Evidence of Dysregulated Cytokine Production by Sinus Lavage and Peripheral Blood Mononuclear Cells in Patients With Treatment-Resistant Chronic Rhinosinusitis

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Background: Treatment-resistant chronic rhinosinusitis (CRS) imposes a clinical challenge. Its pathogenesis may be associated with dysregulated immune/inflammatory responses in the sinus.

Objective: To evaluate production of types 1 and 2 T cytokines (interferon gamma [IFN-γ] and interleukin [IL] 5/IL-4, respectively) and regulatory/inflammatory cytokines (IL-10, IL-12, and IL-18) by sinus lavage (SL) cells and peripheral blood mononuclear cells (PBMCs) in patients with treatment-resistant CRS.

Methods: Sample SL cells and PBMCs obtained from 19 patients with treatment-resistant CRS were cultured with or without stimuli, and cytokine levels in the supernatant were measured using enzyme-linked immunosorbent assay. Control PBMC samples were obtained from 26 children.

Results: Chronic otitis media was found in 15 patients. Neutrophils and/or epithelial cells were dominant in SL cells. IFN-γ, IL-12p40, and IL-10 (>100 pg/mL) were detected in SL cell cultures from 12, 9, and 8 patients, respectively. Production of IL-12p40 and IL-18 by SL cells correlated positively with phytohemagglutinin and IL-12p70 stimuli. In 12 patients, we detected IL-18 (>100 pg/mL) in SL cell cultures without stimuli, whereas PBMCs produced little IL-18, irrespective of stimuli. There was no correlation between cytokine levels produced by SL cells and PBMCs, except for IL-12p40 produced using IL-18. Decreased IFN-γ production by PBMCs was observed in 6 patients with CRS compared with controls, but 4 of them had elevated IFN-γ production by SL cells. Production of IL-12p40 by PBMCs was higher in 10 patients with CRS than in controls, and 7 of these patients had lower IL-10 production, resulting in an increased IL-12p40/IL-10 ratio.

Conclusions: There is a role for locally produced regulatory cytokines in IFN-γ production in the sinus in patients with treatment-resistant CRS. However, aberrant cytokine production patterns by PBMCs can be detected at high frequency in these patients, indicating that this can be used as a prognostic marker for patients with CRS.

PARTICIPANTS, MATERIALS, AND METHODS

STUDY POPULATION

We recruited 19 patients with treatment-resistant CRS (aged 3-18 years; 7 girls and 12 boys) who underwent sinus surgery, pressure equalization tube placement plus sinus tap, or both at the Fairview University Medical Center, Minneapolis, Minn. All patients had clinical signs of rhinosinusitis (facial pain, sinus headache, nasal congestion [engorgement or swelling of the inferior turbinates], discolored rhinorrhea, cough, etc) for more than 3 months; failed more than 2 courses of intravenous antibiotics (>14 days) and at least 1 sinus surgery; and had positive findings on multiple computed tomographic scans of the sinus. All the participants underwent adenoidectomy before sinus surgery or at the time of the first sinus surgery. However, sinusitis did not resolve or recurred within 3 months of surgery in all patients. The clinical features are summarized in Table 1. Antibiotic therapy was discontinued 1 week before surgery. The study protocol was approved by the institutional review committee of the University of Minnesota, Minneapolis, and a signed written consent form was obtained before surgery. The presence of asthma and allergic rhinitis was evaluated by history, physical examination, prick skin test reactivity, or IgE Ab levels against common aeroallergens (dust mites, grass/tree/ragweed pollens, cats, and molds, including Alternaria, Cladosporium, Aspergillus fumigatus, Epicoccum, and Penicillium), and pulmonary function tests, including responses to histamine and metacholine challenge (and pulmonary function tests, including responses to sporium blue dye exclusion in a hemocytometer. Then, 1 to 2

The number of cells in SL samples was measured by trypan blue dye exclusion in a hemocytometer. Then, 1 to 2 x 10^5 cells in 200 µL of phosphate-buffered saline solution were cyto-

