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Origin and immunophenotype of aberrant IEL in RCDII patients

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ABSTRACT

Objectives: Aberrant intra-epithelial lymphocytes (IELs) are the hallmark of refractory coeliac disease type II RCDII and considered a premalignant cell population from which aggressive enteropathy-associated T cell lymphoma (EATL) can evolve. The aim of this study was to gain further insight in the origin and characteristics of aberrant IELs by analysing T-cell receptor (TCR) rearrangements, and by immunophenotypic analysis of aberrant IELs.
Design: Duodenal biopsies from 18 RCDII patients and three RCDII cell lines were analysed for the presence of TCR delta, gamma, and beta rearrangements. In addition, IELs isolated from biopsies derived from RCDII patients were phenotypically analysed.
Results: Aberrant IELs showed an upregulated expression of granzyme B and decreased expression of PCNA. TCR rearrangements in the aberrant IEL population in biopsies of RCDII patients were heterogenic, which is most likely due to a variation in maturity. Similarly, RCDII cell lines displayed a heterogenic TCR rearrangement pattern.
Conclusion: Aberrant IELs originate from deranged immature T lymphocytes and display clear differentiation to a cytotoxic phenotype. Aberrant IELs displayed different stages of maturity between RCDII patients, of which only the patients harbouring the most mature aberrant IEL population developed an EATL.

1. Introduction

Coeliac disease (CD) is a common small intestinal enteropathy induced by dietary gluten proteins as well as other undefined environmental factors, affecting genetically predisposed individuals of all ages. A permanent state of intolerance to gluten-containing food leads to a chronic auto-immune mediated inflammatory response with subsequent remodelling of the proximal small bowel mucosa and nutrient malabsorption (Green and Jabri, 2003). Withdrawal of dietary gluten usually leads to prompt healing of the damaged small-intestinal mucosa and improvement of nutrient absorption.

Although a substantial group of adult-onset CD patients lacks histological recovery after 2 years on a gluten-free diet (GFD) only a small subgroup of patients (Lanzini et al., 2009; Rubio-Tapia et al., 2010), especially those diagnosed above the age of 50 years, develop primary or secondary resistance to a GFD with intestinal villous atrophy and persisting or reoccurring symptoms of malabsorption (Daum et al., 2005). After evaluation of diet-compliance by a dietitian and exclusion of other underlying diseases known to cause villous atrophy, subjects are considered to suffer from refractory coeliac disease (RCD) (Rubio-Tapia and Murray, 2010). Based on the immunophenotype of intraepithelial lymphocytes (IELs), RCD can be subdivided into type I lacking a substantial aberrant IEL population (CD3 -,CD45 +,CD103 +,CD7 +,CD4 -,CD8 -,cytCD3 + cells) and type II in which an aberrant IEL population is present (Cellier et al.,

Abbreviations: APC, allophycocyanin; CD, coeliac disease; EATL, enteropathy associated T-cell lymphoma; EMA-A, endomysial IgA antibodies; FACS, fluorescence activated cell sorter; FITC, fluorescein isothiocyanate; GFD, gluten free diet; IEL, intraepithelial lymphocyte; MFI, mean fluorescence intensity; NKG2D, natural killer associated receptor; NK, natural killer; PCNA, proliferating cell nuclear antigen; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; RCD, refractory coeliac disease; TCR, T-cell receptor; TCRA, T-cell receptor alpha chain; TCRB, T-cell receptor beta chain; TCRD, T-cell receptor delta chain; TCRG, T cell receptor gamma chain; TdT, terminal deoxynucleotidyl transferase; tTGA-A, tissue transglutaminase IgA antibodies; sCD25, soluble IL-2R-alpha.

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Summary box
What is already known:

- Aberrant intra-epithelial lymphocytes (IELs) are the hallmark of refractory coeliac disease type II (RCDII) and considered a premalignant cell population from which an aggressive enteropathy-associated T cell lymphoma (EATL) can evolve.
- Aberrant IELs lack expression of the T cell receptor (TCR)-CD3 complex on the cell surface, yet do contain intracellular TCR and CD3. Currently, the cells from which these aberrant IELs originate, as well as all their phenotypical characteristics, have not been identified.
- Despite standardized treatment, one half of the RCDII patients develop an EATL whereas the other half does not, possibly indicating heterogeneity between aberrant IEL populations in RCDII patients.

New findings:
In a relatively large series of RCDII patients, aberrant IEL populations

- displayed a cytotoxic phenotype based on an upregulated expression of granzyme B.
- indicated to originate from deranged developing precursor T-lymphocytes.
- showed to be heterogeneic between RCDII patients: (more) mature aberrant T cell populations were at higher risk for developing EATL.

Clinical future impact:

- Our results confirmed and provided an explanation for our previous findings that TCRG gene rearrangement analysis, as often used in the workup of RCD, misses at risk monoclonal populations, and shows that phenotypical identification of aberrant IEL populations is a superior predictor of EATL development.
- Our results also suggest that TCR-beta gene rearrangement analysis in addition to phenotypical identification of aberrant IELs could be useful to identify at risk aberrant IEL population.

