Patients

Two duodenal biopsy specimens were obtained during clinically indicated endoscopy from each of 11 non-celiac disease (NCD) and 7 CD patients (CD). The former were on gluten-containing diet and on average 11 (1-16) years old, 5 were females. In this group, 2 patients suffered from type B gastritis, 2 from type C gastritis, 2 from eosinophilic esophagitis, 1 from ulcerative colitis (UC) and 4 individuals had diseases without bowel affection. All NCD patients showed no signs of histological inflammation in duodenal samples. The CD group (median: 14, 6-16 years, 6 females) consisted of 4 individuals on regular gluten containing diet (median: 8, 6-14 years, 3 females, Marsh IIIa-c) and 3 patients on gluten-free diet (median: 16, 14-16 years, 3 females). Concerning the CD patients on gluten-free diet, one patient showed low adherence to the therapy and consequently high TG2-antibody titers and Marsh IIIa. The other 2 patients showed histological and serological remission under gluten-free diet (Marsh 0-I). The study was approved by the ethics committee of the Justus-Liebig-University Giessen (reference number 119/16) and written informed consent was obtained.

Slot Blot

Immunogenicity of various concentrations of mTG after heat-treatment was analyzed by a slot blot method using a polyclonal rabbit antibody against mTG (1:1000, Zedira). In brief, native mTG, mTG heated at 65°C for 20 min and mTG heated at 95°C for 1 min were transferred onto a nitrocellulose membrane by vacuum. Subsequently, incubation with the primary antibody against mTG and with the secondary, HRP-conjugated antibody (horseradish peroxidase, 1:100, Invitrogen, Karlsruhe, Germany) was performed. Visualization was carried out using AEC-Kit (3-Amino-9-Ethylcarbazol, Invitrogen).

Quantitation of RACE cells

To investigate the intracellular distribution of mTG and gliadin within this special cell type, 10 RACE cells from 2 CD patients and 2 controls were quantified. Surface density (SD) and labelling density (LD) of the ER within RACE cells and enterocytes were determined according to Griffiths (*23*). In brief, 10 electron microscopic images of RACE cells and enterocytes from each patient were taken. The evaluation was performed using a standardized grid as well as the following formula with ISm for *Intersections ER membrane*, ISlu for *Intersections ER lumen*, GP for *gold particles*, a *magnification* M of 24.000 and a *grid factor* GF of 4000 µm.

$$SD\ (\mu m^{-1}): \frac{ISm \times M}{ISlu \times GF} \qquad LD\ (GP/\mu m^2): \frac{(GP \times M)^2}{(ISlu \times GF)^2}$$