Reduced Chemokine Receptor 9 on Intraepithelial Lymphocytes in Celiac Disease Suggests Persistent Epithelial Activation

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Background & Aims: **Celiac disease is caused by an inappropriate immune response to dietary gluten, with increased epithelial lymphocyte infiltration in the duodenum/jejunum as a hallmark. The chemokine receptor 9 (CCR9) is a small intestinal homing receptor normally found on most mucosal T cells in this organ. Because CCR9 expression appears to be activation dependent, we examined CCR9 on duodenal T cells from untreated and treated (gluten-free diet) patients with celiac disease and healthy controls.** ***Methods:*** **Duodenal biopsy specimens and blood samples were obtained for histologic analysis and flow-cytometric CCR9 analysis of isolated lymphocytes. CCR9 expression after activation was studied in peripheral blood T cells from healthy volunteers.** ***Results:*** **The median number of CCR9$^+$ cells among CD3$^+$ T cells in epithelium and lamina propria, respectively, was 56% and 48% in controls, 11% and 40% in treated patients, and 1% and 8% in untreated patients. Significant differences occurred between controls and treated or untreated patients in the epithelium but only between controls and untreated patients in the lamina propria ($P = .008$, all comparisons). No such differences were seen in peripheral blood, but stimulation with phorbol myristate acetate and ionomycin and, to a lesser extent, stimulation via NKG2D reduced the CCR9 expression on blood T cells.** ***Conclusions:*** **CCR9 expression is reduced on epithelial and lamina propria T cells in untreated celiac disease. Down-regulation of CCR9 persists in intraepithelial T cells from well-treated patients. This suggests ongoing immune activation preferentially within the epithelium.**

Celiac disease is a common disorder[1–4] characterized by loss of immunologic tolerance to wheat gluten in genetically susceptible individuals.[5] HLA-DQ2– or HLA-DQ8–restricted responses to gliadin peptides from gluten have been shown for mucosal CD4$^+$ T cells isolated from celiac lesions.[6,7] The duodenal/jejunal lamina propria is considered to be the major tissue compartment where the specific T cells are triggered.[5,8] This event is presumed to provide help for cytotoxic T-cell responses. An increased number of intraepithelial T-cell receptor (TCR) $\alpha\beta^+$ and $\gamma\delta^+$ T cells[9] is a hallmark of celiac disease, and such intraepithelial lymphocytes (IELs) are believed to exhibit cytotoxic and regulatory functions,[10,11] although their biological role admittedly is poorly understood.

Ongoing proliferation of IELs and activation of lamina propria lymphocytes appears to take place even in patients treated with a gluten-free diet (GFD).[12] In fact, long-term follow-up of treated patients with celiac disease has shown slow and incomplete histologic recovery.[13] Such persistent mucosal changes do not appear to be related to ingestion of trace amounts of gluten because they are seen at the same frequency in patients on an ordinary GFD and on a GFD that contains no detectable gluten.[14] Therefore, circumstantial evidence suggests ongoing T-cell activation in both mucosal compartments in a large fraction of treated patients with celiac disease. This activity might involve the innate TCR-independent interleukin (IL)-15–induced NKG2D signaling pathway, which has been shown to be important in celiac disease,[15] or TCR-dependent adaptive responses within the epithelium. The extent and degree of ongoing T-cell activation in the treated lesion is difficult to assess and depends partly on the method used.[12]

Chemokine receptor 9 (CCR9) is the only known receptor for the chemokine ligand 25, also called thymus-expressed chemokine.[16–18] CCR9-expressing cells migrate toward chemokine ligand 25, and both molecules colocalize in the thymic cortex and in the small intestinal mucosa.[16–22] In peripheral blood, CCR9$^+$CD45RA$^+$ T cells seem to represent recent thymic emigrants with unknown homing properties.[23] Because most intraepithelial and lamina propria T cells of the small intestinal

Abbreviations used in this paper: APC, allophycocyanin; CCR9, chemokine receptor 9; GFD, gluten-free diet; IEL, intraepithelial lymphocyte; IL, interleukin; mAb, monoclonal antibody; MFI, mean fluorescence intensity; PBMC, peripheral blood mononuclear cell; PMA, phorbol myristate acetate; TCR, T-cell receptor; TG, transglutaminase.

