Recurrent respiratory papillomatosis (RRP), characterized by the recurrent growth of premalignant tumors of the upper respiratory tract, is caused by infection with human papillomavirus (HPV), predominantly types 6 and 11. Surgical removal of these lesions can be required as frequently as every 3 to 4 wks to maintain a patent airway. There is no approved medical treatment for this disease. In this study, we have characterized the TH2-like chemokine profile (CCL17, CCL18, CCL20, CCL22) in patients with RRP and asked whether it was modulated in patients who had achieved significant clinical improvement. CCL17, CCL18 and CCL22 messenger RNAs (mRNAs) were increased in papillomas compared with clinically normal laryngeal epithelium of the RRP patients. Overall, CCL20 mRNA expression was not increased, but there was intense, selective CCL20 protein expression in the basal layer of the papillomas. Patients with RRP expressed more CCL17 (p = 0.003), CCL18 (p = 0.0003), and CCL22 (p = 0.007) in their plasma than controls. Plasma CCL18 decreased over time in three patients enrolled in a pilot clinical trial of celecoxib, and the decrease occurred in conjunction with clinical improvement. There was a significant correlation between sustained clinical remission in additional patients with RRP and reduced levels of CCL17 (p = 0.01), CCL22 (p = 0.002) and CCL18 (p = 0.05). Thus, the change in expression of these three plasma TH2-like chemokines may, with future studies, prove to serve as a useful biomarker for predicting disease prognosis.
guide leukocyte movement, support angiogenesis (12) and participate in the balance of T₄₁-like versus T₄₂-like responses maintained by macrophages (13,14). We had also previously identified a robust expression of cyclooxygenase-2 (COX2) and its downstream product prostaglandin E₂ (PGE₂) throughout the airway tissues of patients with RRP compared with controls, mediated by constitutive activation of the EGFR/Rac1 pathway (15). PGE₂ can bias the adaptive immune response away from an effective T₄₁-like pattern (16), and can enhance expression of T₄₂-like chemokines by innate immunocytes (16,17). Therefore, both viral and host factors could modulate the innate response in these patients.

In this study, we have characterized the T₄₂-like chemokine profile in patients with RRP, asked whether the profile correlated with disease severity, and asked whether that profile changed when severity changed. We found an elevated T₄₂/T₄₁-like chemokine balance in patients with RRP that correlated with disease severity. The inducible T₄₂-like chemokine CCL20 was expressed selectively in the basal keratinocyte layer of papillomas, where infiltrating immunocytes would first gain access to HPV antigen-expressing cells. We also found that plasma levels of the T₄₂-like chemokines CCL17, CCL18 and CCL22 were reduced in concert with sustained clinical remission.

MATERIALS AND METHODS

Patients

Studies were approved by the North Shore-LIJ Health System Institutional Review Board. Biopsies were collected from papillomas and autologous clinically normal airway epithelium (adjacent tissue) from patients with RRP and from control airway tissues from patients without RRP undergoing surgery at Long Island Jewish Medical Center. Blood was drawn prior to induction of anesthesia. Disease severity scores were calculated as described previously (5,18) and classified as either mild/moderate (score <0.06), or severe (score ≥0.06 or tracheal involvement). Severity has been associated previously with altered immunologic responses in RRP, while age of disease onset, gender, or infection with HPV6 versus HPV11 has not correlated (7,8).

Celecoxib Studies

Design of the double-blinded placebo-controlled celecoxib studies for treatment of RRP has been described previously (15). Briefly, patients are randomized to either drug or placebo for 1 year and then switched to the other drug for a second year. The pilot study has been completed, and the blind broken. The Phase IIb trial (ClinicalTrials.gov identifier NCT00571701) (19) is ongoing and the blind has not been broken. At the time of this study, 38 patients were enrolled, 23 patients had sufficient clinical data to assess changes in disease status, and seven were free of disease for at least two 3-month intervals. Multiplex plasma samples, at irregular intervals, were obtained from the three patients enrolled in the pilot study. Plasma samples were obtained at regular 3-month intervals in the Phase IIb study. All samples were stored at −80°C. T₄₂-like chemokine levels in plasma samples were measured as described below.

