<sup>+</sup> T-cells expressing a Th1-like cytokine profile [45]. Contrary, only few studies have been able to study the disease in vivo due to the fact that the pancreas is located close to several vital organs. Cytotoxic actions of IFN- $\alpha$  [46] and IFN- $\gamma$  [47] have previously been observed on human islets in vivo of patients with recent-onset T1D. It was recently observed that IFN- $\alpha$ , - $\beta$  and - $\gamma$  as well as IL-18 and CXCL10 were found in both  $\alpha$  and  $\beta$  cells in autopsied patients, who died from diabetic ketoacidosis within 2-5 days after onset of fulminant T1D [48, 49]. It was also recently shown that enterovirus infection induced pro-inflammatory cytokine genes of TNF- $\alpha$ , IL-1 $\alpha$  and IL-1 $\beta$  from isolated human pancreatic islets [50].

Recently, an attempt to collect pancreatic resection biopsies from living newly onset T1D patients resulted in an unexpectedly high complication rate [51]. In this rare in vivo study of human pancreas, mean glucose-stimulated insulin secretion was reduced in islets from T1D patients [52]. Also the genes involved in production and release of insulin as well as genes involved in secretion of insulin for example the insulin gene INS were less expressed in patients than in controls [52].

Instead, the majority of immunological studies of T1D are on the peripheral immune system of patients with recent onset T1D. Several studies have indicated a fluctuation in the balance between Th1 and Th2 cells. Studies of the peripheral immune system has shown significantly increased levels of for example IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\beta$ , and IL-2 in newly diagnosed T1D patients [53, 54]. It is suggested that the insulinitis lesion is  $\beta$  cell destructive when IFN- $\gamma$ , TNF- $\alpha$  and IL-2 (Th1-like cytokines), produced by islet-infiltrating lymphocytes, dominate over IL-4 (Th2-like cytokine) [55]. Contrary, if Th2-type cytokines dominate and downregulate Th1-type cytokine production this may prevent  $\beta$  cell

destruction in NOD mice as well as in humans [56]. An increased cellular response, especially by Th1 cytokines, to the autoantigens insulin [57], glutamic acid decarboxylase (GAD<sub>65</sub>) [58] and tyrosinophosphatase (IA-2) [59] has been found in T1D patients. Our research group has however previously reported a dominant Th1-associated immune profile during the pre-diabetic phase [60-62]. At the onset of disease, this Th1 dominance is instead downregulated in favor of a temporary increase of an inflammatory immune profile (TNF- $\alpha$ , IL-6 and the chemokines CCL2 and CXCL9 (monokine upregulated by IFN- $\gamma$  (MIG)) together with increase of a Th3/Tr1-immune profile (TGF- $\beta$  and IL-10), spontaneously as well as by GAD<sub>65</sub> stimulation [63, 64].

Besides activation of Th1 cells, autoimmune diseases are thought to have also a Th17/Th22 bias. However, the role of Th17/Th22 cells for progression to T1D is still unclear. In vivo activated monocytes from T1D subjects are shown to induce more IL-17-secreting cells from memory T cells compared with monocytes from healthy controls [65]. An upregulation of Th17 immunity in peripheral blood T cells from children with T1D has also been shown by increased IL-17 secretion and expression of IL-17 and IL-22 [66]. Interleukin-17 and IL-22 overlap in several aspects regarding both biological function and structure and IL-23 is furthermore essential for human Th17 differentiation. The observed increased percentages of both Th17 and Th22, especially during the early stages of T1D (<5 years), may indicate the contribution of these Th cell subsets in the pathogenesis of T1D [67].

Studies into the specificity of infiltrating CD8<sup>+</sup> T cells in the  $\beta$  cell lesion are still in an early phase. So far, it has been shown that CD8<sup>+</sup> T cells take a center role in the destructive process that contribute to sustained islet inflammation leading to a gradual decrease in  $\beta$  cell mass [68]. Due to the limited possibility to study human pancreas most studies are performed in the NOD mice model. B cells, high fraction of macrophages and especially CD8<sup>+</sup> T cells have been observed in pancreas from human as well as from NOD mice [69]. Recent observations indicate for example elevated Tc1 (IFN $\gamma$ <sup>+</sup>IL-17<sup>-</sup>) and Tc17 (IL-17<sup>+</sup>) cells in patients with T1D [67, 69].