NONSPECIFIC INFLAMMATORY PARAMETERS

Sinus lavage cells were filtered through coarse gauze to remove mucins and tissue debris, spun down briefly, and washed once with phosphate-buffered saline solution. Peripheral blood mononuclear cells were obtained by centrifuging cells with Ficoll-Hypaque density gradient at 1500 rpm for 30 minutes at room temperature. Sinus lavage cells (2 x 10^5 cells/mL) as well as PBMCs (10^6 cells/mL) were cultured with or without stimuli in RPMI 1640 supplemented for 4 days in a 5% carbon dioxide incubator, as reported previously. We used mitogens (phytohemagglutinin [PHA], 2 mg/mL, and concanavalin A, 1 mg/L) (Sigma-Aldrich Corp, St Louis, Mo) and dust mite extract (a mixture of Dermatophagoides farinae and Dermatophagoides pteronyssinus [3 mg/mL each]) (Greer Laboratories Inc, Lenoir, NC) as polyclonal and recall Ag stimuli, respectively. Dust mite Ag was selected as a recall Ag because most patients demonstrate responses to dust mite Ag in the assays of cytokine production by PBMCs without seasonal variation (Prescott et al and H.J., unpublished observations, 2000). Levels of IFN-γ and IL-5 were measured as representative T1 and T2 cytokines, respectively. Levels of IL-10, IL-12p40, and IL-18 were also measured as representative regulatory cytokines for IFN-γ production; IL-12/IL-18 augments IFN-γ production, and IL-10 suppresses it. Levels of IL-4 were also measured as a representative T2 cytokine, but we detected little in the cultures of PBMCs and SL cells, partly owing to autocrine consumption of IL-4 by T cells. Interleukin 12p70, a biologically functional IL-12, was not measured because it is rapidly degraded into IL-12p40 and IL-12p35 and is difficult to detect when PBMCs are cultured with these stimuli. Cytokines (IFN-γ, IL-4, IL-5, IL-10, IL-12p40, and IL-18) added to the culture medium without cells are stable, and we recovered more than 90% of cytokines after 4 days' incubation. All the cytokine levels except IL-18 were measured using enzyme-linked immunosorbent assay sets (OptEIA; BD PharMingen, San Diego, Calif). Levels of IL-18 were measured by using standards and Abs from R & D Systems, Minneapolis.

BACTERIAL AND FUNGAL CULTURE

Samples of SL cells were sent to the clinical microbiology laboratory at the University of Minnesota for bacterial and fungal culture; bacteria culture results were expressed as light, moderate, and heavy growth when the bacterial growth was detected on the first, second, or third agar plate, respectively, on which samples were streaked consequently without reapplying the sample to an applicator.

STATISTICS

The equality of 2 sets of data values was evaluated using the Mann-Whitney test (2 sets of independent samples) or the Wilcoxon weighted ranks test (2 sets of related samples). Comparison of multiple values was performed using the Kruskal-Wallis test. Correlation of 2 variables was assessed using the Kendall τ test. Differences with P < .05 were considered statistically significant.
IgE Abs.7-9 Type 1 and T2 responses counter-regulate each other by producing T2 cytokines (IL-4, IL-5, and IL-13) and IgG4/2 Abs. Increased production of these cytokines and Abs, especially IL-4 and IgG4, in patients with sinusitis may play a role in the persistence of sinus inflammation.5,6 Type 1 and T2 responses are distinguished cytokine production patterns: type 1 and type 2 (T1/T2) T cells.7,8 Type 1 responses induce phagocytic cell-mediated immune responses by producing T1 cytokines (IFN-γ, interleukin [IL]-2, and tumor necrosis factor α) and IgG1/IgG3 Abs that enhance opsonization.7,8 Type 2 responses induce eosinophil-mediated inflammatory responses by producing T2 cytokines (IL-4, IL-5, and IL-13) and IgG4/IgE Abs.7,9 Type 1 and T2 responses counter-regulate each other, and imbalance of T1/T2 responses is implicated in the various disorders.8,9 Previously, we reported T1-dominant inflammatory responses in the sinus in patients with nonatopic CRS as evidenced by increased production of IFN-γ by sinus lavage (SL) cells.10 However, inappropriate IFN-γ production could lead to persistent sinus inflammation. In patients with lacuna immunodeficiency involving regulatory mechanisms of IFN-γ production, clinical features may include treatment-resistant CRS.11