Quantitative PCR

Expression of the T₄₂-like chemokines CCL17 (TARC) (20), CCL18 (DC-CK-1, PARC, AMAC-1, MIP-4) (21,22), CCL20 (LARC, MIP-3α) (23–27), and CCL22 (MDC, STCPI, ABCD-1) (17,28–32) and the T₄₁-like chemokines CCL19 (MIP-3b, eotaxin-3) and CCL21 (6Ckine) (33–36) were measured by quantitative reverse transcriptase PCR (qPCR) with gene specific primers and an 8-mer oligonucleotide probe from the Universal Probe Library Set (Roche, Mannheim, Germany) (37) as shown in Table 1. Samples were amplified according to the manufacturer’s directions with the BioRad for Probes PCR kit (Bio-Rad Laboratories, Hercules, CA, USA), using ROX as a normalization standard. Real-time PCR was performed using an Applied Biosystems 7900 HT thermocycler (Life Technologies, Carlsbad, CA, USA) with SDS 2.4 software, and results were analyzed using the ΔΔCt method (38). Six to 19 samples of each type of tissue, derived from different patients, were analyzed for each chemokine.

Immunohistochemistry

Eight-µm sections of paraffin blocks of papillomas and “adjacent” normal laryngeal epithelium were obtained from five patients with severe RRP, processed using standard methods and stained to identify the location of CCL18 and CCL20. Briefly, sections were incubated with either anti-CCL18 polyclonal goat-biotin or anti-CCL20 polyclonal goat-biotin antibodies (R&D Systems, Minneapolis, MN, USA; 1:50 dilution), and detected with Streptavidin-Cy3–conjugated secondary anti-biotin antibodies (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA; 1:200 dilution). Slides were mounted with UltraCruz Mounting medium (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA), observed through a Zeiss Axiovert 200M-inverted microscope, images analyzed using the AxioVision V4.7.2 software (Carl Zeiss Microscopy LLC, Thornwood, NY, USA), and pseudocolor images produced with ImageJ 1.46o (NIH, Bethesda, MD, USA; http://rsb.info.nih.gov/ij/) and Photoshop 7.0 (Adobe, San Jose, CA, USA) software.

Enzyme-Linked Immunosorbent Assay (ELISA)

Chemokines were measured in the plasma of RRP patients and controls by DuoSet (CCL18) or Quantikine (CCL17, CCL20, CCL21 or CCL22) ELISA (R&D Systems) according to the manufacturer’s directions.

Multiplex ELISA

Chemokines were measured in plasma from the subset of patients enrolled in the Phase IIb celecoxib trial with a highly sensitive, customizable, multiplex, cytokine/chemokine array (Aushon Biosystems, Billings, MA, USA) (39). All assays were performed by the vendor in duplicate each time, on at least two different runs, to validate this assay.
patients that did/did not maintain a sustained clinical remission. This contingency table was analyzed by Fisher exact test.

RESULTS

Chemokine Expression by Laryngeal Tissues from RRP Patients and Controls

To further study the possible bias in T_1,2-like chemokine mRNA expression in papillomas suggested in our earlier mRNA expression array study (6) and ask if it represented an HPV-induced change or also was characteristic of the airway of patients with RRP, we quantitatively compared the T_1,1/T_1,2-like chemokine mRNA repertoires expressed by papillomas (papilloma) and clinically-normal laryngeal tissues from RRP patients (adjacent) to laryngeal tissues from controls without RRP (true normal) (Figure 1). Approximately 50% of individual biopsies of clinically normal airway of RRP patients contain latent HPV DNA (41). Therefore we cannot exclude the possibility that latent HPV infection could contribute to altered chemokine expression in adjacent tissue. However, latency is not expected to alter cellular functions since essentially no viral expression is observed (42).

Surprisingly, expression of the T_1,2-like chemokines CCL17 and CCL18 was markedly reduced in “adjacent” tissue of patients compared with control “true normal” tissues, while CCL20 and CCL22 levels were essentially comparable (Figure 1). This suggests that laryngeal tissues of RRP patients may be polarized away from expressing at least some T_1,2-like chemokines, and also that the constitutive expression of PGE_2 does not induce chemokine expression by laryngeal keratinocytes. In contrast, active HPV infection in the papillomas increased the levels of CCL17 and CCL22 markedly and possibly increased the level of CCL18, counteracting the anti-T_1,2 bias. CCL20 mRNA levels were not increased. The expression of the T_1,1 chemokine CCL19 was reduced markedly in papillomas compared with adjacent tissues from RRP patients and normal tissues from controls. Expression of CCL21, another T_1,1-like chemokine, was not detectable in any of the tissues (data not shown). The net effect of these results suggests that active HPV infection shifts the local T_1,1/T_1,2-like chemokine balance toward a T_1,2-like state in RRP patients.

