Celecoxib Up-Regulates the Expression of the ζ Chain of T Cell Receptor Complex in Tumor-Infiltrating Lymphocytes in Human Cervical Cancer

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Celecoxib Up-Regulates the Expression of the \( \zeta \) Chain of T Cell Receptor Complex in Tumor-Infiltrating Lymphocytes in Human Cervical Cancer

Gabriella Ferrandina,1 Franco Oreste Ranelletti,2 Francesco Legge,1 Vanda Salutari,1 Enrica Martinelli,1 Andrea Fattorossi,1 Domenica Lorusso,4 Gianfranco Zannoni,3 Valerio Vellone,3 Amelia Paglia,1 and Giovanni Scambia4

Abstract

**Purpose:** We evaluated the effects of celecoxib treatment on tumor-infiltrating lymphocyte (TIL) subsets [CD3\(^+\), CD4\(^+\), CD8\(^+\), and CD25\(^+\)] and tryptase-positive mast cells in cervical tumors. Circulating levels of cytokines [interleukin (IL)-1\( \beta \), IL-10, tumor necrosis factor-\( \alpha \), IL-6, and IL-12] and angiogenesis-modulating factors (vascular endothelial growth factor and endostatin) have also been analyzed.

**Experimental Design:** Cervical tumor biopsies and blood samples were obtained at the time of diagnosis and after 10 days of celecoxib treatment (400 mg b.i.d., at 8:00 a.m. and 8:00 p.m.) in 27 cases. Immunohistochemistry and ELISA assays were used to assess the expression of biological factors in tumor tissue and circulating levels of cytokines and angiogenic molecules.

**Results:** We showed a statistically significant increase in the percentage of TIL expressing the TCR-\( \zeta \) chain after celecoxib treatment: indeed, in cases exposed to celecoxib, the percentage of TCR-\( \zeta \)-expressing cells ranged from 5.0 to 50.0 (median, 22.5) with respect to baseline expression (range, 3.0–50.0; median, 10.0; \( P = 0.0016 \)). There was no significant treatment-related difference in the percentage of CD3\(^+\), CD4\(^+\), CD8\(^+\), and CD25\(^+\) TIL as well as in tryptase-positive cells. IL-12 levels were significantly reduced in posttreatment samples with respect to baseline levels (\( P = 0.002 \)). We also found a reduction in the circulating levels of vascular endothelial growth factor, and a statistically significant increase of serum endostatin levels (\( P = 0.035 \)).

**Conclusions:** We reported the first evidence in humans that celecoxib restores \( \zeta \) expression by TIL in primary cervical tumors, suggesting that a positive modulation of immune function may serve as an additional mechanism supporting the antitumor effect of this class of drugs.

Much attention has been recently focused on the role of cyclooxygenase-2 (COX-2), one of the three isozymes catalyzing the rate-limiting step in the conversion of arachidonic acid to prostaglandins, in several aspects of tumor biology (1, 2). In particular, COX-2 overexpression has been involved in the inhibition of apoptosis and increased metastatic potential and neoangiogenesis, as well as impairment of host immune responses (2–5).

In humans, the vast majority of the data consistently showed an unfavorable prognostic role of high COX-2 levels in several tumors (6–9).

We also recently showed that the expression of COX-2 in cervical cancer cells is inversely related to the expression of COX-2 in the stroma compartment and that both tumor and stroma COX-2 levels can effectively predict chemotherapy and radiotherapy responsiveness and clinical outcome in this neoplasia (10, 11).

Interestingly enough, high COX-2 content in cervical cancer cells was also shown to be associated with a scarce lymphocytic infiltrate, a lower proportion of CD3\(^+\), CD4\(^+\), and CD25\(^+\) T cells, and a high proportion of tryptase-positive mast cells (10), the latter being involved in the promotion of tumor angiogenesis (12); thus, suggesting that high COX-2 levels can play a major role in inhibiting host immune functions and sustaining tumor neoangiogenesis.

In preclinical models, COX-2 inhibition leads to the inhibition of tumor growth and to tumor chemoprevention (13, 14). In humans, the use of the selective COX-2 inhibitor (coxib) rofecoxib in precancerous esophageal lesions has been suggested to decrease tumor-proliferative potential as evaluated by the reduction of intermediate markers (15). We also recently showed that a short-term treatment with celecoxib is able to reduce the expression of COX-2, Ki67, and microvesSEL density (MVD) in patients with primary cervical cancer (16).