In this study, we address production of IFN-γ and its regulatory cytokines by SL cells and peripheral blood mononuclear cells (PBMCs) in patients with treatment-resistant CRS. The hypotheses to be tested were that we could detect changes in the production of T1/T2 cells and their regulatory cytokines by SL cells or PBMCs in patients with treatment-resistant CRS and that this could serve as a prognostic biomarker. We assessed the production of IFN-γ (a T1 cytokine), IL-4/IL-5 (T2 cytokines), IL-10 (a counter-regulatory cytokine), and IL-12p40/IL-18 (cytokines that induce IFN-γ production) by SL cells and PBMCs using findings from SL cell cytologic examination, the results of microbial cultures, and clinical features.

RESULTS

CLINICAL FEATURES

Clinical features revealed little atopic components in the study patients. Although 4 of 19 patients had elevated IgE levels (>24 mg/dL) (Table 1), 2 of them were skin test nonreactive. Fifteen of 19 patients experienced chronic otitis media (effusion >8 weeks), with no response to pressure equalization tube before the development of CRS. Four patients with chronic otitis media underwent mastoidectomy owing to mastoiditis.

CULTURE RESULTS

Bacterial cultures were positive in 18 of 19 patients tested. Bacterial culture revealed heavy growth of Staphylococcus aureus or coagulate-negative Staphylococcus species. Light and moderate growth of a mixed bacterial flora (S aureus, coagulate-negative Staphylococcus species, Moraxella catarrhalis, α-hemolytic streptococci, Haemophilus influenzae, Corynebacterium, Neisseria species, Streptococcus pneumoniae, Haemophilus haemolyticus, and Haemophilus influenzae) were found in 12 of 18 patients with bacterial culture–positive CRS (Table 1). Fungal cultures were negative in all the study patients.

CYTOLOGIC FINDINGS

The number of SL cells recovered was variable (median, 0.30 cells; range, 0.10-5.76 × 10⁶ cells). Cytologic examination showed that neutrophils were most common (median, 69.6%; range, 11.3%-95.4%). Epithelial cells were also found (median, 14.8%; range, 1.5%-74.5%). Eosinophils were found in SL cell samples from 4 of 19 patients (maximum, 3.4%), but none of them had elevated serum IgE levels.

CYTOKINE PRODUCTION BY SL CELLS

We previously reported that IFN-γ, IL-4, IL-10, and IL-12p40 production levels by SL cells obtained from healthy adults were below the detectable levels of the assay. We also found that SL cells from healthy adults produce little IL-5 or IL-18 (<3.9 pg/mL). In contrast, despite the low number of SL cells cultured (2 × 10⁵ cells/mL), IFN-γ (>100 pg/mL) was detected with PHA or IL-12p70 in 14 (74%) of 19 patients (Table 2). Interleukin 12p40 and IL-10 (>100 pg/mL) were detected in 12 (63%) and
8 (42%) patients, respectively, with any of these stimuli. Of these patients, 7 and 5 revealed spontaneous IL-12p40 and IL-10 production (>100 pg/mL), respectively. Eighteen of 19 patients produced IL-18, greater than 50 pg/mL, and 12 patients (63%) produced IL-18, greater than 100 pg/mL, spontaneously (Table 2 and Figure 1). The IL-5 and IL-4 levels were under the detectable levels. We found low IFN-γ production (<3.9 pg/mL) in 4 patients despite elevated IL-12/IL-18 production (>100 pg/mL).

Levels of IL-18 produced by SL cells correlated positively with IL-12p40 levels in SL cell cultures stimulated with PHA and IL-12p70 (P<.02) (Figure 2). There was no correlation between levels of other cytokines, irrespective of the stimuli used. There was also no correlation between the cytokine levels produced by SL cells and PBMCs, except for IL-12p40 levels stimulated by IL-18 (r=0.67; P<.02).

