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Decreased circulating iNKT cell numbers in refractory coeliac disease

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Abstract

Introduction: A small proportion of coeliac disease (CD) patients become refractory (RCD) to a gluten-free diet (GFD) showing uncontrolled immune-mediated enteropathy. Some of these patients exhibit a high risk to develop enteropathy-associated T-cell lymphoma (EATL).
Aim: The aim of the study was to find whether a lack of circulating homeostatic T cells, such as Treg, $T\gamma\delta^+$ or iNKT cells would be associated with the development of RCD or EATL.
Patients and methods: We investigated in a total of 137 CD patients [28 untreated, 80 responsive to GFD and 29 RCD (14 type I and 15 type II)] and 73 age-matched healthy volunteers the frequency of Treg, $T\gamma\delta^+$ and iNKT lymphocytes by flow cytometric analysis of peripheral blood.
Results: Circulating $T\gamma\delta^+$ cell and Treg frequencies in RCD were comparable to those in healthy controls. However, RCD patients had significantly reduced numbers of circulating iNKT cells, as compared to age-matched patients responding to a GFD. This reduction was similar in RCD patients with and without aberrant intraepithelial lymphocytes and in patients with EATL. Circulating iNKT cell numbers were not reduced in untreated coeliac patients. GFD treated patients had a significantly increased proportion of $CD4^+$ iNKT cells.
Conclusion: Follow-up studies are necessary to determine whether $CD4^+$ iNKT cells control the immune response against gluten and their absence contributes to the progression to RCD and EATL.

Introduction

Coeliac disease (CD) is an immune-mediated enteropathy caused by the ingestion of wheat and other gluten-

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containing cereals (rye, barley and probably oats) in genetically predisposed individuals [1] leading to intestinal villous atrophy. This is characterized by crypt hyperplasia and increase of intraepithelial lymphocytes (IELs). The current treatment is a life-long strict gluten-free diet (GFD) resulting in a complete remission of the symptoms and returning to a normal small intestinal mucosa. However, a small proportion of CD patients becomes unresponsive to a GFD, a stage known as refractory CD (RCD) [2]. This is characterized by an uncontrolled, gluten-independent, intestinal immune reaction. As a result, the IEL of the diseased epithelium can continue to undergo NK-like reprogramming [3] with T cell receptor (TCR)-independent IFN-γ production and cytotoxicity. Additionally, an aberrant IEL population, lacking surface expression of CD3 and CD8, appears in a subgroup of RCD patients (type II) which have a high risk to develop an enteropathy-associated T-cell lymphoma (EATL), whereas RCD type I patients show normal expression of T-cell antigens and have a better prognosis [2,4].

$CD4^+$ $CD25^+$ regulatory T cells [Tregs; $CD3^+$, $CD4^+$, $CD25^+$ and intracellular transcription factor Forkhead Box $P3^+$ ($FoxP3^+$)], invariant NKT cells (iNKT; $CD3^+$, TCR $V\alpha24^+V\beta11^+$) and to a lesser extent TCR $\gamma\delta^+$ lymphocytes (T$\gamma\delta$; $CD3^+$, TCR $\gamma\delta^+$) are lymphocyte populations that help to maintain immune homeostasis [5–10]. Tregs [11] elicit their function by suppressing IL-2 production and T-cell proliferation [12,13]. Intraepithelial T$\gamma\delta$ cells appear to play a key role in oral tolerance by inducing Tregs [14,9]. After TCR-triggering, T$\gamma\delta$ cells rapidly but transiently express the lymph node-homing receptor CCR7. Once in the lymph nodes, they may act as professional antigen presenting cells inducing proliferation and differentiation of naïve T cells [15].

Human iNKT cells express classical NK cell markers as well as an invariant T cell receptor (TCR $V\alpha24^+V\beta11^+$), which recognizes antigens presented by the MHC class I-like molecule CD1d [13,16–19]. iNKT cells can also be sub-divided in $CD4^+$ and $CD4^-$ (most of these $CD4^-CD8^-$) cells. $CD4^-CD8^-$ iNKT cells predominantly produce TH1 cytokines (IFNγ and TNFα) whereas $CD4^+$ iNKT cells can produce both TH1 and TH2 (IL-4 and IL-13) cytokines [20,8]. Because of their unique capacity to rapidly produce large quantities of both TH1 (IFNγ) and TH2 (IL-4) cytokines upon stimulation [21,22], iNKT probably play a key role either in the protection against tumors or in preventing autoimmune disease [18,23,24].

