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Chemokine Production by Buccal Epithelium as a Distinctive Feature of Pediatric Crohn Disease

*Gerard M. Damen, *Jeroen Hol, *Lilian de Ruiter, *Jan Bouquet, *Maarten Sinaasappel, †Janneke van der Woude, ‡Jon D. Laman, §Wim C. J. Hop, *Hans A. Büller, *Johanna C. Escher, and *Edward E. S. Nieuwenhuis

*Department of Pediatric Gastroenterology and Laboratory of Pediatrics, Erasmus MC–Sophia Children’s Hospital, University Medical Center, †Department of Gastroenterology, Erasmus MC, University Medical Center, ‡Department of Immunology, Erasmus MC, University Medical Center, §Department of Epidemiology and Biostatistics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

ABSTRACT

Objectives: Inflammatory bowel diseases (IBD) represent an aberrant immune response by the mucosal immune system to luminal bacteria. Because the oral mucosa harbors the first epithelial cells that interact with microorganisms, we assessed the immunologic activity of buccal epithelium in children with IBD and adults with Crohn disease.

Methods: Buccal epithelial cells were obtained from 17 children and 14 adults with Crohn disease, 18 children with ulcerative colitis, and 40 controls. Cells were cultured with and without microbial stimulation. Chemokine levels were determined in culture supernatants by cytometric bead array and enzyme-linked immunosorbent assay. CXCL-8 production was studied by immunohistochemical analysis of these cells. CXCL-8 production by lipopolysaccharide stimulated monocyte-derived dendritic cells from these patients was determined.

Results: Compared with controls, pediatric ulcerative colitis patients, and adult Crohn disease patients, only in children with

Crohn disease did buccal epithelial cells exhibit enhanced production of CXCL-8, CXCL-9, and CXCL-10. In vitro stimulation with lipopolysaccharide or zymosan resulted in a further increase of chemokine levels only in cells from pediatric Crohn disease patients. CXCL-8 production by stimulated monocyte-derived dendritic cells from children with Crohn disease was equal to that of children with ulcerative colitis.

Conclusions: Buccal epithelium of children with Crohn disease is immunologically active, even in the absence of oral lesions. The enhanced chemokine production is associated with pediatric Crohn disease and appears restricted to cells derived from the epithelial barrier. Assessment of chemokine production by buccal epithelial cells may become a new, rapid, noninvasive test for screening and classification of IBD in children. *JPGN* 42:142–149, 2006. **Key Words:** Chemokines—

Buccal epithelium—Dendritic cells—Pediatric Crohn disease—Pediatric ulcerative colitis. © 2006 Lippincott Williams & Wilkins

INTRODUCTION

Crohn disease (CD), ulcerative colitis (UC), and indeterminate colitis represent diseases of chronic intestinal inflammation, also called inflammatory bowel diseases (IBD). Genetic susceptibility, environmental triggers, and immune dysregulation have been described as the main factors involved in the establishment and development of IBD (1). The aberrant response of the mucosal immune system associated with IBD is thought to be directed toward microorganisms that are present within the intestinal lumen (2,3). This hypothesis is supported by the evidence of a mutation of the bacterial

sensing gene NOD2 being strongly associated with susceptibility to CD (4,5).

Intestinal epithelial cells (IECs) can play a role in initiating and regulating mucosal innate and acquired immune responses through the secretion of cytokines and chemokines. In IBD patients, epithelial cells derived from colonic specimens are able to produce significant amounts of chemokines including CXCL-1 (Gro α), -2 (Gro β), -5 (ENA-78), -8 (interleukin [IL]-8), and CCL-2 (monocyte chemoattractant protein-1 [MCP]-1), -3 (MIP-1- α), -4 (MIP-1- β), -7 (MCP-3), -8 (MCP-2) (6–15). These studies indicate that IECs are an important source of chemokines that play a role in the recruitment of neutrophils and T lymphocytes to the epithelial layer, which may initiate or promote intestinal inflammation in IBD.

CD can be localized throughout the entire digestive tract. In up to 40% of children with CD, biopsies from the upper gastrointestinal tract may reveal granulomas, even in mucosa that appears normal on endoscopy (16,17).

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Address correspondence and reprint requests to Dr. Edward Nieuwenhuis, Department of Pediatric Gastroenterology and Laboratory of Pediatrics, Erasmus MC–Sophia Children’s Hospital, University Medical Center, Dr. Molewaterplein 60, 3015 GJ Rotterdam, The Netherlands (e-mail: e.nieuwenhuis@erasmusmc.nl).

