TH2-like Chemokine Patterns Correlate with Disease Severity in Patients with Recurrent Respiratory Papillomatosis

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Recurrent respiratory papillomatosis (RRP), characterized by the recurrent growth of benign tumors of the 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.

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INTRODUCTION

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. Extension into the lower airway occurs in approximately 17% of patients (1,2). Malignant conversion occurs in approximately 3% of patients with RRP (3,4), and is much more likely in those with pulmonary involvement. Recurrence frequency is variable between patients, but relatively constant within most patients (5). In severe disease, surgical removal of the lesions can be required as frequently as every 3 to 4 wks, leading to a lifetime requirement of greater than 150 surgical procedures to maintain a patent airway. There is no approved medical treatment for this disease.

We reported previously that RRP is characterized by a biased adaptive immune response, with a TH2-like predominance (6–8). Both IL-10 and IL-4 are upregulated in papillomas and in peripheral blood mononuclear cells exposed to HPV-11 E6 protein, and the tissues and cells express a concomitant decrease in IFN-γ, IL-12 and IL-18 (7). However, the immunologic mechanism(s) that governs the variation in disease severity and the TH2-like bias to HPVs remains unresolved. Of note, 6.9% of men and women aged 14 to 69 have oral or airway HPV infection (9) yet the vast majority of these individuals never develop RRP. The incidence in the United States among children (under age 14) is estimated to be 4.3/100,000 (10) and among adults, 1.8/100,000 (11). This suggests that the HPV-specific, TH2-like bias may be unique to these patients.

Toward understanding the immune mechanism(s) that prevents patients with RRP from clearing or controlling their HPV infection, we previously performed a paired messenger RNA (mRNA) microarray study that characterized the repertoires of genes expressed in papillomas versus those expressed by autologous clinically normal laryngeal tissues (6). Among the results was evidence that there was differential chemokine mRNA expression by the papilloma tissues, which suggested that the virus might be able to polarize the patients’ innate immune responses. Chemokines elicit and
guide leukocyte movement, support angiogenesis (12) and participate in the balance of Th1-like versus Th2-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 Th1-like pattern (16), and can enhance expression of Th2-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 Th2-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 Th2-like chemokine balance in patients with RRP that correlated with disease severity. The inducible Th2-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 Th2-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 of 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 Ib 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 Ib study. All samples were stored at –80°C. Th2-like chemokine levels in plasma samples were measured as described below.

Quantitative PCR

Expression of the Th2-like chemokines CCL17 (TARC) (20), CCL18 (DC-CK-1, PARC, AMAC-1, MIP-4) (21,22), CCL20 (LARC, MIP-3a) (23–27), and CCL22 (MDC, STCPI, ABCD-1) (17,28–32) and the Th1-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 Axiosvert 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 Ib 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.
Statistical Methods

Descriptive statistics and analysis of variance (ANOVA) were performed with InStat 3.01 (GraphPad Software, San Diego, CA, USA). Plasma chemokine levels were compared by a nonparametric ANOVA, Kruskal-Wallis test. Chemokine multiplex ELISA data from those patients in the Phase IIb clinical trial who achieved remission for at least two successive 3-month intervals (n = 7) were analyzed via mixed model repeated measures (MMRM) analyses, using SAS 9.1 (SAS Institute, Cary, NC, USA), where each of the chemokines (CCL17, CCL18 or CCL22) was modeled as a function of time, with the severity score group (mild/moderate versus severe) serving as a time-dependent covariate. Data was assumed to have compound symmetry covariance structure, and was validated with several other covariance structures. Model estimation was performed using restricted maximum likelihood (REML), and balanced design was determined using the Kenward-Roger method (40) to calculate fixed effects and degrees of freedom. The variance–covariance matrix was used to determine the correlation between the relative number of days since clinical remission, the disease score and a given chemokine. To correlate CCL18 plasma levels to sustainability of remission, a 2 × 2 contingency table was constructed containing: a) the number of patients from the two clinical trials that did/did not maintain a downward slope in CCL18 expression after achieving clinical remission, as defined above; and b) the number of 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_{1,2}/T_{1,1}-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 PGE2 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
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 T\textsubscript{h}2-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.** T\textsubscript{h}2-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, \(p = 0.007\)). 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.

**Change in Disease Severity Modulates Systemic T\textsubscript{h}2-like Chemokine Expression**

We then asked whether the T\textsubscript{h}2-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/m 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 (\(p = 0.007\)) than patients without RRP (Figure 3). Neither CCL20, which is not constitutively expressed, nor the T\textsubscript{h}1-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).
clinical improvement. A marked decrease in plasma CCL18 was noted in all three patients (Figure 4). The decrease occurred in conjunction with clinical improvement, but patients B and C were both disease free well before the CCL18 levels reached their lowest levels, suggesting that 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 TH2-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 score (gray bars), correlated with decreasing CCL18 concentrations.