1998). The distinction between RCD I and II is defined by a clinically validated cut-off of 20% aberrant IELs (Verbeek et al., 2008a).

As a consequence of a generally good response to immunosuppressive therapy, RCDI has a less dismal prognosis compared to patients suffering from RCDII, reflected in a 5-year survival rate of approximately 90% and 44–58%, respectively (Al-Toma et al., 2007a; Daum et al., 2009; Malamut et al., 2009; Rubio-Tapia et al., 2009). More importantly, approximately 40–50% of all RCDII patients develop an aggressive enteropathy-associated T-cell lymphoma (EATL), which is considered to arise from the clonal expansion of the premalignant aberrant IEL population (Bagdi et al., 1999; Cellier et al., 2000; Daum et al., 2001). EATL is one of the main causes of death in RCD patients, due to its aggressive nature and unresponsiveness to currently available therapies (Al-Toma et al., 2007a; Daum et al., 2009; Malamut et al., 2009; Rubio-Tapia et al., 2009). Despite standardized treatment, one half of the RCDII patients develop an EATL whereas the other half does not, which could indicate that aberrant IEL populations between patients are heterogeneous and might be accompanied with a variable risk to develop an EATL. Even though RCDII and EATL patients are associated with HLA-DQ2 homozygosity (Al-Toma et al., 2006), currently no histopathological or immunophenotypic features have been identified that have a prognostic value in the evolution of aberrant IELs into an EATL. Therefore it is of utmost importance to gain more insight in the origin and characteristics of aberrant IELs in RCDII, which will enable a better identification of high risk patients and the development of new therapeutic options.

Recently, elegant work addressing the expansion and function of aberrant IELs has been performed (Malamut et al., 2010; Mention et al., 2003), nevertheless, the exact role of aberrant IEL in the mucosa of the small intestine still remains unclear. Furthermore, the cells from which monoclonal aberrant IELs originate is currently under debate. Although aberrant IELs found in RCDII do not express the T-cell lineage-specific surface CD3-TCR complex, these cells do contain cytoplasmatic CD3 antigen and display T-cell receptor (TCR) rearrangements, indicative of T-cell lineage commitment. It has been suggested that the TCR-CD3 complex is internalized due to overstimulation of IELs, implying that aberrant IELs originate from mature TCR+ IELs (Cellier et al., 2000). More specifically, it is hypothesised that these cells derive from gamma-delta T-lymphocytes based on the observed inversed correlation between aberrant IELs and gamma-delta cells in RCD II (Verbeek et al., 2008b; Cerf-Bensussan and Azogui, 2004).

Alternatively, a small, unique CD3-CD7+ population considered to be NK/T-cell precursors, which is found in the intestine of healthy individuals, is suggested to represent the physiological counterpart of aberrant IELs (Eiras et al., 1998; Leon and Roy, 2004; Tjon et al., 2010). The presence of an immature lymphoid precursor population in the gut mucosa, which could hypothetically serve as origin for aberrant IELs, is emphasized by the ongoing extrathymic maturation of T-cells in the intestinal mucosa throughout life (Bas et al., 2009).

Therefore, in this study we isolated lymphocytes from duodenal biopsies collected from RCDII patients and compared the expression of markers representing activation, proliferation, DNA-repair and lymphocyte development on aberrant lymphocytes to the expression on normal lymphocytes within the same patient. To elucidate the origin of aberrant IELs, we assessed these biopsies for the presence of TCR delta (TCRD), gamma (TCRG) and beta (TCRB) rearrangements. In addition, extensive analysis of TCR rearrangements in RCDII cell lines was performed.

2. Patients and methods

2.1. Patients

RCDII patients included in this study visited the out-patient department of gastroenterology at the VU University Medical Centre, Amsterdam, The Netherlands for diagnostic work-up or regular follow-up. The diagnosis of RCDII was based on persisting or reoccurring symptoms and small intestinal villous atrophy after a former good response despite strict adherence to a gluten-free diet for at least 1 year. Furthermore, the clinically validated cut-off value of 20% aberrant IELs as detected by flow cytometry was predominantly used to distinguish RCD type I and type II (Verbeek et al., 2008a). A lower percentage of aberrant T-cells was allowed in the presence of ulcerative jejunitis.

2.2. Small intestinal biopsies

During upper gastrointestinal endoscopy multiple large spike forceps biopsies were taken from the second part of the duodenum. For TCR rearrangement analysis, 1–2 biopsy specimens were stored in liquid nitrogen until analysis. For flow cytometric evaluation, 6 biopsy specimens were collected from various locations in the duodenum, from which IELs were isolated and pooled before immediate analysis. All biopsy specimens were obtained for diagnostic purposes and the procedures were in accordance with the ethical guidelines of our institution.