**CYTOKINE PRODUCTION BY PBMCs**

Peripheral blood mononuclear cells from children with CRS produced less IFN-γ than those from controls in the presence of concanavalin A, partly because the cells of 5 patients with CRS produced lower IFN-γ levels than the range observed in controls. One patient with CRS who had low IFN-γ production had a signal transduction defect through the IL-12 receptor.13 However, we found elevated IFN-γ production by SL cells in 4 of these 5 patients. In contrast, 6 (32%) of 19 patients with CRS revealed elevated IFN-γ production with PHA or IL-12p70 (Table 3), but their IFN-γ production by SL cells was not elevated, except in 1 patient. These results indicate a discrepancy in IFN-γ production by SL cells and PBMCs.

Production of IL-12p40 was higher in children with CRS than in controls with medium, PHA, and IL-12, partly because PBMCs produced IL-12p40 higher than its range in controls with PHA and IL-12 in 7 and 8 patients with CRS, respectively (Table 3). Peripheral blood mononuclear cells from patients with CRS produced less IL-10 with PHA than those from controls, and 7 children with CRS produced IL-10 less than the range in controls with PHA and IL-12 (Table 3). As a result, the IL-12p40/IL-10 ratios produced with PHA and IL-12p70 stimuli were higher in patients with treatment-resistant chronic rhinosinusitis. As given in Table 2, several patients spontaneously produced IL-12p40 and IL-18 at high levels. There was no correlation between percentage of neutrophils or endothelial cells and the cytokine levels produced spontaneously by sinus lavage cells.

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**Table 2. Summary of Cytokine Production Patterns by SL Cells in Children With Treatment-Resistant CRS**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>SL cells</th>
<th>Medium</th>
<th>Con A</th>
<th>PHA</th>
<th>Dust mite</th>
<th>IL-12p70</th>
<th>IL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>Median (Range), pg/mL</td>
<td>11.9 (0.8-943.4)</td>
<td>33.6 (1.8-1377.0)</td>
<td>73.6 (3.9-1544.0)</td>
<td>42.0 (0.8-1000.0)</td>
<td>60.3 (3.9-729.0)</td>
<td>40.3 (3.9-2950.0)</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>Median (Range), pg/mL</td>
<td>16.6 (3.9-1095.0)</td>
<td>21.7 (1.6-1134.0)</td>
<td>65.6 (3.9-2468.0)</td>
<td>4.5 (3.9-1241.0)</td>
<td>24.7 (3.9-1020.0)</td>
<td>26.1 (3.9-1727.0)</td>
</tr>
<tr>
<td>IL-10</td>
<td>Median (Range), pg/mL</td>
<td>114.5 (3.9-1229.0)</td>
<td>117 (3.9-1351.0)</td>
<td>120.3 (&lt;3.9-2014.0)</td>
<td>121.8 (3.9-1663.0)</td>
<td>130.0 (&lt;3.9-1519.0)</td>
<td>121.8 (&lt;3.9-1663.0)</td>
</tr>
</tbody>
</table>

† Values as compared with values obtained in control cultures without stimuli. IL-12p40 and IL-18 levels did not differ with or without stimuli, irrespective of the kinds of stimulants, because of significant spontaneous production of IL-12p40 and IL-18 in several patients, as given in the second row.

‡ Data are given as the number (percentage) of patients whose SL cells produced greater than 100 pg/mL of cytokines with medium, PHA, or IL-12p70 among 19 study patients.

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**Figure 1.** Spontaneous interferon gamma (IFN-γ), interleukin (IL) 10, IL-12p40, and IL-18 production by cultured sinus lavage cells from patients with treatment-resistant chronic rhinosinusitis. As given in Table 2, several patients spontaneously produced IL-12p40 and IL-18 at high levels. There was no correlation between percentage of neutrophils or endothelial cells and the cytokine levels produced spontaneously by sinus lavage cells.