Accordingly, we speculate that even in the absence of oral lesions buccal epithelial cells from children with CD may display pro-inflammatory immune responses.

We studied the chemokine production by buccal epithelial cells in pediatric IBD patients, in adult CD, as well as in healthy controls. CXCL-8 (i.e., IL-8) production was assessed because it represents the most commonly produced chemokine by the epithelial cells. The production of related chemokines, such as CXCL-9 (i.e., monokine induced by interferon-gamma) and CXCL-10 (interferon-inducible protein-10) was also determined. In addition CCL-2 (MCP-1) and CCL-5 (regulated upon activation, normal T-cell expressed, and secreted [RANTES]) were determined because several studies have indicated enhanced production of these molecules in colonic biopsies from IBD patients. In addition to the evaluation of spontaneous production of these chemokines, we also established an in vitro assay to determine whether these molecules could be induced by microbial stimuli. Finally, we determined the response of monocyte-derived dendritic cells (moDCs) to lipopolysaccharide (LPS). MoDCs were included because they represent a nonepithelial cell type that is involved in mucosal microbial-host interactions (3,18,19).

MATERIALS AND METHODS

Patient Characteristics

All children with (suspected) IBD that were admitted to the clinic or outpatient clinic of the department of Pediatric Gastroenterology at the Sophia Childrens Hospital from September 2003 to September 2004 were included. The included adult CD patients visited the clinic or outpatient clinic of the department of Gastroenterology at the Erasmus MC in September 2003. The study was approved by the Medical Ethical Committee of the Erasmus MC and the Central Committee on Research Involving Human Subjects.

In children with UC, disease activity was assessed by the modified Truelove and Witts score (20). This score is based on clinical symptoms (number of stools a day and amount of bloodloss in the stools), laboratory parameters (hemoglobin levels and erythrocyte sedimentation rate [ESR]), and physical examination (axillary temperature and pulse rate). On a scale of 6 to 18 points, a score of 6 indicates inactive disease, 7 to 10 mild disease, 11 to 14 moderate disease, and 15 to 18 severe disease. In children with CD, disease activity was expressed by means of the Paediatric Crohn Disease Activity Index (PCDAI) (21). The PCDAI is a disease activity index based on symptoms, laboratory parameters (hematocrit, ESR, and albumin), and physical examination, including changes in linear growth. On a scale of 0 to 100 points, a score less than 15 indicates inactive disease, 15 to 30 mild disease, and greater than 30 moderate to severe disease.

Buccal Epithelium

Buccal epithelial cells were collected by gently rubbing a Cytobrush Plus (Medscand Medical AB, Malmö, Sweden) over the inside of the cheeks. Cells were washed twice in RPMI

1640 (Invitrogen, Merelbeke, Belgium). In a 96-well flat bottom plate 3.5×10^4 cells per well were incubated in 200 μ L medium RPMI 1640 supplemented with 10% fetal calf serum (FCS; Integro, Leuvenheim, the Netherlands), HEPES 15 mmol/L, L-glutamine 2 mmol/L, penicillin 100 U/mL, streptomycin 100 μ g/mL, amphotericin B 500 μ g/mL, and mercaptopurine 50 μ mol/L. When more than 10.5×10^4 cells were obtained, LPS (from *E. coli* serotype 005:B5, Sigma-Aldrich, Zwijndrecht, the Netherlands) or zymosan A (from *Saccharomyces cerevisiae*, Sigma-Aldrich) were added at different concentrations (as indicated), starting from February 2004. Buccal epithelial cells do not proliferate in vitro and have limited viability. In a pilot experiment, CXCL-8 was produced already within the first hours of incubation and reached a plateau at 8 to 12 hours. This is in agreement with another publication concerning the production of CXCL-8 by buccal epithelial cells upon microbial stimulation (10). Therefore, we decided to collect (and store at -80°C) the supernatants of the cell cultures at 24 hours of incubation. The remaining cells were washed three times with phosphate-buffered saline (PBS) and suspended in 50 μ L PBS and 50 μ L 10% human serum albumin. Cytospins were prepared by centrifugation (50 g, 7 min) of the cell suspension. Cytospins were air-dried on silica gel overnight and either used directly or stored at -20°C .