The role of Treg cells i.e. changes in expression and function for the development of human T1D is still not clearly elucidated [70]. Expression of CD25 on CD4<sup>+</sup> T cells is found to be normally expressed in patients with T1D [71]. Despite the observed ability to express high amounts of CD25 on CD4 (CD4<sup>+</sup>CD25<sup>hi</sup>) the capacity of Treg cells to suppress T cell proliferation during in vitro co-cultures with anti-CD3 antibodies is markedly reduced in T1D patients [71]. Defect in regulation of Treg cells seems to result in increased

secretion of IFN- $\gamma$  together with decreased release of IL-10 [71]. In NOD mice model, it has been observed that retroviral transduction of polyclonal CD4 T cells with FoxP3 is not effective in interfering with established T1D [72]. Interestingly, it has been found that Foxp3/scurfin gene is located on chromosome Xp11.23, which includes one of the T1D susceptibility loci [73]. Tumor necrosis factor- $\beta$  is found at high levels at inflammatory sites playing an essential role for the generation of FoxP3<sup>+</sup> inducible Treg cells as well as an important effect on natural Treg cells [74, 75]. Also increased IL-10 secretion has been observed in longstanding T1D patients (6 months to 5 years after diagnosis) [76]. However, even if the Th3/Tr1 response is found to be high at onset of T1D, an immune regulatory defect by reduced function of Treg cells has been observed [71].

We have previously shown a significantly lower percentages of CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>lo/-</sup> among CD4<sup>+</sup> T cells and lower CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>lo/-</sup> to CD4<sup>+</sup>CD25<sup>-</sup> cell ratio in T1D children compared to healthy individuals [77]. Recently, we also found low expression of CD39<sup>+</sup> and CD45RA<sup>+</sup> on CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>CD127<sup>-</sup> T reg cells in T1D children that may indicate loss of suppressive function [78]. Defective regulation is suggested as a feature of T1D regardless of disease duration and an impaired ability of responder T cells to be suppressed contributes to this defect [79]. However, there is still no precise definition of human Treg cells even though lower expression of CD127 is a good complement to expression of CD4 and CD25 [42]. Furthermore, nearly all studies on the importance of Tregs for the development of T1D are performed on peripheral blood, suggesting that T cell populations in the periphery reflect the immune activity at site i.e. pancreas [70]. This may explain the divergent results from studies on the suppressive function of Treg cells indicating either normal [42, 80], decreased [81, 82] or increased [83] suppressive function in the pathogenesis of T1D. It is suggested that once autoimmunity is triggered, the progressive decrease in  $\beta$  cell function may be caused by subsequent exposure events [84]. Unfortunately, many trials fail to demonstrate clinical response even when Treg treatment successfully boosts Tregs [70]. Actually, it has been indicated that the effector T cell population can resist the activity of Treg cells in patients with T1D [79, 80].

### Celiac disease

Celiac disease is considered a multi-factorial autoimmune-like disease, triggered by the ingestion of gluten in genetically susceptible subjects [85], with an increasing frequency [86]. Besides gluten also tissue transglutaminase (tTG) is suggested as an autoantigen for the development of celiac disease [87]. It has been shown that tTG selectively

deamidates gluten peptides, which results in strongly enhanced T cell stimulatory activity [88].

A cross-reactivity between the immune system and the mucosa is initiated when gluten peptides, which are partly resistant to enzymatic processing, cross the intestinal epithelium leading to an inflammatory reaction, resulting in crypt hyperplasia and villous atrophy. After gluten withdrawal the diseased mucosa heals and autoantibodies disappear.

Within the gluten protein, many different epitopes can activate intestinal CD4<sup>+</sup> T cells. A trend towards a superior accumulation of IFN- $\gamma$  in vivo [89] promoting inflammatory effects and a non-proliferative activation of CD4<sup>+</sup> lamina propria T cells, especially by activation of Th1-like cells secreting IFN- $\gamma$  [90] have been shown by gluten. Besides IFN- $\gamma$  also for example IL-2 and TNF- $\beta$  mRNA expression have been observed in biopsies from active celiac patients [91]. In contrast, Th2-associated IL-4 is found equally expressed in patients as in controls [89, 91-93].

Interferon regulatory factor (IRF) and STAT-1 together with reduced IL-2 expression are also found to be pronounced in small intestinal biopsies from untreated celiac disease in children [94]. One year after gluten free diet downregulation of IFN- $\gamma$  mRNA is observed even though the signaling pathway for this interferon still is upregulated [94]. T-regulatory as well as Th17 cells have also recently been shown to be involved in the pathogenesis of untreated celiac disease. In fact, it has been shown that, at baseline, Th1 as well as Th17 and Treg cells are significantly higher in active celiac disease patients observed both in tissue-infiltrating lymphocytes as well as in peripheral blood. Recently, we observed that children with coeliac disease show signs of CD4<sup>+</sup>CD25<sup>hi</sup> Treg cells expression also CD101<sup>+</sup> and CD129<sup>+</sup> that may indicate suppressor activity [78].