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 TH2-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_{H}1-like and T_{H}2-like chemokines in patients with RRP paralleled the increased T_{H}2-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_{H}2-like chemokines CCL17 and CCL22 was increased significantly in papilloma tissues compared to autologous clinically normal tissues (Figure 1). The T_{H}2-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_{H}1-like cytokine IFN-γ (absent in papillomas) suppresses CCL22 expression by monocytes, macrophages, and dendritic cells (DCs) (49). CCL17 is expressed by alternatively activated macrophages (AAMΦs) and DCs, and the same T_{H}2-like cytokines that induce
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/PGE2 pathway affect T_{h2}-like chemokine expression (53,54). However, PGE2 does not appear to have the same effect on laryngeal keratinocytes, since concentrations of PGE2 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_{h2} 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_{h2}-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_{h2}-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).

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-

Table 2. Relationship between chemokine pattern and sustained clinical response.

| Patient number | Slope of CCL17 | Slope of CCL22 | Slope of CCL18 | Sustained clinical response |
|---------------|---------------|---------------|---------------|-----------------------------|
| Pt # 3        | +++           | +++           | +++           | Yes                         |
| Pt # 5        | +++           | +++           | +++           | Yes                         |
| Pt # 2        | +            | +            | +            | Yes                         |
| Pt # 6        | +            | +            | –            | Yes                         |
| Pt # 1        | +            | –            | –            | No                          |
| Pt # 7        | +            | +            | +/−           | No                          |
| Pt # 4        | +/−           | –            | −            | No                          |

*aPatient number refers to the patient identifier numbers in Figure 5.
*b+++ Steep downward slope; +, moderate downward slope; +/−, flat slope; −, inverse slope upward.*
bated, strong evidence supports CCL18 as an antiinflammatory chemokine \(22,54,60,68\). Thus, we conclude that RRP is indeed a \(T_h2\)-like chemokine disease with a strong bias away from effective control of HPV infection, and that the bias correlates with disease severity.

The systemic \(T_h2\)-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 rather than regulation by PGE2 since initiation of treatment and clinical improvement clearly preceded the decline in chemokine expression to baseline for two of the three patients (Figure 4). Expansion of this research using seven patients from our current celecoxib study provided additional insights (Figure 5). There was a highly significant correlation between achievement of clinical remission and decline in plasma CCL17 and CCL22 levels, but not CCL18 levels. Rather, a sustained decline in CCL18 levels appeared to predict continued remission (Table 2). We postulate that immunologic events that contribute to clinical improvement involve modulation of the expression of CCL17 and CCL22, and that changes in CCL18 reflect the likelihood of sustaining the improvement. Monitoring plasma levels of the three \(T_h2\)-like cytokines (CCL17, CCL22 and CCL18) may, with further study, prove to be a useful tool for predicting disease prognosis in RRP.