Monocyte-Derived Dendritic Cells

Peripheral blood mononuclear cells were isolated by density gradient centrifugation of anticoagulated human blood from IBD patients using Lymphoprep (Nycomed, Oslo, Norway). CD14⁺ cells were isolated by applying MACS CD14 MicroBeads and the Magnetic cell separator MidiMACS (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified cells were typically greater than 95% CD14⁺ as determined by flow cytometry. To obtain MoDCs (22), CD14⁺ cells (5×10^5 cells/well) were cultured in RPMI 1640 with L-glutamine (Invitrogen), 10% FCS (Integro), gentamycin 56 mg/mL, recombinant human granulocyte macrophage colony stimulating factor (molgramostim) at 500 U/mL (Novartis, Arnhem, the Netherlands), and recombinant human IL-4 at 250 U/mL (PeproTech, Rocky Hill, NJ). At day 6, LPS was added to a subset of wells at indicated concentrations. Supernatants were collected at day 7.

Measurement of Chemokines in Cell Culture Supernatants

CXCL-8 levels in the cell culture supernatants were determined by enzyme-linked immunosorbent assay according to the manufacturers protocol (BD Biosciences, San Diego, CA). CXCL-9 and -10 and CCL-2 and -5 were detected by application of the standard protocol of the Cytometric Bead Array using the human chemokine-I kit specific for these chemokines (BD Biosciences).

Immunohistochemical Staining of Buccal Epithelial Cells for CXCL-8

For immunohistochemical detection of CXCL-8, the peroxidase-labeled avidin-biotin method was used. Buccal epithelial cells of the cytopins were fixed by immersion into fresh

acetone containing 0.02% (vol/vol) H₂O₂. Slides were then air-dried for 10 minutes. Histochemical revelation of endogenous peroxidase activity was performed with 4-chloride-1-naphthol (4-Cl-1-naphthol). A solution of 80 mg 4-Cl-1-naphthol in 1 mL of ethanol 100% was added to 200 mL Tris-HCL buffer together with 6.2 μ L 30% H₂O₂. This solution was filtered. Slides were immersed into this solution for 15 minutes at room temperature. Slides were washed with PBS 1 \times 1 min and with PBS/Tween 20 0.05% 1 \times 10 minutes. Subsequently, cells were incubated with the primary mouse antihuman CXCL-8 antibody (BD Biosciences) overnight at 4°C in a humidified atmosphere or with the irrelevant isotype-match antibody (IgG2b; Dako, Glostrup, Denmark). The next morning, cells were incubated with the secondary biotinylated rabbit anti-mouse antibody (Dako) for 30 minutes at room temperature. Between incubation steps, slides were washed twice with PBS/Tween20 0.05%. Subsequently, cells were incubated with avidin-biotin complex labeled horseradish peroxidase-conjugated (Dako) for 1 hour at room temperature. Slides were washed twice in PBS. The peroxidase activity was revealed by incubation with 3-amino-9-ethyl-carbazole (Sigma-Aldrich) for 10 minutes at room temperature, leading to a bright red precipitate. Again slides were washed twice with PBS. Finally, cells were counterstained using Mayer's hematoxylin (Merck) and embedded in glycerol gelatin. The same method was used for detection of CD45 (using a primary mouse anti-human CD45 antibody; BD Biosciences).

Statistical Analysis

To obtain approximate normal distributions, patient groups were compared using the *t*-test after logarithmic transformation. Outcomes less than the lower limit of detection were set at this limit, and the resulting value was analyzed as a left-censored observation using STATA software (proc CNREG). Paired data (LPS 0.01 vs. LPS 0.1 μ g/mL) were compared using the Wilcoxon signed-rank test. Correlation coefficients given are Spearman's (r_s). $P = 0.05$ (two-sided) was considered the limit of significance.

RESULTS

Patient Characteristics and Yield of Buccal Epithelial Cells

In September 2004, 35 children with IBD were included, as well as 14 adult CD patients and 40 controls

(children and adults). Patient demographics and disease characteristics are shown in Table 1. In IBD patients as well as in adult CD patients, disease activity ranged from mild to severe. Six children with CD and six children with UC were newly diagnosed with IBD and had not received anti-inflammatory medication yet. None of the adult CD patients was newly diagnosed (they were all tertiary admitted). Treatment strategies of the IBD patients, children, as well as adults were very different. Five adolescents stopped their medication without consulting the pediatric gastroenterologist. Other patients used 5-ASA, prednisone, azathioprine, methotrexate, anti-tumor necrosis factor, or a combination of these. Most of the controls (children and adults) were healthy. Others were admitted under the suspicion of IBD but turned out not to have IBD.

The median yield of buccal epithelial cells for the pediatric CD patient was 30×10^4 cells per patient (ranging from 3.5 to 81×10^4 cells per patient). The median yield of buccal epithelial cells for the pediatric UC patient was comparable (24×10^4 cells per patient, ranging from 3.5 to 85.6×10^4 cells per patient). The yield of buccal epithelial cells for the adult CD patients and the controls was also within the same range.