Treatment with a gluten-free diet however influences the immunological pattern and decreases Th1-, Th17 and Treg cells [95]. Gluten-free diet has shown to also normalize the expression of Th2 gene markers for example IL-4R, IL-13RA1, IL-5 and STAT6 [94] and the production of IL-10 by intraepithelial lymphocytes [89]. Recombinant human IL-10 has even been shown to induce a long term hypo-responsiveness of gliadin specific mucosal T cells [96].

T-regulatory cells are thought to contribute to the immunological failure in celiac disease. In duodenal biopsy samples from patients with an active celiac disease a higher density of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells has been observed in comparison to patients with treated celiac disease and healthy controls [97]. In co-culture, these Treg cells are shown to be functionally suppressive, but their activity is impaired by for

example IL-15<sup>[97]</sup>. Contrary, a low grade inflammation has been detected in individuals with potential risk of developing celiac disease who still have a normal small intestinal mucosa but with a positive CD-associated serology<sup>[98]</sup>. Intestinal CD4<sup>+</sup>CD25<sup>+</sup> T cells with suppressive effects on T responder cell may however prevent this low grade inflammation<sup>[98]</sup>.

### Combination of type 1 diabetes and celiac disease

Type 1 diabetes and celiac disease are two immune-mediated diseases sharing common susceptibility factors including for example environment and genetics. In 85-90% of all cases of these two diseases in combination, T1D is diagnosed before celiac disease and most cases of celiac disease are diagnosed within 5 years of T1D diagnosis<sup>[99]</sup>. Complications for type 1 diabetic patients with also celiac autoimmunity are higher HbA1c, more hypoglycaemic episodes, higher prevalence of iron and vitamin B12 deficiency, lower insulin-growth factor-1 (IGF-1) and also lower bone mineral density<sup>[100]</sup>.

#### *Prevalence of celiac disease in type 1 diabetic patients*

The overall incidence of both T1D and celiac disease individually is increased<sup>[86, 101]</sup>. Comparison with prevalence of coeliac disease in the general population reveals an at least 10-fold increase in patients with T1D. Patients with manifest T1D may have had a latent celiac disease, which is activated parallel to the anti-islet immune reactivity during the development of T1D. Out of a cohort of approximately 28.000 T1D patients, 10.7% produced as well tTG autoantibodies<sup>[102]</sup>. A recent systematic review of more than 26.000 T1D patients revealed that one in twenty patients with T1D have biopsy-verified celiac disease, thus a prevalence of 6.0%<sup>[103]</sup>. Contrary, a low prevalence of  $\beta$ -cell autoimmunity is found in young patients with celiac disease<sup>[104]</sup>.

#### *Genetics in common for celiac disease and type 1 diabetes*

Celiac disease has an autoimmune characteristic with known HLA (HLA-DQA1 and HLA-DQB1) association<sup>[105]</sup>. Homozygous carriers of DQB1\*02 have higher risk of developing celiac disease than heterozygote individuals<sup>[106]</sup> leaving a larger window for additional risk factors outside the HLA haplotypes in heterozygote individuals. Among T1D, approximately 30-50% of all patients are heterozygotes for HLA DR3-DQ2 and DR4-DQ8 genotype.

Since the genotype DR3-DQ2 shows as well a strong associations with celiac disease, this indicates a genetic similarity in these two diseases. Thus, both diseases are associated with HLA class II genes on chromosome 6p21 and that can, to some extent, explain the co-segregate of

these two diseases in the population. Actually, T1D patients homozygous for DR3-DQ2 carry a 33% risk for the presence of tTG autoantibodies<sup>[107]</sup>.

Furthermore, a higher permeability has been found in type 1 diabetics with a DQB1\*02 allele that may predispose for an abnormal immune responses against food antigens<sup>[108]</sup>. Contrary, it was recently suggested that DPB1\*04 may reduce the risk of tTGAs autoantibodies and thus was inversely associated with coeliac disease autoimmunity<sup>[109]</sup>. Also non-HLA genes for example CTLA-4, tyrosine-protein phosphatase non-receptor type 2 (PTPN2) and CCR5 genes, are pointed out as predicting factors for both T1D and celiac disease<sup>[110]</sup>.