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**COMMENT**

We previously showed that elevated IFN-γ production was more likely to be found in the sinus of patients with CRS without atopy along with increased frequency of positive microbial cultures, indicating microbial-triggered T1 responses. This study determined production of IFN-γ and its regulatory cytokines, including IL-10, IL-12p40, and IL-18, by SL cells and PBMCs in patients with treatment-resistant CRS. The results revealed substantial production of IFN-γ, IL-18, IL-12p40, and IL-10 in the sinus in patients with treatment-resistant CRS. Sinus lavage cells produced IL-18 (>100 pg/mL) without stimuli in these patients at high frequency, whereas...
control cultures without stimuli. Blood mononuclear cells produced little IL-18 with or without stimuli, and IL-18 levels produced with stimuli did not differ significantly from those produced in PHA, phytohemagglutinin.

ease outcome and complications; this is particularly true for children with CRS. Postulated risk factors for CRS include atopic disorders, asthma, peripheral eosinophilia, aspirin sensitivity, specific Ab deficiency, and age (>50 years), but risk factors may vary depending on the study population. For example, peripheral eosinophilia has been frequently reported in adults with CRS, but we did not find considerable peripheral eosinophilia in children with CRS in the previous study.10 Our previous results suggest that elevated IFN-γ production by SL cells likely indicates absence of atopy.

PBMCs produced little IL-18. In addition, an aberrant cytokine production pattern by PBMCs was found in a subset of patients with treatment-resistant CRS.

Pathogenesis of CRS seems to be heterogeneous, and a subset of patients with CRS undergoes multiple courses of antibiosis and surgical procedures despite appropriate medical and surgical treatments.1 It is important to identify patients with CRS resistant to conventional treatments in the early stage of the disease to improve disease outcome and complications; this is particularly true for children with CRS. Postulated risk factors for CRS include atopic disorders, asthma, peripheral eosinophilia, aspirin sensitivity, specific Ab deficiency, and age (>50 years), but risk factors may vary depending on the study population. For example, peripheral eosinophilia has been frequently reported in adults with CRS, but we did not find considerable peripheral eosinophilia in children with CRS in the previous study.10

Our previous results suggest that elevated IFN-γ production by SL cells likely indicates absence of atopy.

Figure 2. Significant positive correlation between interleukin (IL) 12p40 and IL-18 levels produced by sinus lavage cells when cells were stimulated with phytohemagglutinin (A) or biologically functional IL-12 (IL-12p70) (B).

Table 3. Summary of Cytokine Production Patterns by PBMCs in 19 Children With Treatment-Resistant CRS and 26 Controls

