Autoimmunity Reviews 8 (2009) 659–662

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Regulatory T cells in diabetes and gastritis ☆

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ARTICLE INFO

Article history:
Received 23 January 2009
Accepted 7 February 2009
Available online 12 February 2009

Keywords:
T1D
Autoimmune gastritis
Regulatory T cells
Pepsinogen I
Foxp3

ABSTRACT

Patients with Type 1 diabetes mellitus (T1D) have an increased prevalence of associated organ-specific autoimmune diseases such as pernicious anemia whose histological substrate is a chronic atrophic gastritis (CAG). Latent pernicious anemia precedes clinically-manifest pernicious anemia and may be difficult to detect solely on simple analytical grounds. We recently described an increased prevalence of clinically-latent pernicious anemia in T1D using low concentrations of pepsinogen I, a zymogen of pepsin present in gastric mucosa, as a useful additional diagnostic marker, besides parietal cell antibodies, for screening latent pernicious anemia in T1D. The failure of peripheral tolerance mechanisms such as regulatory T cells (Treg) might be involved in CAG development in T1D patients. Indeed, functional defects in Tregs have been described in T1D patients. To this end, the percentage of Tregs in peripheral blood of T1D-CAG patients was analyzed and compared with those of a group of T1D without associated autoantibodies and a healthy control group. Tregs levels were also analyzed in gastric biopsies of T1D-CAG patients. The results obtained have led to new questions regarding the pathogenic mechanisms implicated in the development of associated autoimmune diseases in T1D.

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1. Type 1 diabetes mellitus and associated autoimmune diseases

Type 1 diabetes mellitus (T1D) is an organ-specific autoimmune disease characterized by T-cell-mediated destruction of pancreatic beta cells, resulting in absolute dependence on insulin for survival and maintenance of health [1]. The incidence of T1D has consistently increased worldwide in recent decades and shows a trend towards

☆ This work was supported in part by Proyecto FIS 05/0150 from the Spanish Ministry of Health to Prof. A. Sanmartí.
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doi:10.1016/j.autrev.2009.02.014

earlier onset [2,3]. The disease has a strong hereditary component, being HLA region genes the maximal contributors, but up to other 50 genes contribute, such as insulin, *PTP*N22, *CTL*A4 and *IL-2* pathway genes [4]. The factors that trigger the onset of clinical disease remain largely unknown [5,6].

Patients with T1D show an increased prevalence of associated organ-specific autoimmune diseases [7]. Fifteen to 30% of subjects with T1D have autoimmune thyroid disease (AITD), 4–9% celiac disease, 0.5–4% pernicious anemia and around 0.5% Addison's disease [8,9]. These associations suggest that T1D and other organ-specific autoimmune diseases may share some causative genetic factors, as has been postulated in several studies [10–14].

Prospective studies have demonstrated that autoimmune disease is preceded by a long preclinical phase in which individuals can be identified by the presence of autoantibodies [15]. T1D patients often carry autoantibodies that can act as markers of other autoimmune diseases that may develop later, i.e., 20–30% carry anti-thyroid peroxidase (TPO) and/or thyroglobulin antibodies markers of AITD, 5–10% anti-endomysial (EMA) and/or anti-transglutaminase antibodies (tTG) markers of celiac disease, 15–20% parietal cell antibodies (PCA) markers of gastric autoimmunity and 0.5–2% anti-21-hydroxylase antibodies, markers of Addison's disease [16]. A simple way to screen for autoimmunity in a susceptible population is to measure autoantibodies that can alert the clinicians and contribute this way to prevent morbidity and reduce mortality. The specific strategy for the follow-up of patients with positive autoantibodies is an area of active debate and research [17]. The American Diabetes Association (ADA) recommendations for AITD are to measure TSH in newly diagnosed T1D patients once metabolic control has been restored, every 1–2 years thereafter and at any time if suggestive symptoms of hypo- or hyperthyroidism appear [18]. Suggested recommendations for the screening of gastric autoimmunity in T1D are to test for PCA at T1D onset, yearly for 3 years, at the 5th year and every 5 years thereafter [19].