Localization of T_1,2-like Chemokine Expression in Papillomas

CCL18 and CCL20 were the only T_1,2-like chemokines we studied whose mRNAs were not expressed at markedly

---

**Table 1.** Real-time PCR primers and probes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primer (5'→3')</th>
<th>Antisense primer (5'→3')</th>
<th>UPL probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cnt1</td>
<td>GAPDH</td>
<td>AGGCTATGAAGTCAAGAAACC</td>
<td>60</td>
</tr>
<tr>
<td>T_1,2</td>
<td>CCL17</td>
<td>GCTGCAGCCAGCTGCAATC</td>
<td>27</td>
</tr>
<tr>
<td>CCL18</td>
<td>AGATCCACACAAAGTCATAGG</td>
<td>GATCTGCGCTGCTGCTCTT</td>
<td>88</td>
</tr>
<tr>
<td>TGAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL20</td>
<td>GTGGCCTTTCTGGAATGAA</td>
<td>CAACCCCAAGAAGGCTCTTT</td>
<td>117</td>
</tr>
<tr>
<td>CCL22</td>
<td>CGTGTGAACACATTCTCTCTG</td>
<td>CCTATCCGGAAGAGTACGAA</td>
<td>51</td>
</tr>
<tr>
<td>T_1,1</td>
<td>CCL19</td>
<td>AGTGGACCAAATGAGCTG</td>
<td>34</td>
</tr>
<tr>
<td>CCL21</td>
<td>AGAAAGGAAGGGGCTCCAAA</td>
<td>AGGCTGACAAGCTGTTG</td>
<td>01</td>
</tr>
</tbody>
</table>

UPL, Universal Probe Library.
higher levels in the papillomas than in the adjacent normal tissues from patients with RRP. We therefore asked whether active virus infection altered CCL18 and CCL20 distribution within the tissues. The CCL18 staining pattern in papillomas showed scattered positive cells in the upper spinous layer (Figure 2A; thin arrow). The frequency of these cells varied within different areas of a given papilloma and between patients. CCL18 expression in the adjacent tissues was barely detectable, but was diffuse throughout the epithelium (Figure 2B). By contrast, there was intense and selective CCL20 expression in the basal layer of the papillomas that extended more weakly into the lower spinous layers (Figure 2C). This pattern of staining was consistent in papillomas from multiple patients with severe RRP. CCL20 in adjacent tissues was diffusely uniform throughout the epithelial layers (Figure 2D). The high spinous cell/basal cell ratio in papillomas compared with normal laryngeal epithelium would explain the reduction in overall level of CCL20 mRNA in the papilloma tissue. Interestingly, we have shown previously that HPV 6/11 viral RNA is expressed strongly in the suprabasal layers (1) (Figures 2E,F), the converse to the CCL20 expression pattern. We were unable to detect the other Th2-like chemokines by immunohistochemistry, which could reflect their immediate release from the cells.

**Figure 2.** CCL18 localizes to scattered cells in the spinous layer of papilloma tissues and CCL20 localizes to the basal layer. CCL18 and CCL20 were detected by immunohistochemistry in papillomas and clinically normal adjacent epithelium of patients with severe RRP. (A) CCL18 stained scattered cells in the papillomas (thin arrow), but (B) showed only faint diffuse staining in normal adjacent tissues. (C) CCL20 was localized predominately to the basal layer of papillomas, adjacent to the basement membrane, while (D) normal adjacent tissue had uniform staining throughout the epithelium. (E,F) In situ hybridization, reprinted by permission from Steinberg, et al., 1988 (1), shows HPV-6/11 viral expression in the suprabasal layer. A–D scale bar = 20 µm; E and F scale bar = 100 µm; large arrows point to the basement membrane.

