BASIC–ALIMENTARY TRACT

Evidence for the Role of Interferon-alfa Production by Dendritic Cells in the Th1 Response in Celiac Disease

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Background & Aims: **Dendritic cells (DCs) play a crucial role in immune responses by controlling the extent and type of T-cell response to antigen. Celiac disease is a condition in which T-cell immunity to gluten plays an important pathogenic role, yet information on DCs is scant. We examined mucosal DCs in celiac disease in terms of phenotype, activation/maturation state, cytokine production, and function.** ***Methods:*** **Mucosal DCs from 48 celiacs and 30 controls were investigated by flow cytometry. In situ distribution of DCs was analyzed by confocal microscopy. Interferon (IFN)-alfa, interleukin (IL)-4, IL-5, IL-12p35, IL-12p40, IL-18, IL-23p19, IL-27, and transforming growth factor-β transcripts were measured by real-time reverse-transcription polymerase chain reaction in sorted DCs. DC expression of IL-6, IL-12p40, and IL-10 was assessed by intracellular cytokine staining. The effect of IFN-alfa and IL-18 blockade on the gluten-induced IFN-γ response in celiac biopsy specimens grown ex vivo also was investigated.** ***Results:*** **Mucosal DCs were increased in untreated, but not treated, celiacs. The majority of them were plasmacytoid with higher levels of maturation (CD83) and activation (CD80/CD86) markers. Higher transcripts of Th1 relevant cytokines, such as IFN-alfa, IL-18, and IL-23p19, were produced by celiac DCs, but because IL-12p40 was undetectable, a role for IL-23 is unlikely. Intracellular cytokine staining of celiac DCs showed higher IL-6, but lower IL-10 expression, and confirmed the lack of IL-12p40. Blocking IFN-alfa inhibited IFN-γ transcripts in ex vivo organ culture of celiac biopsy specimens challenged with gluten.** ***Conclusions:*** **These data suggest that IFN-alfa–producing DCs contribute to the Th1 response in celiac disease.**

Celiac disease is an enteropathy in genetically susceptible individuals mediated by a T helper cell type (Th)1 immune response to gluten.[1] In celiac mucosa, activated T cells produce abundant interferon (IFN)-γ, and express the Th1 transcription factor T-bet.[2] Th1 polarization is driven by many cytokines, including interleukin (IL)-12, IL-18, IL-23, and IFN-alfa.[3] Both IL-18 and IFN-alfa have been shown to be increased in untreated celiac mucosa although the cellular source is not known, but there appears to be no IL-12p40.[4–6]

Mucosal T-cell activation is thought to depend on the delivery of costimulatory signals from intestinal dendritic cells (DCs), which act as sentinels for incoming food or bacterial antigens.[7] DCs achieve this through a number of different mechanisms including their level of maturation at the time of antigen presentation,[8] signaling via receptor-ligand interactions,[9] and secretion of immunosuppressive (IL-10, transforming growth factor [TGF]-β)[10–12] vs proinflammatory cytokines (IL-12 or IL-23).[13,14]

Despite increasing evidence for the importance of DCs in maintaining the balance between immune activation and tolerance in the gut,[7,14,15] the only study exploring DCs in celiac disease is that of Ráki et al,[16] who proposed a role for CD11c-positive myeloid DCs in activating gluten-reactive T cells in the celiac lesion. However, there was an unexpected absence in active celiac mucosa of CD123-positive plasmacytoid DCs, which are known to

Abbreviations used in this paper: DC, dendritic cell; IFN, interferon; IL, interleukin; LPMC, lamina propria mononuclear cell; PT-gliadin, peptic tryptic digest of gliadin; RT-PCR, reverse-transcription polymerase chain reaction; TGF, transforming growth factor; Th, T helper cell type; TLR9, Toll-like receptor-9; TNF, tumor necrosis factor.

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exert a crucial role in inflammation by linking innate with adaptive immunity, and elicit Th1 polarization by secreting IFN-alfa.[17]

In the present study, we combined 2 different methodologic approaches for the phenotypic characterization of DCs in celiac duodenal mucosa (confocal microscopy and flow cytometry), and investigated DC function in terms of cytokine production and activation/maturation behavior in response to immunodominant and nonimmunodominant gliadin peptides.