CXCL-8 Production by Buccal Epithelial Cells Exclusively Enhanced in Children with Crohn Disease

Figure 1 shows the levels of CXCL-8 in the culture supernatants of buccal epithelial cells. In children with CD, CXCL-8 production was significantly higher than in children with UC ($P = 0.001$) or controls ($P < 0.001$). Four of six children with CD were newly diagnosed, had a moderate to severe disease activity, and were not receiving medication yet. These four patients presented with a high CXCL-8 production (of more than 300 pg/mL). CXCL-8 production by buccal epithelial cells derived from adult CD patients was comparable with that of controls. In children with CD, the CXCL-8 production by buccal epithelial cells was correlated with ESR ($r_s = 0.61$; $P = 0.016$), as is shown in Figure 2. In these children, CXCL-8 production did not correlate with the C-reactive

TABLE 1. Patient characteristics and patient demographics

	Pediatric UC	Pediatric CD	Adult CD	Controls
Number	18	17	14	40
Male/female	10/8	10/7	4/10	18/22
Age at diagnosis (yr)	1.5–15 (median 11)	1.2–16 (median 10)	>18	n.a.
Age at study entry (yr)	4–17 (median 13)	1.7–17 (median 12)	29–63 (median 42)	1–50 (median 17)
Newly diagnosed at study entry	6/18	6/17	0/14	n.a.
PCDAI for children	n.a.	7.5–55 (median 24)	n.a.	n.a.
CDAI for adults	n.a.	n.a.	47–325 (median 164)	n.a.
Modified Truelove-Witts score	7–16 (median 10)	n.a.	n.a.	n.a.

UC, ulcerative colitis; CD, Crohn disease; n.a., not applicable; PCDAI, Pediatric Crohn Disease Activity Index; CDAI, Crohn Disease Activity Index.

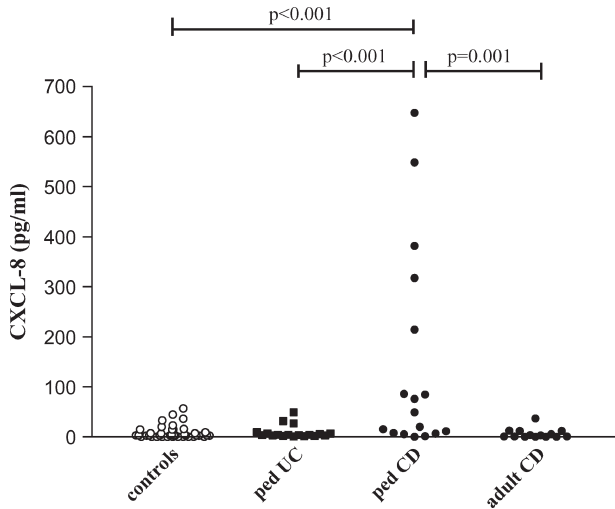


FIG. 1. CXCL-8 is exclusively enhanced in pediatric Crohn disease (CD) patients. CXCL-8 levels in the culture-supernatants of buccal epithelial cells of controls (adults and children), children with ulcerative colitis (ped UC), children with CD (ped CD), and adults with CD (adult CD). Samples were taken at 24 hours of incubation. In children with CD, CXCL-8 production was significantly enhanced compared with children with UC ($P < 0.001$) and controls ($P < 0.001$) and adults with CD did not show enhanced CXCL-8 production.

protein ($r_s = -0.13$; ns), the hemoglobin ($r_s = -0.37$; ns), the thrombocytes ($r_s = -0.06$; ns), the leukocytes ($r_s = -0.10$; ns), or the albumin ($r_s = -0.42$; ns) in the blood (data not shown). Finally, the CXCL-8 production was not related to clinical disease activity expressed as PDAI ($r_s = 0.33$; ns) (data not shown).