<table>
<thead>
<tr>
<th>PBMCs</th>
<th>IFN-γ, Median (Range), pg/mL</th>
<th>IL-12p40, Median (Range), pg/mL</th>
<th>IL-10, Median (Range), pg/mL</th>
<th>IL-18, Median (Range), pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>6.3 (1.1-2211.0)</td>
<td>7.9 (3.9-160.0)</td>
<td>21.3 (3.9-326.0)</td>
<td>8.6 (1.3-23.9)</td>
</tr>
<tr>
<td>Controls</td>
<td>571.1 (3.9-6118.0)</td>
<td>755.5 (62.9-4285.0)</td>
<td>934.0 (138.4-1645.0)</td>
<td>14.4 (2.9-92.0)</td>
</tr>
<tr>
<td>Con A‡</td>
<td>1420.0 (65.0-6768.0)</td>
<td>1231.0 (472.0-3273.0)</td>
<td>1087.0 (185.8-7660.0)</td>
<td>1285.0 (47.1-7405.0)</td>
</tr>
<tr>
<td>PHA†</td>
<td>925.5 (3.9-7317.0)</td>
<td>1420.0 (65.0-6768.0)</td>
<td>511.0 (78.0-1800.0)</td>
<td>3.9-4612.0</td>
</tr>
<tr>
<td>Dust mite‡</td>
<td>1285.0 (47.1-7405.0)</td>
<td>1420.0 (65.0-6768.0)</td>
<td>401.0 (21.3-1753.0)</td>
<td>3.9-4612.0</td>
</tr>
<tr>
<td>IL-12p70†</td>
<td>1285.0 (47.1-7405.0)</td>
<td>1420.0 (65.0-6768.0)</td>
<td>1231.0 (472.0-3273.0)</td>
<td>1420.0 (65.0-6768.0)</td>
</tr>
<tr>
<td>IL-18</td>
<td>513.1 (3.9-1373.0)</td>
<td>1018.0 (28.8-4242.0)</td>
<td>755.2 (54.9-1413.0)</td>
<td>1144.0 (32.9-8421.0)</td>
</tr>
<tr>
<td>Controls</td>
<td>502.5 (67.7-1561.0)</td>
<td>1008.0 (122.0-3180.0)</td>
<td>934.0 (138.4-1645.0)</td>
<td>1144.0 (32.9-8421.0)</td>
</tr>
<tr>
<td>PBMCs</td>
<td>&lt;Range§ (IL-12p40)</td>
<td>&gt;Range§ (IL-12p40)</td>
<td>&lt;Range§ (IL-10)</td>
<td>&gt;Range§ (IL-18)</td>
</tr>
<tr>
<td>Medium</td>
<td>0</td>
<td>3 (16)</td>
<td>0</td>
<td>3 (16)</td>
</tr>
<tr>
<td>Con A</td>
<td>5 (26)</td>
<td>1 (5)</td>
<td>0</td>
<td>5 (26)</td>
</tr>
<tr>
<td>PHA</td>
<td>3 (16)</td>
<td>4 (21)</td>
<td>7 (37)</td>
<td>7 (37)</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>2 (11)</td>
<td>4 (21)</td>
<td>0</td>
<td>5 (26)</td>
</tr>
<tr>
<td>IL-18</td>
<td>0</td>
<td>5 (26)</td>
<td>1 (5)</td>
<td>4 (21)</td>
</tr>
</tbody>
</table>

*PBMC indicates peripheral blood mononuclear cell; CRS, chronic rhinosinusitis; IFN, interferon; IL, interleukin; Con A, concanavalin A; and PHA, phytohemagglutinin.
†Cytokine levels obtained are higher than those obtained in control cultures without stimuli in patients with CRS and control children (P<.005). Peripheral blood mononuclear cells produced little IL-18 with or without stimuli, and IL-18 levels produced with stimuli did not differ significantly from those produced in control cultures without stimuli.
‡The number (percentage) of patients whose PBMCs produced lower levels of cytokines than the ranges observed in control children.
§The number (percentage) of patients whose PBMCs produced higher levels of cytokines than the ranges observed in control children.
and positive microbial cultures. However, IL-10/IL-12p40 production by SL cells did not reveal any close association with IFN-γ production by SL cells in the previous study.10 The present study was an extension of our previous study addressing regulatory mechanisms of IFN-γ production in the sinus of children with treatment-resistant CRS. We hypothesized that growth of microbes triggers IFN-γ production by activating constitutive cells to produce IL-12/IL-18 in the sinus.14-16,21

In this study, 18 of 19 patients had positive bacterial cultures of SL cell samples. We also revealed elevated IFN-γ production (>100 pg/mL) by SL cells with PHA or IL-12p70 in 14 (74%) of 19 patients with CRS. This is striking given the low numbers of SL cells cultured (2 × 10^6 cells/mL). We also observed substantial IL-12p40 and IL-18 production (>100 pg/mL) in many patients (Table 2). Moreover, 7 (37%) and 12 (63%) of 19 patients showed spontaneous IL-12p40 and IL-18 production (>100 pg/mL) by SL cells, respectively. This is in contrast to the minimal amount of IL-18 produced by PBMCs, although the concentration of PBMCs cultured (10^6 cells/mL) was 5-fold higher than that of SL cells. These findings indicate that in children with treatment-resistant CRS, SL cells are likely activated to produce IL-12 and IL-18 spontaneously.