Although several in vitro and preclinical evidences have reported that COX-2 inhibition could overcome tumor-induced...
immunosuppression by altering the balance of immunoregulating cytokines (5), restoring monocyte and dendritic cell functions (17, 18), and reducing the number and activity of suppressor CD4+ CD25+ T cells (19), very few data are available on the effects of coxibs on the variables of immunomodulation in humans (20).

The present study was aimed at evaluating the effects of a short-term celecoxib treatment on tumor-infiltrating lymphocyte (TIL) subsets (CD3+, CD4+, CD8+, and CD25+ cells) and tryptase-positive mast cells in cervical tumors. We also focused on the proportion of T cell receptor (TCR)-γ-ζ-expressing cells in TILs before and after celecoxib treatment, given the key role of the γ-ζ chain of TCR complex in the intracellular signaling pathway of T cell activation (21), and the notion that this molecule is frequently down-regulated in human cancer (22–24), implying a reduced T cell activity. Moreover, the activity of celecoxib on circulating levels of immunoregulatory cytokines [interleukin (IL)-1β, IL-10, tumor necrosis factor-α, IL-6, and IL-12] and two key angiogenesis-modulating factors [vascular endothelial growth factor (VEGF) and endostatin] have been analyzed. Finally, we extended and confirmed our previous observations in the present, larger series (16), on celecoxib-induced down-regulation of tumor COX-2, Ki67, and MVD.

**Patients and Methods**

**Patients and study design.** The study was aimed at evaluating the effects of a short-term celecoxib (200 mg tablets; Celebrex, Pfizer, Rome, Italy) treatment on TIL subsets and expression of surrogate markers of proliferation and neangiogenesis in cervical tumor biopsies. The circulating levels of cytokines and angiogenesis-regulatory molecules before and after celecoxib treatment of the same patients were also investigated (16).

The protocol was approved by the Institutional Ethics Committee. The inclusion criteria were: women (ages 18-65 years) with histologic diagnosis of cervical cancer, required to have normal physical examination and normal laboratory values for hepatic and renal functions, and Karnofsky score ≥80. Subjects were excluded in case of pregnancy/breast-feeding, history of gastric/duodenal ulcer or inflammatory intestinal disease, use of aspirin or other nonsteroidal anti-inflammatory drug and/or sulfamidics, known personal/familial hypersensitivity to these drugs, history of heart failure, hypertension, cardiac dysfunction, venous thromboembolism.

During the screening period, all subjects signed a written informed consent and provided complete medical history. Subjects underwent physical examination, electrocardiographic evaluation, and laboratory tests including hematologic and chemical measurements, urinalysis, and serum pregnancy test.

Subjects that were admitted into the study underwent blood sample collection for baseline hematologic/chemical evaluation and analysis of circulating levels of cytokines, VEGF, and endostatin. Moreover, cervical tumor biopsy under colposcopic examination was done to confirm the diagnosis and provide pretreatment tissue samples for immunohistochemistry.

The duration and dosage of treatment were chosen on the basis of pharmacokinetic data (25). Treatment subjects received celecoxib from days 1 to 10 (400 mg b.i.d., at 8:00 a.m. and 8:00 p.m.). Completion of staging procedures (gynecologic exam, imaging, chest X-ray, and additional exams) were completed during the treatment period. Samples for the assessment of hematology, biochemistry, and cytokine assessments were collected at the end of treatment (day 11). On the same day, tumor biopsy was done in the context of gynecologic exam under anesthesia. Cervical tumor biopsies as well as blood samples were also obtained in patients not receiving celecoxib, and were used as control in order to investigate the possibility that phlogistic and reparative processes could affect the expression of the factors analyzed.

**Immunohistochemical analysis.** Tissue specimens were fixed in 10% neutral buffered formalin and paraffin-embedded according to standard procedures (7, 10). Four-micrometer-thick sections of representative blocks from each case were deparaffinized in xylene, rehydrated, and treated with 0.3% H2O2 in methanol for 10 minutes to block endogenous peroxidase activity. All sections were subjected to heat-induced epitope retrieval in a microwave oven.