To conclusively identify the epithelial cell as the source for the CXCL-8, we performed immunohistochemical

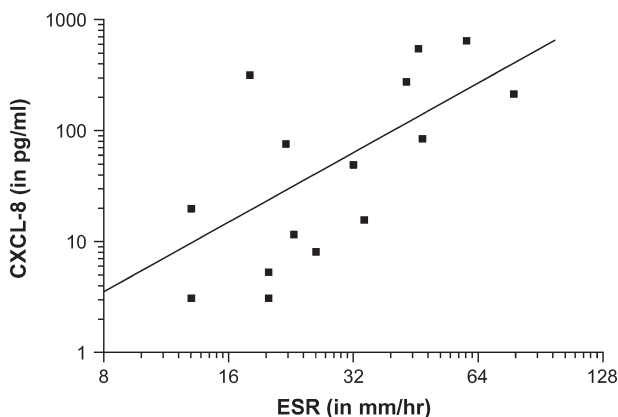


FIG. 2. In children with Crohn disease (CD), CXCL-8 production by buccal epithelium correlates with erythrocyte sedimentation rate (ESR). CXCL-8 levels in the culture supernatant of buccal epithelial cells derived from children with CD correlate with ESR in blood of these patients ($r_s = 0.61$; $P = 0.016$). Line represents least-squares regression line after logarithmic transformation of both axes.

analysis of these cells. Figure 3 shows representative examples of CXCL-8 production by buccal epithelial cells. CXCL-8 was particularly detected at the periphery of the cells and was located in granules. As shown, buccal epithelial cells were not contaminated with cells positive for the hematopoietic marker CD45 (i.e., monocytic cells or macrophages).

Enhanced Production of Other Chemokines by Buccal Epithelial Cells from Pediatric CD Patients

In addition to CXCL-8, the levels of CXCL-9, CXCL-10, CCL-2, and CCL-5 were determined in the culture supernatant of the buccal epithelial cells at 24 hours of incubation by using a cytometric bead array. Figure 4 shows that in children with CD, CXCL-9 production was significantly higher compared with children with UC ($P = 0.008$). In children with CD, CXCL-10 production was also higher compared with children with UC ($P = 0.024$). The production of CCL-2 and CCL-5 were equally low in both patient groups.

Stimulation of Buccal Epithelial Cells with LPS or Zymosan

To determine whether the buccal epithelium of pediatric CD patients produced more CXCL-8 as a result of a lower threshold for microbial stimulation, we next performed an in vitro stimulation assay. In this assay, buccal epithelial cells were cultured in the presence of LPS or zymosan at different concentrations. In 4 of 10 children with CD, the production of CXCL-8, CXCL-9, or CXCL-10 increased more than 50 pg/mL. Figure 5 shows a representative example of enhanced chemokine production upon microbial stimulation. No induction (either by LPS or zymosan) of chemokine production (CXCL-8, CXCL-9, CXCL-10, CCL-2, or CCL-5) was found in any of the buccal epithelial cells that were derived either from children with UC ($n = 10$) or in cells from healthy controls ($n = 20$).

Response of Monocyte-Derived Dendritic Cells to Lipopolysaccharide

Next, we determined whether the enhanced chemokine production by buccal epithelium demonstrated in pediatric CD could be extended to other immunocompetent nonepithelial cells. As such, CXCL-8 production by moDCs in response to LPS was measured in pediatric IBD patients. MoDCs were derived from children with CD and UC and stimulated with different concentrations of LPS, as described. As is illustrated in Figure 6, CXCL-8 production in response to LPS 0.1 $\mu\text{g/mL}$ generally was higher than in response to LPS 0.01 $\mu\text{g/mL}$ ($P = 0.004$ for CD; $P = 0.04$ for UC). In response to LPS