Cytologic examination revealed that the main cells found in SL were neutrophils and epithelial cells. These cells could be the main source of IL-12 and IL-18, 2 IFN-γ-inducing cytokines in the sinus; both lineage cells are known to produce these cytokines.14-16,21 Moreover, IL-12p40 and IL-18 levels produced by SL cells correlated when cells were stimulated with PHA and IL-12p70. These results point to the synergistic actions of these 2 cytokines for IFN-γ production in the sinus.

In this study, we also found 4 patients with CRS who had little IFN-γ production by SL cells despite elevated IL-12p40 and IL-18 levels. This might be explained by increased production of counter-regulatory cytokines such as IL-10 and transforming growth factor β.22,23 However, we did not find any negative correlation between the IFN-γ and IL-10 levels produced by SL cells. Production of IL-10 by SL cells was generally low compared with IL-12p40/IL-18 production. In 2 patients with low IFN-γ production by SL cells, we did not find considerable transforming growth factor β production (H.J., S.S., H.L., F.L.R., unpublished observation, 2001). In our patients with high IL-12p40/IL-18 but low IFN-γ production by SL cells, it is unlikely that excessive production of IL-10 transforming growth factor β caused low IFN-γ production by SL cells. Interferon gamma is crucial for eradicating certain microbial pathogens. Our results thus indicate that in children with treatment-resistant CRS, dysregulation of IFN-γ production in the sinus could be associated with persistent sinus inflammation.

Abnormalities involving regulatory cytokines for IFN-γ production do not result in abnormal Ab production or changes in PBMC phenotypes or lymphocyte proliferative responses. Nevertheless, such a defect could cause inefficient microbial clearance in the sinus and middle ear. Thus, we reasoned that although all the study patients revealed normal immune functions with conventional immune workup, we could still detect aberrant cytokine production by PBMCs in some children with treatment-resistant CRS. Many study patients (15/19; 79%) had chronic otitis media requiring surgical procedures for the middle ear before the development of CRS, further indicating lacuna immunodeficiency in these patients.

This study revealed IFN-γ, IL-12p40, and IL-10 production by PBMCs higher or lower than the ranges observed in control children in subsets of patients with CRS (Table 3). In 5 (26%) of 19 patients, we found IFN-γ production lower than the reference range for children despite normal to elevated IL-12p40 production. Production of IL-10 was not elevated in these patients. One of these patients was later found to have a signal transduction defect through IL-12R, resulting in a partial defect in IFN-γ production.11 In our assay system, T cells are likely the major source of IFN-γ. Other patients with treatment-resistant CRS may also reveal other types of lacuna immunodeficiency involving IFN-γ production by T cells. However, we still found substantial IFN-γ production by SL cells in 4 of 5 patients with low IFN-γ production by PBMCs. These results indicate other cellular sources for IFN-γ in the sinus in these patients, may be compensating for defective IFN-γ production by T cells.

In 6 (32%) of 19 patients with CRS, we also found IFN-γ production by PBMCs higher than the reference range along with higher IL-12p40 production and lower IL-10 production than the ranges in controls. Interleukin 12 promotes IFN-γ production, whereas IL-10 suppresses it.14-16,22 Elevated IFN-γ production by PBMCs in these children with CRS could be partly due to unbalanced production of regulatory cytokines for IFN-γ production. Only 1 of these 6 patients revealed elevated IFN-γ production by SL cells. These results again indicate the importance of microenvironmental factors for IFN-γ production in the sinus. It may be worthwhile to include the assays of T1/T2 cytokine production and its regulatory cytokines by PBMC as a second-line workup in children with treatment-resistant CRS if conventional immune workup does not reveal any abnormalities. If such abnormality is detected, intervention treatments such as exogenous IFN-γ can be considered an option.

This study presents evidence of dysregulated regulatory mechanisms for IFN-γ production in the sinus in a subset of children with treatment-resistant CRS. More-
over, we also revealed the possibility that in a subset of children with treatment-resistant CRS, we may detect abnormal production of IFN-γ and its regulatory cytokines by PBMCs. Further analysis of the dysregulated mechanisms of IFN-γ production may reveal prognostic biomarkers for treatment-resistant CRS.

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