Effect of Elemental Diet on Mucosal Immunopathology and Clinical Symptoms in Type 1 Refractory Celiac Disease

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Background & Aims: **Patients with celiac disease (CD) who do not improve or exhibit villous atrophy on a gluten-free diet may have type 1 refractory CD (RCD) with a polyclonal mucosal T-cell infiltrate, or type 2 RCD with a monoclonal infiltrate, also termed *cryptic T-cell lymphoma*. Both conditions are difficult to treat. Here we describe the effects of a nonimmunogenic elemental diet on clinical symptoms and mucosal immunopathology in type 1 RCD.** ***Methods:*** **Ten CD patients on a strict gluten-free diet were diagnosed with type 1 RCD after extensive clinical evaluation in a tertiary referral hospital. A 4-week amino-acid–based liquid elemental diet regimen was given with no other treatment, except in 1 patient who also received methotrexate. Duodenal biopsy specimens were obtained before and after treatment for histologic assessment, immunophenotyping of intraepithelial lymphocytes, T-cell receptor clonality, mucosal interleukin (IL)-15 expression, flow-cytometric analysis of interferon (IFN)-γ–secreting T cells, and whole biopsy specimen IFN-γ messenger RNA determination.** ***Results:*** **Nine patients completed the treatment; however, 1 patient did not tolerate the diet. Histologic improvement and reduced epithelial IL-15 were seen in 8 patients, whereas IFN-γ–secreting mucosal T cells and IFN-γ messenger RNA levels decreased in 4 and 7 patients, respectively. Clinical improvement was noted in 6 patients, with 1 patient showing normalization of hypoalbuminemia. Three patients could discontinue their total parenteral nutrition.** ***Conclusions:*** **Persistent mucosal IFN-γ and IL-15 production often occurs in type 1 RCD despite conventional treatment. Elemental diet is a therapeutic option that can provide long-term immunopathologic and clinical improvement of this difficult condition.**

Celiac disease (CD) occurs in genetically susceptible individuals and is driven by immunopathologic mechanisms triggered by wheat gluten peptides or similar prolamins.[1] The small intestinal lesion shows an increased frequency of intraepithelial lymphocytes (IELs), crypt hyperplasia, and various degrees of villous atrophy that can be graded according to the Marsh classification.[2,3] Strict adherence to a gluten-free diet (GFD) will normalize the clinical and histopathologic features in most cases, although in adults morphologic recovery often takes a long time or may be incomplete.[4] Indeed, in some patients severe symptoms and/or a mucosal lesion persists even when dietary noncompliance, overt lymphoma, or other disorders are ruled out. This condition, called refractory CD (RCD),[5,6] can be divided into type 1 RCD with a normal IEL population, or type 2 RCD with an aberrant or premalignant IEL population, based on clonality analysis of T-cell receptors (TCRs) and immunophenotyping.[7–9] Type 2 RCD can progress from cryptic to overt enteropathy-associated T-cell lymphoma,[6,7,10] also called *enteropathy-type intestinal T-cell lymphoma*, which may present as ulcerative jejunitis.[7]

Treatment of type 1 RCD mainly is empiric because few adequate clinical trials have been performed; steroids, immunosuppressive agents, chemotherapy, or a combination of such drugs[11] have been used as supplemental therapy with variable results. There is often a concern about the detrimental effects of these drugs; importantly, steroids may lead to osteoporosis in CD patients.[12] Elemental diet as an optional treatment has been suggested[5] and anecdotally tried with success in a young woman who wanted to avoid harmful side effects from traditional drugs,[13] although no substantiating immunopathologic investigation was performed. A limited beneficial effect of elemental diet was also reported in another woman who later was treated successfully with antibodies to tumor necrosis factor.[14]

Abbreviations used in this paper: BMI, body mass index; CD, celiac disease; cDNA, complementary DNA; FACS, fluorescent-activated cell sorting; GAPDH, glyceraldehyde phosphate dehydrogenase; GFD, gluten-free diet; IEL, intraepithelial lymphocyte; IFN, interferon; Ig, immunoglobulin; IL, interleukin; mRNA, messenger RNA; RCD, refractory celiac disease; TCR, T-cell receptor.

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The aim of the present investigation was to study the efficacy of elemental diet in a consecutive series of type 1 RCD patients based on histopathologic and immunopathologic criteria in addition to clinical evaluation. This combined approach provided promising results with regard to long-term improvement of type 1 RCD.

Materials and Methods

Patients and Clinical Material

Consecutive patients with type 1 RCD were recruited during the period from 1995 to 2004 in our tertiary referral hospital that serves the whole southeast region of Norway (approximately 2 million inhabitants). Eligibility for this study was based on clinical and internationally accepted histologic criteria for the diagnosis of RCD.[5,6] Other possible causes of absent responsiveness to GFD such as dietary noncompliance, inadvertent gluten ingestion, or separate gastrointestinal disorders were ruled out. Clinical assessment of the patients included clinical investigation, interview by a clinical dietician, gastroduodenoscopy, and small-bowel follow-through examinations.