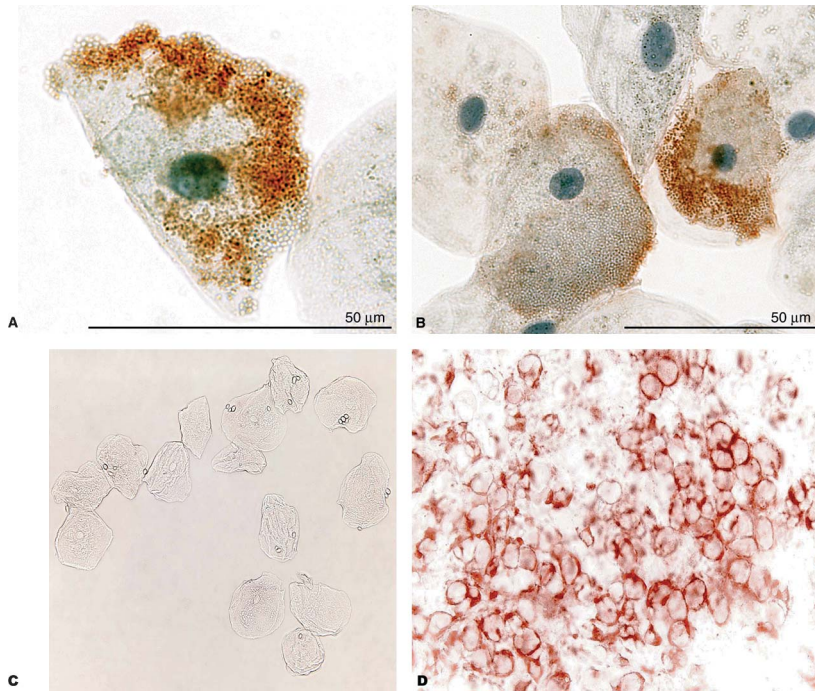


FIG. 3. Representative examples of CXCL-8 production by buccal epithelial cells. Immunohistochemical analysis for CXCL-8 performed on cytopins of buccal epithelial cells. (A and B) buccal epithelial cells of a child with CD identified as source for CXCL-8. Orange-red pigment indicates positive CXCL-8 staining. In contrast with bone marrow cells, positive for CD45 (red staining, D), buccal epithelial cells were all negative for the hematopoietic marker CD45 (C), which excludes contamination with monocytic cells or macrophages.

0.01 $\mu\text{g}/\text{mL}$, moDCs of children with CD produced the same amounts of CXCL-8 as those of children with UC ($P = 0.64$; ns). In response to LPS 0.1 $\mu\text{g}/\text{mL}$, moDCs of children with CD also produced comparable amounts of CXCL-8 compared with that of children with UC ($P = 0.89$; ns). In conclusion, in response to LPS, moDCs of children with CD did produce comparable amounts of CXCL-8 as did moDCs of children with UC.

DISCUSSION

One hypothesis on the etiology of IBD is that these diseases represent an aberrant immune response by the mucosal immune system to either pathogenic or resident luminal bacteria. According to this hypothesis, resident bacteria can persistently stimulate the mucosal and systemic immune system, thereby perpetuating the inflammatory response. IECs and DCs are among the

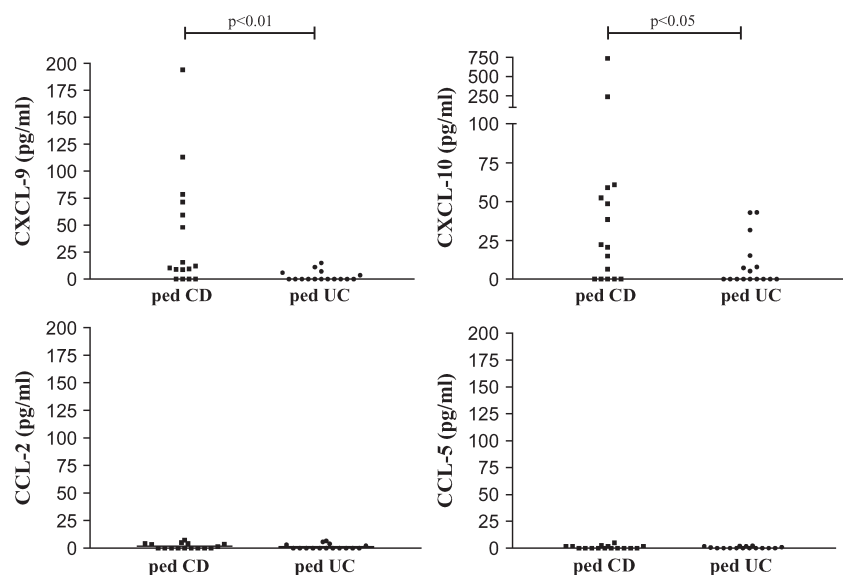


FIG. 4. Levels of CXCL-9, CXCL-10, CCL-2, and CCL-5 in the culture supernatants of buccal epithelial cells of children with Crohn disease (ped CD) and ulcerative colitis (ped UC). Samples were taken at 24 hours of incubation. CXCL-9 and CXCL-10 production was significantly enhanced in CD patients compared with UC patients ($P < 0.01$ and $P < 0.05$, respectively). CCL-2 and CCL-5 levels were very low in both CD and UC patients.