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mucosa express CCR9,[19,20,22,24] it is considered to be a homing molecule for this part of the gut where its ligand is preferentially expressed by the epithelium. Even so, the dependency of CCR9 for gut homing is uncertain because only a slight decrease of intraepithelial TCR$\gamma\delta^+$ T cells was noted in CCR9 knockout mice.[25] Up-regulation of CCR9 can be induced by pre-TCR signaling,[16] retinoic acid,[26] and dendritic cells from Peyer's patches and mesenteric lymph nodes.[27,28] Thus, mucosal CCR9 induction may play an important role in the maintenance of CCR9 on gut T cells. Profound down-regulation of CCR9 by TCR cross-linking was shown in persistently activated Th1 cells generated from umbilical cord $CD4^+$ T cells stimulated with IL-2 and IL-12 together with antibodies to IL-4.[22] Interestingly, this effect was reversible because CCR9 reappeared after removal of the stimulus. Thus, the level of CCR9 expression on T cells might be modulated by the degree of T-cell activation.

An increased frequency of $CCR9^+$ T cells was observed in peripheral blood of untreated patients with celiac disease,[24] but expression of this receptor in the mucosal lesion has to our knowledge not been previously studied. We therefore examined CCR9 expression on epithelial and lamina propria T cells of untreated and GFD-treated patients with celiac disease. We found that both untreated and treated patients with celiac disease had reduced frequencies of $CCR9^+$ intraepithelial T cells compared with control subjects, whereas a reduction of lamina propria $CCR9^+$ T cells was seen only in untreated patients. We also showed in peripheral blood mononuclear cells (PBMCs) that down-regulated CCR9 expression was associated with T-cell activation. Although we did not include intestinal dendritic cells in these in vitro experiments, our results suggested that in situ activation of intraepithelial T cells persists even in well-treated patients with celiac disease.

Patients and Methods

Patients and Sampling

During diagnostic or follow-up gastroduodenoscopy, multiple duodenal biopsy specimens (n = 4–6) and concomitant blood samples were obtained from consecutively recruited patients with treated (n = 7; age range, 22–70 years) and untreated (n = 7; age range, 29–62

Table 1. Subject Characteristics Including Diagnoses, Duodenal Histopathology According to the Marsh Classification, IgA Tissue Transglutaminase Antibodies (Anti-TG2), and Duration of GFD for Patients With Celiac Disease

Patient no.	Age (*yr*)	Sex	Diagnosis	Marsh category	Anti-TG2 <5 U/mL	Duration of GFD (*yr*)
1	28	F	Dyspepsia	0	Negative[a]	NA
2	28	F	Dyspepsia	0	Negative	NA
3	33	F	Dyspepsia	0	Negative	NA
4	40	F	Dyspepsia	0	ND	NA
5	49	F	Dyspepsia	0	ND	NA
6	69	F	Peptic ulcer	0	ND	NA
7	22	F	Treated celiac disease	3A	Negative	1
8	35	M	Treated celiac disease	0	Negative	12[b]
9	50	F	Treated celiac disease	3A[c]	Negative	7[d]
10	52	F	Treated celiac disease	0	ND	6[e]
11	66	M	Treated celiac disease	0	Negative	1
12	68	F	Treated celiac disease	2	Negative	1
13	70	F	Treated celiac disease	0	Negative	4[f]
14	29	F	Untreated celiac disease	3C	>100	NA
15	30	F	Untreated celiac disease	3C	70	NA
16	30	F	Untreated celiac disease	3C	6[g]	NA
17	35	F	Untreated celiac disease	3C	18[h]	NA
18	41	F	Untreated celiac disease	3B	>100	NA
19	44	F	Untreated celiac disease	3C	>100	NA
20	62	F	Untreated celiac disease	3C	>100	NA

NA, not applicable; ND, not done.

[a]Multiple food allergies: positive IgE test to wheat, rye, barley, oats, peas, peanuts, and hazelnuts; clinical reaction to kiwis, oranges, and pears. The subject had normal duodenal mucosa, and an endomysium IgA antibody test was negative before diet restriction 5 years earlier.

[b]Endoscopy for duodenal ulcer follow-up.

[c]Subject accidentally ate gluten-containing bread 2 weeks before biopsy, which was followed by immediate symptoms recovering completely 2 days later.

[d]Endoscopy for variable stools.

[e]Endoscopy for dyspepsia. Also treated with low-dose prednisone for systemic lupus erythematosus diagnosed 17 years earlier.

[f]Endoscopy for nausea and loose stools.

[g]Subject presented with meteorism, borborygmus, iron anemia (hemoglobin level, 10.2 g/dL), and low ferritin (8 μg/L) and vitamin B_{12} (137 pmol/L) values and showed good response to a GFD.

[h]Subject had a low ferritin value (6 μg/L) at presentation and showed good response to a GFD.