**Figure 3.** Th2-like chemokines are overexpressed in the plasma of patients with RRP and correlate with disease severity. (A) CCL17, (B) CCL18 and (C) CCL22 were measured in plasma of patients with RRP and controls. The concentration (mean ± SEM) of each chemokine is shown. Concentrations of all three chemokines are higher in patients with RRP: CCL17 pg/mL (severe 317.9 ± 84.0, mild/mod 169.7 ± 22.2, control 98.0 ± 14.3, p = 0.003); CCL18 ng/mL (severe 81.9 ± 12.8, mild/mod 72.7 ± 6.7, control 36.2 ± 8.8, p = 0.0003); and CCL22 pg/mL (severe 686.5 ± 105.1, mild/mod 512.8 ± 31.7, control 411.4 ± 34.0, p = 0.0007). Levels are significantly different among patients with severe RRP (n = 10), patients with mild/moderate RRP (n = 16) and controls (n = 14), as determined by ANOVA, Kruskal-Wallis test.

**Th2-like Chemokines Are Enriched in the Plasma of RRP Patients, and Levels Correlate with Disease Severity**

We then asked whether the Th2-like chemokine expression bias present locally in airway tissues of RRP patients was also seen systemically. We measured chemokines in the plasma of 16 patients with mild/moderate disease and 10 patients with severe disease compared with 10 controls without the disease. Patients with RRP expressed more CCL17 (p = 0.003), CCL18 (p = 0.0003) and CCL22 (p = 0.0007) than patients without RRP (Figure 3). Neither CCL20, which is not constitutively expressed, nor the Th1-like chemokine CCL21, was expressed differentially in plasma of RRP patients and controls (data not shown). The assays were repeated in five subjects (three RRP patients with stable ongoing disease and two controls) over six time points spread out over 2 years, with less than 4% variability in the results for each individual when repeated measures were obtained over time. This supports our findings that intrapatient immunologic parameters do not vary significantly over time independently of disease status (8), but we have seen a change in immune parameter when a therapeutic intervention changed the course of disease (43).

**Change in Disease Severity Modulates Systemic Th2-like Chemokine Expression**

We used plasma samples from the three patients who had been enrolled in a pilot clinical trial of celecoxib as a novel medical treatment for RRP (15) to ask if treatment with the selective COX-2 inhibitor celecoxib would modulate CCL18 levels and whether changes would correlate with
TH2-like chemokines, CCL18 and also changed. We measured levels of three time their disease changed. However, we patients were receiving celecoxib at the inception of therapy or even know if the chemokine reduction reflected, but did not drive, clinical response.

To further investigate the correlation between change in disease severity and change in chemokine levels, we analyzed the plasma from the seven patients enrolled our current Phase IIb celecoxib trial who were in complete remission (absence of disease) for at least two consecutive 3-month time intervals. Since the ongoing clinical trial remains blinded, we could not correlate chemokine levels with inception of therapy or even know if the patients were receiving celecoxib at the time their disease changed. However, we did know that their disease status had changed. We measured levels of three T\textsubscript{H2}-like chemokines, CCL18 and also CCL17 and CCL22, at multiple sequential time points surrounding the time at which remission occurred. We correlated the chemokine levels with the number of months since onset of remission, using a test of repeated measures with severity group at the time of each sample as a covariant. Data were depicted graphically and linear regression lines of best fit were determined for each patient (Figure 5).

We noted that patients followed one of two patterns of chemokine change. In pattern I, patients had downward slopes for all three chemokines (patients #2, #3 and #5). In contrast, those with pattern II (patients #1, #4, #6 and #7) had no decline in CCL18 despite clinical improvement, coupled with a shallow downward or level slope for one or more of the other two chemokines. These patterns associated with sustainability of response (Table 2). All three patients with pattern I had sustained remission, while three of the four patients with pattern II had recurrence of disease by the last time point analyzed. The correlation between sustained clinical remission and CCL18 pattern was significant \((p = .05)\). Thus, analyzing the change in expression of these three T\textsubscript{H2}-like chemokines may be able to predict sustained improvement.

**DISCUSSION**

Chemokines have multiple functions. They attract leukocytes to sites of inflammation, regulate leukocyte homing, and have a role in angiogenesis and tumor growth (44). RRP is a virally induced disease characterized by growth of premalignant tumors, with an apparent failure of the host immune system to control the infection. We have found that the pattern of expression of T\textsubscript{H1}-like and T\textsubscript{H2}-like chemokines in patients with RRP paralleled the increased T\textsubscript{H2}-like cytokine milieu that we have shown previously (7,8,45). We also found that changes in chemokine expression correlated with change in disease severity, consistent with the hypothesis that chemokines play a role in the HPV-specific immune dysregulation of this disease.