The following antibodies were used: anti-COX-2 rabbit polyclonal antibody (1:300; Cayman, Ann Arbor, MI), anti-Ki67 monoclonal antibodies (1:50; clone MIB1; Ylem, Rome, Italy), and anti-COX-3 monoclonal antibodies (1:50; clone J/70 A; DAKO, Glostrup, Denmark). In the studies of TIL characterization, the following antibodies were used: anti-CD3 (1:100; clone PS1), anti-CD4 (1:50; clone 1F6), anti-CD8 (1:50; clone 4B11), anti-CD25 (1:100; clone 4C9), all from Ylem (Avezzano, Italy), and anti-TCR-γ-ζ (1:100; clone 2H2D9; TIA2); Beckman Coulter, Miami, FL] which recognizes an intracytoplasmatic epitope of the γ-ζ chain of the TCR complex (26). Tryptase-positive mast cells were identified by using the monoclonal antibody anti-mast cell tryptase (1:100; clone AA1; DAKO). Controls were done using nonimmunized rabbit or mouse serum instead of primary antibodies. Positive controls for each variable were always run in the assay.

For COX-2, the intensity of immunohistochemical staining was evaluated as previously reported (7, 10) and expressed as integrated density values (IDV), i.e., the product of the mean density value of the immunoreactive regions by the percentage of the immunostained tumor or stroma components. The evaluation of MVD was done as reported by West et al. (27) with minor modifications. Ki67 positivity was expressed as the percentage of tumor cells with nuclear staining (16).

Quantification of CD3+, CD4+, CD8+, CD25+, and TCR-γ-ζ as well as tryptase-positive mast cells was done by choosing five corresponding ×20 fields, from each of six serial tissue sections (one for each antibody, i.e., anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-TCR-γ-ζ, and anti-mast cell tryptase). Both immunostained and negative cells within a superimposed grid of 0.0225 mm2 were counted. Two cell counts for each digital image were done by moving the grid over representative stromal areas. The total cell number was calculated by averaging cell counts from each section and from the six consecutive sections (n = 60). The number of immunostained cells, relative to each phenotype, was calculated by averaging cell counts from two grid areas from five ×20 fields (n = 10). The results are reported as the percentage of immunostained cells per total number of stromal cells.

The computerized image analysis of all tissue sections were done by three different pathologists (F.O. Ranelletti, V. Vellone, and G.F. Zannoni) blinded as to whether the sections were from pretreated or posttreated samples and without any prior knowledge of the clinical and biological variables.

**Assessment of circulating levels of cytokines and angiogenesis regulatory molecules.** Plasma samples were collected in vacutainer tubes containing heparin and serum was obtained using a serum separator tube. All samples were aliquoted and stored at −80°C until assay.

Plasma levels of IL-1β, IL-10, and IL-12 and serum levels of tumor necrosis factor-α, IL-6, endostatin, and VEGF were measured by a solid phase chemiluminescent ELISA assay (R&D Systems, Abingdon, United Kingdom), according to the manufacturer’s protocol.

**Statistical analysis.** Wilcoxon’s signed rank sum test for paired samples was used to analyze the results of immunohistochemistry and cytokine levels in pretreatment versus posttreatment samples. Statistical analysis was carried out by using SOLO (BMDF Statistical Software, Los Angeles, CA).

**Results**

**Patient enrollment, compliance, and toxicity.** Since January 2002, 35 subjects entered the study at the Gynecologic Oncology Unit, at the Catholic Universities of Rome and Campobasso.

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Nineteen patients, which have previously been reported (16), are included in this study. The clinicopathologic characteristics of the enrolled patients are summarized in Table 1. There was no difference in the clinicopathologic features of patients receiving (n = 27) or not receiving (n = 8) celecoxib treatment. The compliance rate to celecoxib was 100% because all patients completed the planned celecoxib assumption. No adverse events were registered with the exception of three patients (11.1%) who experienced a mild cutaneous rash which spontaneously resolved. There were no treatment-related modifications of chemistry and hematology values (data not shown).