#### *Effect of gluten-free diet*

Among patients with both T1D and celiac disease, the majority of patients are diagnosed with T1D before the diagnosis of celiac disease. It has been a controversy between the suggestion that gluten can be the driving antigen and immunological trigger able to induce T1D in silent, untreated celiac disease<sup>[111]</sup> or instead, that gluten-free diet can have a diabetes-protective effect<sup>[112]</sup>.

It has been shown that the pathogenesis of T1D is influenced by diet<sup>[113]</sup> and that a gluten-free diet reduces the incidence to 6% in animals model (NOD mice)<sup>[114]</sup>. However, in humans the results are more conflicting. Insulin sensitivity is shown to improve after gluten-free diet in first-degree relatives of T1D patients<sup>[115]</sup>. However, this improvement has not been seen to influence on humoral autoimmunity since autoantibodies do not change during the period of gluten-free diet<sup>[115]</sup>. Likewise, islet autoantibody levels are not changed in islet-antibody-positive first degree relatives of patients with T1D placed on gluten-free diet for 12 months<sup>[116]</sup>. Thus, a gluten-free diet has not been shown able to delay or prevent development of T1D<sup>[116, 117]</sup>. Likewise, in an intervention study in which infants with high genetic risk for T1D were randomized to either early or late introduction of gluten, no benefit in delaying gluten exposure could be found in respect to celiac disease autoimmunity or T1D up to the age of three years<sup>[118]</sup>. Neither could the same intervention study, at a follow-up at the age of 8 years, indicate that delayed gluten introduction can decrease the risk of developing autoimmune T1D<sup>[119]</sup>. Also, in a 2-year prospectively followed cohort of subjects with already diagnosed T1D, with and without celiac autoimmunity, no significant adverse outcome was found in children with a delayed introduction of gluten<sup>[120]</sup>.

Thus, there is still a controversial regarding the effect of gluten-free diet in T1D patients with celiac disease. At first it

was suggested that glycemic control in patients with both T1D and celiac disease was improved by gluten-free diet [121]. More recent studies instead suggests that a gluten-free diet can lead to increased weight gain and raised body mass index (BMI) due to high saturated fat and high glycemic index carbohydrate [122].

#### *T cell response to gluten and also other dietary antigens*

A limited T cell response to gluten has been able to detected in newly diagnosed T1D patients [123]. It has also been shown that approximately 50% of tTG autoantibody negative T1D patients display an increased CD3<sup>+</sup> T cell proliferation to dietary wheat polypeptides [124]. In these T1D patients also a mixed cytokine response of for example TNF, IFN- $\gamma$  and IL-17A were observed whereas the pro-inflammatory secretion was inhibited by anti-DR antibodies [124]. The degree of celiac disease in T1D patients has also in another study been correlated to the densities of IFN- $\gamma$  mRNA positive cells as well as IFN- $\gamma$ , IL-2 and TNF- $\alpha$ -positive cells [125]. This may indicate a diabetes-related inflammatory state in the gut immune tissues associated with defective oral tolerance and possibly gut barrier dysfunction [124]. Actually, involvement of gut immune system has been implicated in the pathogenesis of T1D. Increased expression of IL-18 mRNA in the small intestinal biopsies [126] and increased  $\alpha 4/\beta 7$ -integrin<sup>+</sup> cells in the lamina propria have been found among T1D patients indicating inflammation [127].

It has also been suggested that newly diagnosed diabetic patients have a non-specific activation of the immune system directed towards several dietary proteins for example bovine serum albumin (BSA) and beta-lactoglobulin ( $\beta$ LG), as a result of a defective immune regulation or loss of immunological tolerance to a variety of ingested antigens [128-130]. The same pattern is noted in celiac disease, where antibodies not only to gluten but also to other dietary proteins are elevated before treatment [131].

On the contrary, in children diagnosed with both T1D and coeliac disease, we have previously shown a diminished Th1-like immune profile. Children with combination of these two diseases showed hardly any IFN- $\gamma$  secretion after in vitro stimulation with either gluten or other food agents ( $\beta$ LG and ovalbumin (OVA)). The same diminished Th1-like profile response was also seen towards for example inhalation allergens (birch and cat extract). This result was very distinct from children diagnosed with either T1D or celiac disease as well as reference children and suggests a suppressed immune response in children with combination of these two diseases [132].