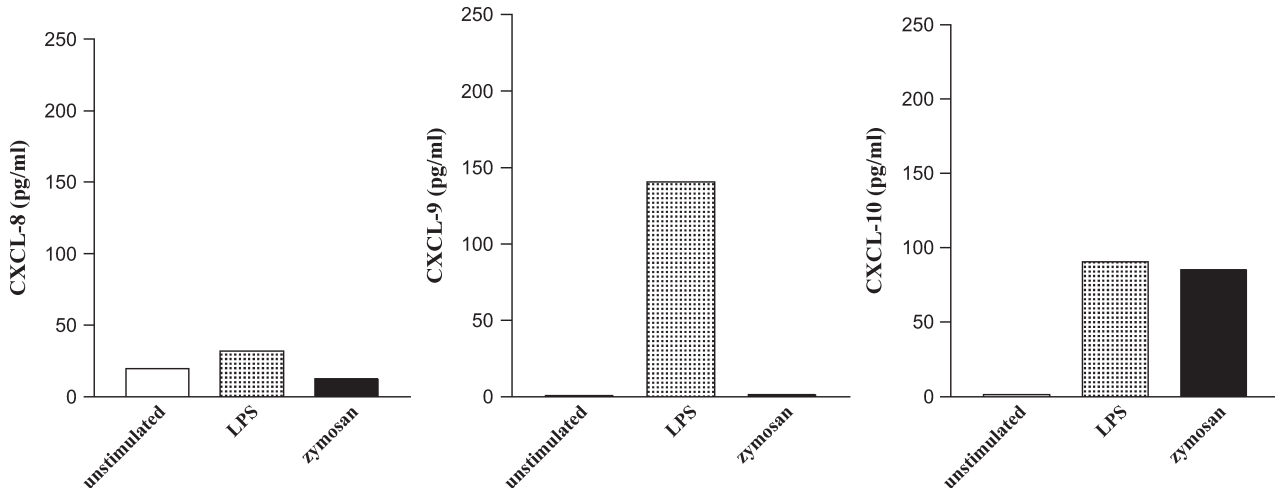


FIG. 5. Representative example of inducible chemokine production by buccal epithelial cells from pediatric Crohn disease patients. Cells stimulated with lipopolysaccharide (LPS) at 0.1 $\mu\text{g/mL}$ or zymosan at 100 $\mu\text{g/mL}$. At 24 hours of incubation, chemokine production was determined in culture supernatants.

first cells that are capable of sensing microbial signals through the expression of pattern recognition molecules such as the Toll-like receptors (TLR). Through the presentation of antigens and the production of chemokines and cytokines, IECs and DCs are involved in the initiation and regulation of the acquired immune response.

Several studies have identified the IECs as a major source of chemokines that play an important role in chemotaxis, adhesion, activation, and degranulation of migratory immune cells. In response to microorganisms or pro-inflammatory cytokines, IECs are capable of producing CXCL-1, -3 (GRO γ), -5, -8, -9, -10, and -11 (I-TAC) as well as CCL2, -3, -4, and -5 (10,23–28). In freshly obtained specimens from IBD patients, epithelial cells of the lower gastrointestinal tract showed an increased expression of CXCL-1, -5, and -8, as well as CCL-2, -3, -4, -7, and -8 (6–15). These studies used in situ hybridization with radio-labeled probes of the chemokine-genes, immunohistochemical analyses, or specific protocols for the isolation of IECs. Importantly, other studies that made use of the same techniques failed to demonstrate such chemokine production by colonic epithelial cells in vivo (29–32). Although buccal epithelial cells appear to be an obvious subject for experimentation because these cells are so readily available without the need for endoscopy or biopsies, to our knowledge this is the first functional study on these cells in IBD patients. In fact, chemokine production or expression by buccal epithelial cells in these patients has never been studied before.

Here, we demonstrate that in children with CD, buccal epithelial cells produce significantly higher levels of CXCL-8, CXCL-9, and CXCL-10, in comparison with children with UC, with controls, or with adults with CD. Interestingly, not all newly diagnosed children with CD presented with an enhanced chemokine production by buccal epithelial cells. Also, no relation was found between the disease activity (PCDAI) or a specific drug that was used and chemokine production. These findings may suggest that the enhanced chemokine production is specifically associated with a subset of patients with a specific (genetic) ethiopathogenesis.