**Immunohistochemistry.** Table 2 shows the values of the percentage of TIL subsets in cervical tumor tissue biopsies before and after celecoxib treatment. There was no significant difference in the percentage of CD3+, CD4+, CD8+, and CD25+ T cells in tissue samples before and after treatment. Figure 1 shows a representative example of TCR-ζ immunostaining and the percentage values of TCR-ζ-positive cells for each patient before and after celecoxib treatment. We showed a statistically significant increase in the percentage of cells expressing the TCR-ζ chain after celecoxib treatment: indeed, in cases exposed to celecoxib, the percentage of TCR-ζ+ cells ranged from 5.0 to 50.0 (median, 22.5) with respect to baseline expression (range 3.0-50.0; median, 10.0; P = 0.0016). There was a decrease in the percentage of TCR-ζ+ cells after celecoxib treatment (Fig. 1B) in only four cases (14.8%). The difference in pretreatment versus posttreatment percentage values of TCR-ζ+ cells was not found to be related to the absolute basal levels of tumor COX-2 IDV (r = +0.1, P = 0.5). As far as the percentage of tryptase-positive mast cells is concerned, we did not find any modification in posttreatment versus pretreatment samples (Table 2).

Table 3 shows the values of tumor and stroma COX-2 IDV, as well as the percentage of Ki67 and MVD positivity in pre-celecoxib and post-celecoxib cervical tumor biopsies. At baseline, COX-2 IDV in tumor compartment ranged from 0.5 to 71.0 (median, 32.8) and were significantly higher than tumor COX-2 IDV after celecoxib treatment (range, 0.1-58.1; median, 22.9; P = 0.012). An increase of tumor COX-2 IDV levels was observed in posttreatment biopsy in only five cases (18.5%). On the other hand, there was no difference in stroma COX-2 IDV in tissue samples before and after celecoxib treatment (P = 0.37).

The percentage of Ki67-positive tumor cells in pre-celecoxib cases ranged from 15.4 to 87.4 (median, 50.3) and was higher than the percentage in the corresponding posttreatment samples (range, 15.5-83.8; median, 42.7; P = 0.032). There was an increase in the percentage of Ki67-positive cells after celecoxib treatment in only three cases (11.1%).

MVD values in pre-celecoxib biopsies ranged from 5.0 to 55.6 (median, 31.1) and were significantly higher than the corresponding values in posttreatment samples (range, 10.0-49.5; median, 23.2; P = 0.037). In five cases (18.5%), higher levels of MVD were found in posttreatment versus pretreatment tumor biopsy.

There was no significant difference in the relative proportions of TIL subsets as well as in the expression of COX-2 IDV, Ki67, and MVD expression in tumor tissue samples from control patients (data not shown). Finally, no correlation between any

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**Table 1. Clinicopathologic characteristics of cervical cancer population**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of cases (%)</th>
<th>No. of controls (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>27</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>51 (30-72)</td>
<td>58 (32-80)</td>
<td>NS</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB-II</td>
<td>19 (70.4)</td>
<td>6 (75.0)</td>
<td>NS</td>
</tr>
<tr>
<td>III-IV</td>
<td>8 (29.6)</td>
<td>2 (25.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Histotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>25 (92.6)</td>
<td>6 (75.0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (7.4)</td>
<td>2 (25.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Grades</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>6 (27.3)</td>
<td>1 (16.7)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16 (72.7)</td>
<td>5 (83.3)</td>
<td></td>
</tr>
<tr>
<td>Not available</td>
<td>3</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>7 (25.9)</td>
<td>1 (12.5)</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>20 (74.1)</td>
<td>7 (87.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: FIGO, Federation Internationale des Gynaecologistes et Obstetristes stage; NS, not significant.

*Calculated by χ² test.

†Calculated by Kruskal-Wallis nonparametric test.