Comparison of papilloma tissues to autologous clinically normal airway tissues as well as to tissues from controls with no history of RRP enabled us to ask which chemokine differences were driven by the papillomavirus infection and which were a reflection of the patient’s local or systemic immune status that might impact on susceptibility to HPV-induced disease. One caveat to this approach is the fact that latent HPV infection is widespread in the airway of patients with RRP, and we cannot exclude the possibility that the latent infection affects chemokine expression, even though viral expression is essentially undetectable in latency (42).

Keratinocytes are known to express many of the same cytokines and chemokines as immunocytes (46), although the regulation of expression has not been studied as extensively in keratinocytes. Expression of the T\textsubscript{H2}-like chemokines CCL17 and CCL22 was increased significantly in papilloma tissues compared to autologous clinically normal tissues (Figure 1). The T\textsubscript{H2}-like cytokines IL-4 and IL-13, which we reported previously are increased in papillomas (7,47), are potent stimulators of CCL22 expression by monocytes (48–50) while the T\textsubscript{H1}-like cytokine IFN-\(\gamma\) (absent in papillomas) suppresses CCL22 expression by monocytes, macrophages and dendritic cells (DCs) (49). CCL17 is expressed by alternatively activated macrophages (AAM\(\phi\))s and DCs, and the same T\textsubscript{H2}-like cytokines that induce

![Figure 4](Image 117x616 to 495x697)

**Figure 4.** Plasma CCL18 levels decrease in concert with improved clinical status in patients treated with celecoxib, a COX-2 inhibitor. Three patients (A,B,C) in the pilot clinical trial had CCL18 levels (solid line) measured by a single-plex ELISA in plasma samples collected at varying times during the study. Clinical improvement, denoted by reduction in severity score (gray bars), correlated with decreasing CCL18 concentrations.
CCL22 expression promote generation of AAMφs (51). Others have suggested that elevated COX-2 expression leads to an overexpression of CCL20 through a PLCP1/PKCα/MEK1/2/ERK1/2-dependent pathway (52) and that alterations in the COX2/PGES/PGE_2 pathway affect T_{h}2-like chemokine expression (53,54). However, PGE_2 does not appear to have the same effect on laryngeal keratinocytes, since concentrations of PGE_2 in papilloma and clinically normal airway tissues of RRP patients are quite comparable (data not shown).

CCL18, a highly abundant and constitutively expressed chemokine in normal plasma (22,55–58), is expressed by many innate immunocytes including DCs (13,59), AAMφs (13) and eosinophils (60). CCL18 is expressed prominently in asthma and other T_{h}2 disorders (54,60). Thus, it was surprising that we did not see a marked increase in CCL18 in the papilloma tissues. The vast majority of mRNA analyzed from the tissues is derived from the epithelial cells, not innate or adaptive immune system cells. Thus, even though we saw some cells in some papillomas that were strongly positive by immunohistochemistry (Figure 2A), the CCL18 mRNA would be diluted by the large amount of mRNA extracted from negative papilloma cells. It appears that the virus, the microenvironment in the tissues, or both acting together, stimulate papilloma cells to upregulate expression of CCL17 and CCL22, but not CCL18.

TH2-like chemokines are important chemoattractants that guide T_{h}2-like cells and regulatory T cells (Tregs) to sites of inflammation (61) through their interaction with CCR4 (48,62). Increased expression of CCL17 and CCL22 could be the mechanism for our previous finding that Tregs are enriched in papillomas (61). CCL22 is also a potent chemoattractant for DCs and natural killer (NK) cells (63–65), which are more abundant in papillomas (47,66). CCL20 mRNA levels did not appear to be upregulated in papillomas when analyzing total biopsy extracts, but the protein was clearly upregulated differentially in the basal layer of this stratified squamous epithelium (Figure 2C). CCL20 is an inducible chemokine that acts as a chemoattractant for NK cells (67) and immature DCs (24), and plays an important role in recruitment and activation of T_{h}2-like T cells. Basal cells are immediately adjacent to the basement membrane, thus CCL20 expression in these cells would form a strategic barrier to selectively admit TH2-like, but not TH1-like, T cells into the tissue. This would restrict access to the more suprabasal and spinous layer of keratinocytes where high-level HPV expression occurs in papillomas (Figures 2E,F) (1).