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DISCLOSURE

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

REFERENCES

1. Steinberg BM, Gallager T, Stoler M, Abramson AL. (1988) Persistence and expression of human papillomavirus during interferon therapy. Arch Otolaryngol. Head Neck Surg. 114:27–32.
2. Weiss MD, Kashima HK. (1983) Tracheal involvement in laryngeal papillomatosis. Laryngoscope. 93:45–8.
3. Lin HW, et al. (2010) Malignant transformation of a highly aggressive human papillomavirus type 11-associated recurrent respiratory papillomatosis. Am J Otolaryngol. 31:291–6.
4. Blumin JH, Handler EB, Simpson CB, Osipov V, Merati AL. (2009) Dysplasia in adults with recurrent respiratory papillomatosis: incidence and risk factors. Ann Otol Rhinol Laryngol. 118:481–5.
5. Abramson AL, Steinberg BM. (1987) Laryngeal papillomatosis: clinical, histopathologic and molecular studies. Laryngoscope. 97:678–85.
6. DeVoti JA, et al. (2008) Immune dysregulation and tumor-associated gene changes in recurrent respiratory papillomatosis: a paired microarray analysis. Mol Med. 14:608–17.
7. DeVoti JA, et al. (2004) Failure of gamma interferon but not interleukin-10 expression in response to human papillomavirus type 11 E6 protein in respiratory papillomatosis. Clin Diagn Lab Immunol. 11:538–47.
8. Bonagura VR, Hatam L, DeVoti J, Zeng FF, Steinberg BM. (1999) Recurrent respiratory papillomatosis: Altered CD8(+) T-cell subsets and TH1/ TH2 cytokine imbalance. Clin Immunol. 93:302–11.
9. Gillison ML, et al. (2012) Prevalence of oral HPV infection in the United States, 2009–2010. JAMA. 307:693–703.
10. Derkay CS. (1995) Task force on recurrent respiratory papillomatosis. A preliminary report. Arch Otolaryngol. Head Neck Surg. 121:896–911.
11. Derkay CS, Wiatrak B. (2008) Recurrent respiratory papillomatosis: a review. Laryngoscope. 118:1236–49.
12. Tamh DE, Longo DL, Murphy WJ. (1996) Human interferon-inducible protein-10 induces monocellular cell infiltration in mice and promotes the migration of human T lymphocytes into the peripheral tissues and human peripheral blood lymphocytes-SCID mice. Blood. 87:1423–31.
13. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. (2002) Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 23:549–55.
14. Mantovani A. (1999) Chemokines. Introduction and overview. Chem Immunol. 72:1–6.
15. Lucas AJ, Wu R, Mullooly V, Abramson AL, Steinberg BM. (2012) Constitutive overexpression of the oncongene Ral1 in the airway of recurrent respiratory papillomatosis patients is a targetable host-susceptibility factor. Mol Med. 18:244–9.
16. Kalinski P. (2012) Regulation of immune responses by prostaglandin E2. J Immunol. 188:21–8.
17. Mclorey A, et al. (2006) Histamine and prostaglandin E regulate the production of Th2-attracting chemokines (CCL17 and CCL22) and down-regulate IFN-gamma-induced CXCL10 production by immature human dendritic cells. Immunology. 117:507–16.
18. Abramson AL, et al. (1992) Clinical effects of photodynamic therapy on recurrent laryngeal papillomas. Arch Otolaryngol Head Neck Surg. 118:25–9.
19. Study of Celebrex (Celecoxib) in Patients With Recurrent Respiratory Papillomatosis [Internet]. 2007 Dec 10 – [study dates]. [Bethesda (MD)]: National Library of Medicine, ClinicalTrials.gov; [cited 2012 Nov 16]. Available from: http://clinicaltrials.gov/ct2/show/record/NCT00571701.
20. Pittsburgh, PA: North Shore Long Island Jewish Health System (sponsor). Study last updated Jan 2012.
21. Sakata T, Miyazaki M, Kobayashi M, Herndon DN, Suzuki F. (2004) CCL17 and IL-10 as effectors that enable alternatively activated macrophages to inhibit the generation of classically activated macrophages. J Immunol. 172:1407–13.
22. Nibbs RJ, et al. (2000) C-C chemokine receptor 3 antagonism by the beta-chemokine macrophage inflammatory protein 4, a property strongly enhanced by an amino-terminal alanine-methionine swap. J Immunol. 164:1488–97.
23. Vaccaro M, et al. (2003) Unique regulation of CCL18 production by maturing dendritic cells. J Immunol. 170:3843–9.
24. He S, Wang L, Wu Y, Li D, Zhang Y. (2010) CCL3 and CCL20-recruited dendritic cells modified by melanoma antigen gene-1 induce anti-tumor immunity against gastric cancer ex vivo and in vivo. J Exp Clin Cancer Res. 29:37.
25. He C, Zhang SL, Hu CJ, Tong DW, Li YZ. (2010) Higher levels of CCL20 expression on peripheral blood mononuclear cells of Chinese patients with inflammatory bowel disease. Immunol Invest. 39:16–26.
25. Kao CY, et al. (2005) Up-regulation of CC chemokine ligand 20 expression in human airway epithelium by IL-17 through a JAK-independent but MEK/NF-kappaB-dependent signaling pathway. J. Immunol. 175:6676–83.

26. Reihman J, Hsu Y, Chen LC, Bleck B, Gordon T. (2005) Airway epithelial cells release MIP-3alpha/CCL20 in response to cytokines and ambient particulate matter. Am. J. Respir. Cell Mol. Biol. 28:648–54.

27. Homey B, et al. (2000) Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. J. Immunol. 164:6621–32.

28. Gobert M, et al. (2009) Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. Cancer Res. 69:2000–9.

29. Manari M, Lang R, Binda E, Panina-Bordignon P, D’Ambrosio D. (2004) Dominance of CCL22 over CCL17 in induction of chemokine receptor CCR4 desensitization and internalization on human Th2 cells. Eur. J. Immunol. 34:231–40.

30. Ritter M, et al. (2005) Elevated expression of TARC (CCL17) and MDC (CCL22) in models of cigarette smoke-induced pulmonary inflammation. Biochem. Biophys. Res. Commun. 334:254–62.

31. Shimada Y, Takehara K, Sato S. (2004) Both Th2 and Th1 chemokines (TARC/CCL17, MDC/CCL22, and Mig/CXCL9) are elevated in sera from patients with atopic dermatitis. J. Immunol. 166:6633–40.

32. Yanai M, et al. (2007) The role of CCL22/macrophage-derived chemokine in allergic rhinitis. Clin. Immunol. 125:291–8.