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**Table 2. T cell characterization in cervical tumor tissue biopsies**

<table>
<thead>
<tr>
<th>Marker*</th>
<th>Pre-celecoxib</th>
<th>Post-celecoxib</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, median (range)</td>
<td>Mean, median (range)</td>
<td></td>
</tr>
<tr>
<td>% CD3+ cells</td>
<td>51.7, 63.1 (25.0-90.5)</td>
<td>50.0, 58.8 (8.3-82.8)</td>
<td>not significant</td>
</tr>
<tr>
<td>% CD4+ cells</td>
<td>201.245 (6.0-51.0)</td>
<td>187.230 (5.0-44.0)</td>
<td>not significant</td>
</tr>
<tr>
<td>% CD8+ cells</td>
<td>116.126 (6.0-40.3)</td>
<td>12.8, 19.7 (14.5-30.5)</td>
<td>not significant</td>
</tr>
<tr>
<td>% CD25+ cells</td>
<td>19.7, 25.3 (4.0-70.4)</td>
<td>15.7, 21.9 (10-41.6)</td>
<td>not significant</td>
</tr>
<tr>
<td>% TCR-ζ+ cells</td>
<td>14.9, 10.0 (3.0-50.0)</td>
<td>23.8, 22.5 (5.0-50.0)</td>
<td>0.0016</td>
</tr>
<tr>
<td>% Tryptase+ cells</td>
<td>10.3, 8.9 (4.2-37.0)</td>
<td>10.9, 9.5 (4.8-39.5)</td>
<td>not significant</td>
</tr>
</tbody>
</table>

*Expressed as the percentage of immunostained cells per total number of stromal cells. See also Patients and Methods.

†Calculated by Wilcoxon’s signed rank sum test for paired samples.
of the variables analyzed and in the clinicopathologic characteristics of the patients was found (data not shown).

Assessment of circulating levels of cytokines and angiogenesis regulatory molecules. Table 4 summarized the results of the analysis of circulating levels of cytokines and angiogenesis-regulating factors before and after celecoxib treatment. Among cytokines, only IL-12 was shown to be affected by celecoxib treatment: IL-12 levels were significantly reduced in posttreatment samples (median, 1.1 pg/mL; range, 0-32.6) with respect to baseline levels (median, 1.5 pg/mL; range, 0-10.9; \( P = 0.002 \)). As far as the angiogenesis-modulating factors are concerned, we found a trend to a reduction in the circulating levels of VEGF after celecoxib treatment, although the statistical significance was not reached (\( P = 0.068 \)). On the other hand, celecoxib treatment was associated with a statistically significant increase of serum endostatin levels (posttreatment levels: median, 57.0 ng/mL; range, 25.0-95.0 versus baseline levels: median, 46.5 ng/mL; range, 17.0-85.0; \( P = 0.035 \)). Indeed, a decrease of endostatin levels after celecoxib treatment was observed in only three cases (11.1%).

Discussion

We showed for the first time in human cervical cancer that short-term treatment with celecoxib at therapeutic doses is associated with the increase of \( \zeta \) chain expression in TILs, whereas no treatment-related modification of other T cell subsets was observed, as already reported for peripheral lymphocyte subpopulations (16). The different extents of \( \zeta \) chain increase across the cohort could be related to tumor biology variability.

These modifications do not seem to be related to phlogistic or repair processes due to biopsy because no change of TCR-\( \zeta \) cells were observed in patients not receiving the drug. Moreover, the observed change in \( \zeta \) chain expression is likely to be predominantly due to the its modulation on T cells rather than on natural killer cells because very few natural killer cells have been found to infiltrate cervical tumor areas (23).

It is well recognized that despite the presence of an activated phenotype, as testified by the presence of activation surface markers, TILs, and circulating T cells in cancer patients show immunologic dysfunctions, such as \( \zeta \) chain alterations, which are likely to translate into failure of antitumor activity (21).

TCR-associated \( \zeta \) chain is a transmembrane protein which participates, after being phosphorylated on tyrosine residues, with the intracellular signaling pathway of TCR-associated complex and to the correct activation of T cells (21). Low expression of \( \zeta \) chain has been consistently observed in TILs and circulating T lymphocytes in patients with solid tumors including cervical cancer (22, 23, 28–30), and associated with T cell apoptosis and functional impairment. Moreover, \( \zeta \) chain down-regulation is significantly more pronounced in cancer lesions with respect to tumor adjacent normal mucosa and peripheral T cells (31), suggesting that immunosuppression exerted by tumor-produced factors may be predominantly active \textit{in situ} and gradually extend beyond tumor microenvironment to ultimately influence circulating T cells. Finally, a low frequency of TCR-\( \zeta \) lymphocytes has been shown to be associated with more advanced stage tumors (22), and with poor clinical outcome (29, 31, 32), suggesting that TCR-\( \zeta \) might be a putative biomarker of prognosis (28–30). In this context, our data on celecoxib-induced \( \zeta \) chain up-regulation leads us to hypothesize that inhibition of tumor COX-2-dependent prostaglandins could help restore TCR function.