#### *T-regulatory associated immunity*

Gluten as a possible antigen to induce peripheral blood mononuclear cell (PBMC) expression of FoxP3 mRNA to a higher extent in children with celiac disease compared to reference children has for example been shown by our research group [133]. Enhanced intestinal expression of FOXP3 mRNA has as well been shown in active celiac disease patients compared to controls [126].

A few percentages of T cells implicated in insulinitis origin from the intestinal. This may explain why signs of mucosal inflammation by increased density of lamina propria CD25<sup>+</sup> mononuclear cells and intraepithelial CD3<sup>+</sup> cells in jejunal biopsies as well as increased CD25<sup>+</sup> mononuclear cells in biopsies exposed to gliadin has been observed in small intestinal biopsies from T1D children [134].

In children diagnosed with both T1D and celiac disease, we have been able to show an increased peripheral expression of phytohaemagglutinin (PHA)-induced FoxP3 mRNA in comparison to children diagnosed exclusively with T1D [133]. Increased frequency of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells together with pronounced expression of FoxP3 mRNA have also been detected in small-bowel biopsy specimens from children diagnosed with both celiac disease and T1D [126, 135]. Further, the density of FoxP3<sup>+</sup> cells are also correlate with histological grade of atrophic changes in the small bowel [136]. Upregulated FoxP3 expression may counterbalance the loss of mucosal integrity by maintaining immune tolerance in the small intestine of patient with both celiac disease and T1D [137]. Contrary, a low expression of the tight junction protein 1 (TJP1) serving as a marker of intestinal mucosa integrity has been found in patients with both T1D and celiac disease [136, 138]. Also gliadin and tTG IgA antibodies were the highest in these patients [138]. These results may reveal that intestinal permeability is most severely impaired in patients with these two immunological diseases in combination [138].

#### **Combination of type 1 diabetes and celiac disease - an immunological challenge**

We have recently shown that children suffering from both type 1 diabetes and celiac disease show higher percentage of terminally differentiated effector cells (TEMRA) CD4<sup>+</sup> cells in contrast to a lower percentages of both early and late effector memory CD8<sup>+</sup> cells compared to references [78]. Previous observation of pancreatic grafts show that a large population of CD8<sup>+</sup> pancreas-infiltrating lymphocytes lacks expression of CD28 [139]. Immune aberrancies have also been shown in the gut, in children who are prone to T1D, suggesting poor development of oral tolerance [125]. This may

reduce the immunological process towards proteins/antigen in general. This is a theory supported by our previous observation that children suffering from these two immunological diseases in combination show a suppressed immune response (IFN- $\gamma$ ) to both food (gluten, milk and egg) and inhalant antigens (birch and cat extract) compared to children with either T1D or celiac disease as well as compared to reference children [132].

These results as well as the previous presented data indicate that the combination of type 1 diabetes and celiac disease is an immunological challenge and it is obvious that we are far from understanding the immunological impact of these two autoimmune diseases in combination. This immunological challenge needs to be elucidated to be able to predict and prevent these common autoimmune diseases.

### Conflicting interests

The author has declared that no competing interests exist.

### Abbreviations

$\beta$ LG: beta-lactoglobulin; BSA: bovine serum albumin; BMI: body mass index; CCL: C-C motif ligand; CCR: C-C motif receptor; CD: cluster of differentiation; CTLA: cytotoxic T lymphocyte associated protein; CXC: C-X-C motif ligand; GAD: glutamic acid decarboxylase; GM-CSF: granulocyte macrophage colony-stimulating factor; FoxP3: forkhead box P3; IA-2: tyrosine phosphatase; IFN: interferon; IGF: insulin growth factor; IL: interleukin; IP-10: IFN- $\gamma$ -inducible protein 10; IRF: interferon response factor; MCP: monocyte chemoattractant protein; MHC: major histocompatibility complex; MIG: monokine upregulated by IFN- $\gamma$ ; MIP: macrophage inflammatory protein; NK: natural killer; NOD: non-obese diabetic; OVA: ovalbumin; PBMC: peripheral blood mononuclear cell; PHA: phytohaemagglutinin; PTPN2: tyrosine-protein phosphatase non-receptor type 2; RANTES: regulated on activation, normal T cell expressed and secreted; ROR-C: RAR-related orphan receptor; STAT: signal transducer and activator of transcription; T1D: type 1 diabetes; T-bet: T-box expressed in T cells; Tc: T-cytotoxic; TEMRA: terminally differentiated effector cells; TGF: transforming growth factor; Th: T-helper; TJP: tight junction protein; TNF: tumor necrosis factor; Treg: T-regulatory; tTG: tissue transglutaminase.

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