2. Type 1 diabetes mellitus and autoimmune gastritis

The prevalence of autoimmune gastritis is 3- to 5-fold increased in T1D compared to the general population [9]. Autoimmune gastritis affects the parietal cell-containing gastric corpus and fundus with sparing of the antrum [20]. PCA targeted against gastric H^+/K^+ ATPase are detected in 60–85% of patients [21]. Fifteen to 20% of T1D patients exhibit PCA, particularly those with GAD-65 antibodies and HLA-DQA1*0501–B1*0301 haplotype [14]. Chronic autoimmune damage to the gastric proton pump, H^+/K^+ ATPase, may result in decreased acid secretion, hypergastrinemia and iron deficiency anemia. At a later stage of the disease, pernicious anemia may result from vitamin B12 deficiency because PC also produces the intrinsic factor required for B12 absorption and/or because blocking intrinsic factor antibodies develop in these patients [22]. The progression of autoimmune chronic atrophic gastritis (type A) to gastric atrophy and clinical anemia is likely to span 20 to 30 years [21]. Latent pernicious anemia in T1D defined as low cobalamin concentrations due to cobalamin malabsorption without anemia have been described in few studies, reporting prevalences ranging from 1.08 to 4% [23].

Our group has recently evaluated possible biochemical markers which could be useful for latent pernicious anemia diagnosis in T1D, among them pepsinogen I and ghrelin. Pepsinogen I is a peptide secreted by zymogenic cells in the body and fundus of the stomach; its low serum concentrations are considered a good non-invasive method to diagnose corpus atrophy [24]. We recently described an increased prevalence of latent pernicious anemia in a group of T1D patients, considering low pepsinogen I concentrations as the main selective parameter for its diagnosis compared with a healthy, age-matched control group [25]. In fact, only higher PCA titers ($\geq$1/640) identified patients with significantly lower levels of pepsinogen I. In contrast to these results, plasma ghrelin, a peptide synthesized mainly in endocrine X/A-like cells of the gastric oxyntic glands, was not decreased in our group of T1D patients with atrophic chronic gastritis and was thus not considered to be a good biochemical marker for gastric atrophy in this group of patients [26].

3. Regulatory T cells in type 1 diabetes mellitus and autoimmune gastritis

A growing body of evidence suggests that an imbalance in the immune system plays a major role in the pathogenesis of autoimmune disease [27]. Regulatory T cells (Tregs) play a major role in modulating the activity of self-aggressive T cells not deleted in the thymus and are one of the pillars for maintaining the immune system in homeostatic balance [28–30]. Most studies investigating the role of Tregs in human T1D found no differences in the peripheral blood frequency of these cells between T1D and control subjects when the expression of transcription factor forkhead box P3 (Foxp3) was used for their identification [31,32]. However, the functional capacity of Tregs in humans with T1D has not been so clearly established. Two reports suggested functional defects in Tregs in patients with T1D [31,33], but others have also reported normal suppressive activity [34]. On the other hand, several studies have shown that pro-inflammatory cytokines present at the inflammation site may abrogate the suppressive activity of Tregs or cause effector T (Teff)-cell to become resistant to suppression [35], this questioning the relevance of the *in vitro* suppressor cell assay. It has recently been suggested that in T1D the source of this "defective regulation" is intrinsic to the Teff compartment due to the resistance of responding T cells to the CD4+FOXP3+ regulatory T cells [36].

Experimental autoimmune gastritis is a well-defined model of organ-specific autoimmunity; in fact, it has played a central role in defining the characteristics of CD4+CD25+ Tregs [37,38]. Surprisingly, to our knowledge, Tregs have not been analyzed in detail in human autoimmune gastritis.