Materials and Methods

Patients and Tissues

Biopsy specimens were obtained from the second part of the duodenum in 34 antiendomysial antibody–positive patients with untreated celiac disease. The histopathologic diagnosis was based on typical lesions with crypt hyperplasia, villous atrophy (19 patients showed a Marsh IIIc lesion and 15 showed a Marsh IIIb lesion), and increased intraepithelial lymphocytes. There was histologic improvement in all 34 patients after gluten withdrawal. Biopsy specimens also were collected from 14 celiac patients in remission on a gluten-free diet for at least 12 months, and negative for antiendomysial antibodies. All celiac patients were HLA-DQ2–positive. Thirty control subjects undergoing endoscopy for functional dyspepsia, negative for antiendomysial antibody, and with normal histology also were studied. Some of the biopsy specimens were processed for routine histology or embedded in OCT Tissue-Tek (Sakura Finetek, Torrance, CA) and snap frozen; others were used for organ culture or to obtain suspensions of purified lamina propria mononuclear cells (LPMCs). All the experiments were performed on biopsy specimens from UK patients, with the exception of the ex vivo challenge that was performed in Italy (Naples and Rome). Each patient who took part in the study gave informed consent and ethics committee approval was obtained.

Cell Isolation

The epithelial layer was removed with 1 mmol/L ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich, Poole, UK). After stirring for 1 hour at 37°C, the supernatant was removed and the remaining tissue was treated with type 1A collagenase (1 mg/mL; Sigma-Aldrich) for 2 hours with stirring at 37°C.[18] Cells were washed twice with RPMI-1640 medium (Sigma-Aldrich) containing 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Numbers of cells isolated from 6–8 biopsy specimens for each patient ranged from 0.9 to 3.1×10^6. The cells were resuspended in 1 mL phosphate-buffered saline (PBS) and kept on ice until used. Cells were not used if viability did not exceed 90%.

Cell Culture

Freshly isolated LPMCs were cultured at 37°C for 24 hours with 20 μg/mL p31-43 (LGQQQPFPPQQPY) or p57-68 (QLQPFPQPQLPY) gliadin peptides or with 10 ng/mL tumor necrosis factor (TNF)-α (R&D Systems, Abingdon, UK) or 1 mg/mL peptic tryptic digest of gliadin (PT-gliadin, Frazer III fraction; Sigma-Aldrich). For assessment of intracellular cytokines, paired cultures of LPMCs, with and without monensin (eBioscience, San Diego, CA) were cultured for 4 hours at 37°C in a humidified atmosphere of 5% CO_2 in air.

Organ Culture

Biopsy specimens were placed on grids in the central well of an organ culture dish and placed in a tight container with 95% O_2/5% CO_2 at 37°C.[19] Biopsy specimens from untreated celiac patients were cultured for 24 hours with p31-43 or p57-68 gliadin peptides (20 μg/mL). Biopsy specimens from treated celiac patients were cultured with 1 mg/mL PT-gliadin, with and without a neutralizing anti–IFN-alfa antibody (10 μg/mL; Immunokontact, Frankfurt, Germany) for 8 hours. Biopsy specimens from untreated celiac patients also were preincubated for 8 hours with a neutralizing anti–IL-18 antibody (5 μg/mL; R&D Systems) before adding 1 mg/mL PT-gliadin. As positive control for the blocking activity of the anti–IL-18 antibody, blood T cells were cultured for 24 hours in anti–CD3-coated 96-well plates (BD Biosciences, Oxford, UK) with or without IL-18 (100 ng/mL; R&D Systems), IL-18 (100 ng/mL) plus anti–IL-18 antibody (10 μg/mL), and IL-18 (100 ng/mL) plus control IgG (10 μg/mL). IFN-γ transcripts were determined by quantitative reverse-transcription polymerase chain reaction (RT-PCR).

Flow Cytometry

Labeling of LPMCs was performed at 4°C for 30 minutes with the following antibodies: fluorescein isothiocyanate–conjugated Lineage Cocktail 1 (CD3, CD14, CD16, CD19, CD20, and CD56; BD Biosciences), phycoerythrin-conjugated anti–HLA-DR (BD Biosciences), APC-conjugated anti-CD11c, allophycocyanin-conjugated anti-CD123 (Miltenyi Biotec, Auburn, CA), PE-Cy5–conjugated anti-CD80, PE-Cy5–conjugated anti-CD83, and PE-Cy5–conjugated anti-CD86 (BD Biosciences). Appropriate isotype-matched control antibodies (BD Biosciences) were included in all experiments. After washing twice with 250 μL buffer (PBS containing 1 mmol/L EDTA and 0.02% sodium azide), cells were fixed in 2% paraformaldehyde, and analyzed by flow cytometry using a FACSCalibur Flow Cytometer (BD Biosciences). DCs were identified as HLA-DR–positive, lineage-negative cells,[20] and within this gate either the CD11c-positive population or the CD123-positive population were analyzed using CellQuest Software (BD Biosciences). Eosinophils and mast cells, which are large granular cells potentially positive for HLA-DR