33. Debes GF, Hopken UE, Hamann A. (2002) In vivo differentiated cytokine-producing CD4(+) T cells express functional CCR7. J. Immunol. 168:5441–7.

34. Flanagan K, Moroziewicz D, Kwak H, Horig H, Bonagura VR. (2004) Papillomavirus-specific CD4(+) T cells exhibit reduced STAT-5 signaling and altered cytokine profiles in patients with recurrent respiratory papillomatosis. J. Immunol. 168:9705–11.

35. Zhang Q, et al. (2003) The role of CCL22/macrophage-derived chemokine in immune responses to human papillomavirus-6 and -11. APMIS 111:145–50.

36. Bonechi R, et al. (1998) Differentiation of chemokine receptors and chemotactic responsiveness of type 1 helper cells (Th1s) and Th2s. J. Exp. Med. 187:129–34.

37. Bonechi R, et al. (1998) Divergent effects of interleukin-4 and interferon-gamma on macrophage-derived chemokine production: an amplification circuit of polarized Th2 helper 2 responses. Blood. 92:2668–71.

38. Andrew DP, et al. (1998) STCP-1 (MDC) CC chemokine acts specifically on chronically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13. J. Immunol. 161:5027–38.

39. Semnani RT, Mahapatra L, Moore V, Sanprasert V, Alaaeddine N, Hilal G, Baddoura R, Antoniou J, DiScipio RG. (2004) Eosinophils and monocytes produce pulmonary and activation-regulated chemokine, which activates cultured monocytes/macrophages. Am. J. Physiol. Lung Cell. Mol. Physiol. 286:L494–501.

40. Hatam LJ, et al. (2012) Immune suppression in premalignant respiratory papillomas: enriched functional CD4(+)/Foxp3(+) regulatory T-cells and PD-1/PD-L1/L2 expression. Clin. Cancer Res. 18:1925–35.

41. Rivino L, et al. (2004) Chemokine receptor expression identifies Pre-T helper (Th1), Pre-Th2, and nonpolarized cells among human CD4(+) central memory T cells. J. Exp. Med. 200:725–35.

42. Chantry D, et al. (1998) Profile of human macrophage transcripts: insights into macrophage biology and identification of novel chemokines. J. Leukoc. Biol. 64:49–54.

43. Godiska R, et al. (1997) Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. J. Exp. Med. 185:1959–604.

44. Chang M, et al. (1997) Molecular cloning and functional characterization of a novel CC chemokine, stimulated T cell chemotactic protein (STCP-1) that specifically acts on activated T lymphocytes. J. Biol. Chem. 272:5229–37.

45. Bonagura VR, et al. (2010) Activating killer cell immunoglobulin-like receptors 3DS1 and 2DS1 protect against developing the severe form of recurrent respiratory papillomatosis. Hum. Immunol. 71:212–9.

46. Stolberg VR, et al. (2011) Cysteine-cysteinyll chemokine receptor 6 mediates invariant natural killer T cell airway recruitment and innate stage resistance during mycobacterial infection. J. Infect. Immun. 79:3858–65.

47. Newton R, et al. (1997) Superinduction of COX-2 mRNA by cycloheximide and interleukin-1beta involves increased transcription and correlates with increased NF-kappaB and JNK activation. FEBS Lett. 418:135–8.

48. Schuetyser E, et al. (2004) Identification of biologically active chemokine isoforms from ascitic fluid and elevated levels of CCL18/pulmonary and activation-regulated chemokine in ovarian carcinoma. J. Biol. Chem. 279:24854–93.

49. de Nadal P, et al. (2006) Involvement of CCL18 in allergic asthma. J. Immunol. 176:2826–93.

50. Lindhout E, et al. (2001) The dendritic cell-specific CC-chemokine DC-CCK1 is expressed by germinal center dendritic cells and attracts CD8-negative mantle zone B lymphocytes. J. Immunol. 166:5284–9.

51. Prasse A, et al. (2006) A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. Am. J. Respir. Crit. Care Med. 173:781–92.

52. Radstake TR, et al. (2004) Increased expression of CCL18, CCL19, and CCL21 by dendritic cells from patients with rheumatoid arthritis and regulation by Fc gamma receptors. Ann. Rheum. Dis. 64:359–67.

53. Gunther C, Carballido-Perrig N, Kopp T, Carballido JM, Pfeifer C. (2009) CCL18 is expressed in patients with bullous pemphigoid and paraprosessus disease. Br. J. Dermatol. 160:747–55.

54. Schrautstatter I, Takamori H, Sikoza L, Srimaroa P, DiSeipio RG. (2004) Eosinophils and monocytes produce pulmonary and activation-regulated chemokine, which activates cultured monocytes/macrophages. Am. J. Physiol. Lung Cell. Mol. Physiol. 286:L494–501.