The study was an open nonrandomized elemental diet intervention trial that was approved by the regional ethics committee. Written informed consent was obtained and clinical assessment, ordinary blood tests, and determination of immunoglobulin A (Ig)A antibodies to tissue transglutaminase were performed before and after the diet regimen. In addition, multiple duodenal biopsy specimens were obtained for evaluation of histology, in situ immunophenotyping, TCR clonality, flow-cytometric determination of interferon (IFN)-γ–secreting mucosal T cells, and polymerase chain reaction–based quantification of mucosal IFN-γ transcripts.

Elemental Diet Treatment

Elemental diet (E028 Extra Liquid) from Scientific Hospital Suppliers International (SHS International, Liverpool, UK) contained a complete mixture of amino acids, dried glucose syrup, blended vegetable oils, minerals, trace elements, and vitamins. A sufficient amount was self-administered at home by the patients and constituted the staple diet for every meal during the 4-week trial period. Energy requirements were calculated from measurements of basal metabolic rate and assessment of average daily activity level. A long-chain triglyceride fat emulsion from highly refined peanut oil was supplemented as an energy supply, and limited amounts of tea, coffee, broth, sweet drinks, and chewing gum were allowed.

Duodenal Biopsy Procedures

Gastroduodenoscopy was performed before and after the elemental diet. Duodenal biopsy specimens were formalin-fixed for ordinary histology (n = 3–5) of well-oriented paraffin wax sections, or embedded in optimum cutting temperature compound (Miles Labs, Elkhart, IN) and snap-frozen in liquid nitrogen for immunohistochemistry (n = 2–3), or stored in RNAlater medium (n = 3–6; Qiagen, Valencia, CA), or transferred for cell isolation (n = 3–6) to a 15-mL centrifuge tube on ice containing RPMI-1640 cell culture medium with 2 mmol/L L-glutamine and 50 μg/mL gentamicin (all from Life Technologies, Paisley, Scotland), and 5% sterile filtered fetal calf serum was added (Biological Industries, Kibbutz Beit Haemek, Israel).

Histopathology and Immunohistochemistry

Formalin-fixed specimens were dewaxed and incubated overnight at 4°C with 2.5 μg/mL monoclonal antibody to IL-15 (MAB647, R&D Systems, Abingdon, UK), followed by a 1-hour incubation at room temperature with indocarbocyanine-conjugated goat anti-human IgG1 (1/1000; Southern Biotechnology, Birmingham, AL). Frozen acetone-fixed tissue sections (4–6 μm) were immunostained with murine monoclonal antibodies to human CD3 (1/40; clone RIV9, IgG3; courtesy of Dr J. Hilgers, Amsterdam, The Netherlands) combined with monoclonal antibodies to CD4 (1/20; clones SK3 and SK4, both IgG1), CD8 (1/20; SK1, IgG1) (both from Becton Dickinson, San Jose, CA), TCRγδ (1/100; 5A6.E9, IgG1; T Cell Sciences, Cambridge, MA), or CD45 (1/40, clones 2B11 and PD7/26, both IgG1; DAKO, Glostrup, Denmark) as previously described.[15] We used leukocyte common antigen (CD45) as a pan IEL marker instead of CD7 because these overlap nearly 100% among IELs (data not shown).[16] All sections were examined with a Nikon Eclipse E800 fluorescence microscope (Nikon, Tokyo, Japan). H&E pictures were obtained with a mounted SPOT camera (Diagnostic Instruments Inc., Brattleboro, VT) and further adjusted with Microsoft PowerPoint 2002 (Redmond, WA) (H&E sections). IL-15 immunohistochemical pictures were obtained with an F-view camera and adjusted with analySIS software (both from Soft Imaging System, Münster, Germany).

Mucosal Cell Isolation

Cells from duodenal specimens were isolated as previously described.[15] Briefly, epithelial cell suspensions were obtained with 2 mmol/L ethylenediaminetetraacetic acid in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline, yielding a median cell viability of 57% (range, 34%–100%) and a median yield of 1.8×10^6 viable cells (range, $.6–8.1 \times 10^6$). Lamina propria cell suspensions then were obtained from the remainder of the tissue with collagenase and dispase giving a median viability of 92% (range, 76%–100%) and a median yield of 3.9×10^6 viable cells (range, $1.3–8.9 \times 10^6$).

Interferon-γ Secretion and Detection Assay

IFN-γ–secreting viable T cells were detected and phenotyped as described.[15,17–19] Briefly, an IFN-γ catch reagent was added to $.04–2.85 \times 10^6$ viable cells that were allowed to secrete IFN-γ for 45 minutes. IFN-γ captured on producer cells was detected with a mouse anti-human IFN-γ–phycoerythrin conjugate, and a fluorescein isothiocyanate-labeled anti-human CD3 or anti-human CD8 conjugate was added to phenotype the positive cells (both from Becton Dickinson). Propidium iodide (.5 μg/mL) was used to exclude dead cells during acquisition ($.3–2.0 \times 10^5$ cells) on a FACScan flow cytometer (Becton Dickinson). Gates

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