In order to better assess the mechanisms involved in the development of a second autoimmune disease, i.e. autoimmune gastritis in T1D patients, we analyzed the presence of Tregs in peripheral blood of T1D patients with autoimmune chronic atrophic gastritis (CAG) (T1D–CAG) and compared them with T1D patients without other associated autoimmune diseases and healthy controls (Alonso et al., *in press*). Autoimmune CAG in this study was described as

the presence of positive PCA, biochemical markers suggestive of gastric atrophy and histological confirmation of gastric atrophy. The results show that T1D patients with autoimmune CAG have an increase in the frequency of Tregs compared to T1D in peripheral blood. In agreement with previous reports, no differences were observed between T1D without associated autoimmunity and controls (Alonso et al., *in press*).

Most studies on Tregs in human autoimmune diseases focused on peripheral blood and only a few evaluated Tregs in the target tissue [39,40], but in fact the levels of Tregs in periphery may be of little relevance to the site of the autoimmune inflammatory process. We have investigated the presence of Tregs in gastric mucosal biopsies of T1D patients with CAG. The immunohistochemical examination demonstrated that Tregs were indeed present in gastric lymphocytic infiltrates of T1D–CAG patients while very few were seen in normal gastric mucosa. Fig. 1 shows a representative case of a T1D–CAG biopsy specimen. Interestingly, the percentage of Tregs in gastric mucosa of T1D–CAG patients was lower than in another chronic inflammatory condition of the gastric mucosa, H. *pylori* gastritis (Alonso et al., *in press*). These findings suggest that infection may be a stronger stimulus than autoimmunity for the recruitment of Tregs. Further studies are required to identify the factors involved in Tregs homing to tissues and the different inflammatory milieux in autoimmune diseases as well as their potential therapeutic manipulation.

In summary, here we describe Tregs expression in a group of T1D patients with a CAG. We observed that these patients have both increased Tregs number in peripheral blood, and that Tregs are present in gastric mucosa infiltrates, probably reflecting the activation of regulatory mechanisms. The levels of Tregs as well as the Tregs:Teffector T cell ratio should be analyzed in future studies including T1D patients with associated autoimmune diseases, other than autoimmune gastritis, such as autoimmune thyroiditis or celiac disease. This would be useful to improve understanding of the importance of peripheral tolerance mechanism failure in their pathogenesis.

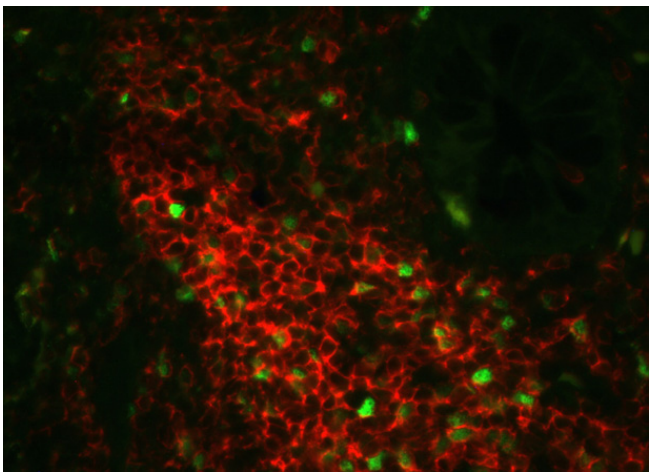

Fig. 1. Immunofluorescence staining of cytoplasmic Foxp3+ (236A/E7 mAb, green) cells among CD3+lymphocytes (red) in a gastric biopsy specimen of a representative T1D-CAG patient (×400).

Take-home messages

- Autoimmune gastritis is 3 to 5-fold increased in T1D compared with healthy subjects.
- Low pepsinogen I concentrations and high parietal-cell antibodies (PCA) titers (≥1/640) can be used for screening latent pernicious anemia in T1D patients.
- Most authors agree that there are no differences in Tregs peripheral blood frequencies between T1D and healthy subjects.
- T1D patients with autoimmune chronic atrophic gastritis show a higher frequency of peripheral blood Tregs compared with T1D patients without other associated autoantibodies and controls.
- Tregs are present in gastric mucosa of T1D patients with autoimmune chronic atrophic gastritis, which indicates the existence of an ineffective counter-regulatory mechanism trying to restore tolerance in these patients.

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