CCL22 expression promote generation of AAMφs (51). Others have suggested that elevated COX-2 expression leads to an overexpression of CCL20 through a PLCP1/PKCα/MEK1/2/ERK1/2-dependent pathway (52) and that alterations in the COX2/PGES/PGE_2 pathway affect T_{h}2-like chemokine ex-expression (53,54). However, PGE_2 does not appear to have the same effect on laryngeal keratinocytes, since concentrations of PGE_2 in papilloma and clinically normal airway tissues of RRP patients are quite comparable (data not shown).

CCL18, a highly abundant and constitutively expressed chemokine in normal plasma (22,55–58), is expressed by many innate immunocytes including DCs (13,59), AAMφs (13) and eosinophils (60). CCL18 is expressed prominently in asthma and other T_{h}2 disorders (54,60). Thus, it was surprising that we did not see a marked increase in CCL18 in the papilloma tissues. The vast majority of mRNA analyzed from the tissues is derived from the epithelial cells, not innate or adaptive immune system cells. Thus, even though we saw some cells in some papillomas that were strongly positive by immunohistochemistry (Figure 2A), the CCL18 mRNA would be diluted by the large amount of mRNA extracted from negative papilloma cells. It appears that the virus, the microenvironment in the tissues, or both acting together, stimulate papilloma cells to upregulate expression of CCL17 and CCL22, but not CCL18.

TH2-like chemokines are important chemoattractants that guide T_{h}2-like cells and regulatory T cells (Tregs) to sites of inflammation (61) through their interaction with CCR4 (48,62). Increased expression of CCL17 and CCL22 could be the mechanism for our previous finding that Tregs are enriched in papillomas (61). CCL22 is also a potent chemoattractant for DCs and natural killer (NK) cells (63–65), which are more abundant in papillomas (47,66). CCL20 mRNA levels did not appear to be upregulated in papillomas when analyzing total biopsy extracts, but the protein was clearly upregulated differentially in the basal layer of this stratified squamous epithelium (Figure 2C). CCL20 is an inducible chemokine that acts as a chemoattractant for NK cells (67) and immature DCs (24), and plays an important role in recruitment and activation of T_{h}2-like T cells. Basal cells are immediately adjacent to the basement membrane, thus CCL20 expression in these cells would form a strategic barrier to selectively admit T_{h}2-like, but not T_{h}1-like, T cells into the tissue. This would restrict access to the more suprabasal and spinous layer of keratinocytes where high-level HPV expression occurs in papillomas (Figures 2E,F) (1).

Plasma chemokine levels reflect a more systemic immune state of the RRP patients than analysis of laryngeal biopsy tissues. Plasma levels of CCL17 and CCL22 were clearly elevated in the RRP patients, in keeping with the papilloma tissue levels, but so was CCL18 (Figure 3). This disconnect suggests that the highly elevated plasma CCL18 levels in these patients are derived from another site, possibly lymphoid tissues. While the role of CCL18 has been de-
bated, strong evidence supports CCL18 as an anti-inflammatory chemokine (22,54,60,68). Thus, we conclude that RRP is indeed a Th2-like chemokine disease with a strong bias away from effective control of HPV infection, and that the bias correlates with disease severity. The systemic Th2-like chemokine bias in these patients is not “hard wired,” since plasma levels changed when disease severity improved.

CONCLUSION
On the basis of the first clinical study (15), we would conclude that celecoxib therapy induces both clinical response and reduction in CCL18 plasma levels and that CCL18 levels reflect clinical state. A recent study (15) showed that celecoxib interferon therapy induces both clinical response and reduction in CCL18 plasma levels, and that bias correlates with disease severity. The improvement in these patients is not “hard wired,” since plasma levels changed when disease severity improved.


DISCLOSURE
BM Steinberg has a research grant of celecoxib and matching placebo from Pfizer for the clinical studies.

ACKNOWLEDGMENTS
This work was supported by grants R01DE017227 from the National Institute of Dental and Craniofacial Research (VRB) and U01DC007946 from the National Institute on Deafness and Other Communication Disorders (BMS and ALA), National Institutes of Health. The authors thank Nick Agostino for assistance with some of the ELISA assays, and Helena Schmidt-mayerova for her assistance in the initial CCL18 ELISAs. We thank Virginia Mullooy for coordinating the collection of patient samples, the residents from the Department of Otolaryngology and the attendings and residents from the Department of Anesthesiology for their assistance. Assistance with statistical analysis was provided by Martin Lesser and Jonathan Levine.

REFERENCES