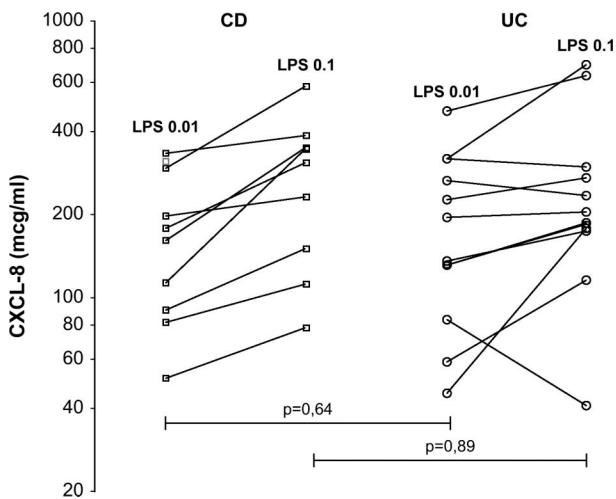


FIG. 6. CXCL-8 production by stimulated monocyte-derived dendritic cells (moDCs) in children with Crohn disease (CD) is comparable with that of children with ulcerative colitis (UC). Levels of CXCL-8 in culture supernatant of moDCs of children with CD ($n = 10$) and UC ($n = 13$) compared. CXCL-8 production in response to lipopolysaccharide (LPS) 0.1 $\mu\text{g/mL}$ generally was higher than in response to LPS 0.01 $\mu\text{g/mL}$ ($P = 0.004$ for CD; $P = 0.04$ for UC). In response to LPS 0.01 $\mu\text{g/mL}$, moDCs of children with CD produced the same amounts of CXCL-8 as those of children with UC ($P = 0.64$; ns). In response to LPS 0.1 $\mu\text{g/mL}$, moDCs of children with CD also produced comparable amounts of CXCL-8 compared with that of children with UC ($P = 0.89$; ns).

In children with CD, the production of CXCL-8 by buccal epithelial cells was correlated with the ESR in the blood. No such correlation was found in children with UC, despite the fact that the range of ESR in these children was comparable with that of the children with CD. The correlation between ESR and CXCL-8 production in CD patients may represent a phenomenon that is associated only with pediatric CD and not with pediatric UC. Because an enhanced ESR is found in active pediatric CD as well as in active pediatric UC, CXCL-8 production by buccal epithelial cells appears the better test to discriminate between the two diseases.

Upon stimulation with LPS or zymosan, only buccal epithelial cells derived from pediatric CD patients show an inducible production of chemokines. On the basis of our preliminary data (11 of 17 children with CD had an enhanced release of CXCL-8, CXCL-9, or CXCL-10 spontaneously, and 4 of 10 children with CD had an increased release upon microbial stimulation), we estimate that over 72% of all children with CD will exhibit an enhanced chemokine production, spontaneously or upon microbial stimulation. These striking differences in response patterns by buccal epithelial cells can be explained by various mechanisms. Alterations in the local (oral) flora of pediatric CD patients may be associated with an enhanced chemokine production. The fact that chemokine production by microbial stimuli could only be induced in pediatric CD patients points to an enhanced ability of these cells to become stimulated. Because we could not show the same differences in moDCs, this mechanism appears to be limited to epithelial cells of pediatric CD patients. The enhanced chemokine production by buccal epithelial cells from pediatric CD patients may be associated with mutations in the NOD2 molecule, such as described (33). A recent paper by Watanabe et al. (34) elucidated how signaling through mutated NOD2/CARD15 molecules may lead to disease by causing an excessive T_H1 response. The authors present a model whereby NOD2 senses muramyl dipeptide (a breakdown product of peptidoglycan) within the cell, which leads to a blockade of TLR2 signaling upon activation by peptidoglycan at the cell surface. This may in fact represent a physiologic mechanism through which the inflammatory response to gut flora is limited. Mutant NOD2 (in CD) will not sense MDP and will be associated with unopposed TLR2 signaling, which leads to enhanced IL-12 production, one of the essential mediators of intestinal inflammation in IBD. Another approach may be that the expression of molecules such as TLR2 and 4 is specifically enhanced in the epithelial cells of pediatric CD patients, as is suggested by various authors (35–37). Finally, the results may also be explained by alterations in the expression of molecules such as TOLLIP. Recently, it was reported that these types of molecules might contribute to a state of hyporesponsiveness of epithelial cells to microbial stimuli (38–40).

Finally, we found a striking difference in epithelial chemokine-response patterns by comparing pediatric with adult IBD patients. None of the adult CD patients in this study were diagnosed in childhood. The question whether pediatric CD patients will also exhibit this enhanced chemokine production into adulthood remains to be elucidated. Interestingly, recent reports suggest that, in comparison with adults, pediatric CD may represent a distinct disease that may be associated with an enhanced incidence of NOD2/CARD 15 mutations (41,42).

A high production of CXCL-8 or CXCL-9 by the buccal epithelium, either spontaneously or upon microbial stimulation, increases the suspicion of CD in nondiagnosed children. In children with indeterminate colitis, an enhanced production of these chemokines may contribute to further discrimination. Determining the chemokine production by these cells can be of great value in making the correct diagnosis and in deciding on a specific treatment modality. Enhanced chemokine production by buccal epithelial cells may well provide us with the first soluble marker that is exclusively linked to pediatric CD and may therefore become a new, rapid, and noninvasive test in children with suspected IBD.

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