Although circulating iNKT, T$\gamma\delta$ and Treg cell numbers are relatively low (approximately 0.02–0.2% of the $CD3^+$ T-cells are iNKT cells [24], 2–10% of the $CD3^+$ cells are T$\gamma\delta$ cells [15] and 2–5% of $CD4^+$ T-cells are Tregs [11]), numeric deficiencies have been reported in autoimmune disease and in malignancy [18,23,25–27]. Reduced circulating numbers of both iNKT cells and T$\gamma\delta^+$ cells have been reported in CD [28,29]. However, these studies have not been performed in relation to the development of uncontrolled autoimmunity as seen in RCD or to the development of malignancies as EATL. In addition, since iNKT can be either regulatory or pro-inflammatory, expression of the regulation-associated proteins FoxP3 and CTLA4 in iNKT cells might provide new information on the actual regulatory function.

The hypothesis proposed in this study is that a lack of regulatory T cells, including $CD4^+$ iNKT cells, Treg and T$\gamma\delta$ cells, predisposes to the development of RCD. To this end, circulating levels of these cells were assayed in a group of RCD patients (both type I and type II), as well as in active (untreated) and GFD-treated CD patients and in age-matched healthy controls. Moreover, the intracellular levels of the regulatory proteins CTLA4 and FoxP3 were determined in iNKT cells.

Materials and methods

We studied a total of 137 CD patients, 28 untreated (mean age 26.5 years; range 1–75 years, 36% males), 80 responsive to GFD (mean age 38.2 years; range 3–76 years 22% males) and 29 refractory CD (RCD) not responding to a GFD (mean age 57.50 years; range 45–68 years 38% males) and 73 age-matched healthy volunteers without known autoimmune diseases or malignancies (mean age 32.2 years; range 2–82 years; 36% males). RCD patients were divided in RCD type I (14 patients, mean age 57.5 years; range 47–68 years; 36% males) and RCD type II (15 patients, mean age: 60.6 years;

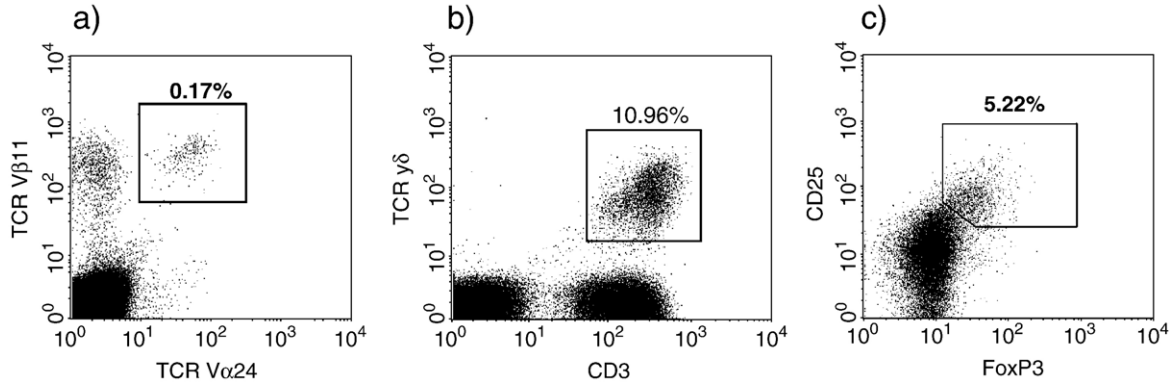

Figure 1 Analysis of circulating regulatory cells from one representative donor. Live lymphocytes were gated based on forward and sideward scatter. (a) iNKT cell analysis. Example of staining of PBMCs for Vα24 and Vβ11 staining on gated $CD3^+$ lymphocytes. iNKTs were defined as $CD3^+$ $V\alpha24^+$ and $V\beta11^+$ (b) TCR T$\gamma\delta^+$ analysis. Example of staining of PBMCs for T$\gamma\delta$ and CD3 expression on gated live lymphocytes. TCR T$\gamma\delta^+$ cells were defined as $CD3^+$ T$\gamma\delta^+$. (c) Treg analysis. Example of staining of gated $CD3^+CD4^+$ T-cells for surface CD25 and intracellular and FoxP3 expression. Treg cells were defined as surface $CD3^+$ $CD4^+$ $CD25^{high}$ and intracellular $FoxP3^+$.