Indeed, evidence suggests that pharmacologic or genetic inhibition of COX-2 might play a relevant role in circumventing immune dysfunction: (a) COX-2 inhibition abrogates the capacity of lung cancer cells to induce the immunosuppressive cytokine IL-10, although reversing the tumor-derived prostaglandin E2–dependent inhibition of the immunostimulating cytokine IL-12 (5); (b) COX-2 inhibition or decreased expression of prostaglandin E2 EP2 receptor prevents tumor-induced suppression of dendritic cell activity (18, 33); (c) in preclinical models, the antitumor effects of COX-2 inhibition were associated with increased numbers of TIL in...
mesothelioma (34), and with the inhibition of CD4⁺CD25⁺ T regulatory cells in lung cancer cells (19).

Several mechanisms have been proposed to be involved in tumor-associated ζ chain down-regulation, such as tumor activation of T cell or granulocytic peptidases mediating ζ chain cleavage (35), granulocyte-derived oxygen radicals (36), or tumor-derived Fas-ligand–containing microvesicles able to activate apoptosis as well as ζ chain degradation (37). It has also been hypothesized that ζ chain down-regulation results from the chronic antigenic stimulation associated with tumor progression, the so-called “immune exhaustion” (21). Finally, compromised dendritic cell functions have been suggested to be possibly involved in loss of the correct assembly of TCR complex (21). In this context, it is conceivable that celecoxib reduction of the immunosuppressive activity of tumor COX-2-derived prostaglandins might relieve T cells of the immunosuppressive influence of the microenvironment (38), thus facilitating the restoration of the physiologic molecular pathway for response, as suggested by the reversible nature of ζ chain suppression in T cells once removed from the tumor or exposed to IL-2 (30, 39). Alternatively, it is possible that celecoxib inhibition of tumor cell growth might directly reduce tumor-derived suppressive factors such as molecules able to impair ζ chain function: for instance, a ζ chain inhibitory protein has been isolated from ascites of patients with ovarian cancer, which selectively interferes with ζ chain mRNA synthesis, thus determining the suppression of the protein, and impairment of T cell response (40). Additional studies aimed at unraveling the biochemical mechanisms involved in or responsible for the association between COX-2 inhibition and up-regulation of ζ chain in TIL of cervical cancer should be planned.

In contrast with observations reported in preclinical models (5, 19), we failed to find any treatment-related modification of circulating levels of proinflammatory as well as antiinflammatory cytokines, with the exception of IL-12, which seemed decreased in celecoxib-treated samples. It has to be taken into account that the assessment of cytokine production within the tumor could provide additional important information in this research field; although tumor tissue is also likely to represent a very heterogeneous microenvironment, which might not fit the straight biochemical relationships seen in in vitro and in vivo models.

Finally, we confirmed and extended our previous observations about the ability of celecoxib to significantly reduce tumor COX-2 expression, proliferative potential, and microvasculature as suggested by the significant reduction of Ki67 and CD31 positivity (16). Importantly, consistent with the known ability of COX-2 inhibitors to interfere with neoangiogenesis (4, 41), in our series, celecoxib treatment was associated with the shifting of the balance between circulating levels of VEGF and endostatin toward an “antiangiogenetic” state, as also reported in a preclinical model of gastric ulcer healing (42).

### Table 3. Expression of COX-2 IDV, Ki67, and MVD in cervical tumor tissue biopsies

<table>
<thead>
<tr>
<th>Marker*</th>
<th>Pre-celecoxib</th>
<th>Post-celecoxib</th>
<th>P ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, median (range)</td>
<td>Mean, median (range)</td>
<td></td>
</tr>
<tr>
<td>Tumor COX-2 IDV</td>
<td>27.0, 32.8 (0.5-71.0)</td>
<td>19.2, 22.9 (01-58.1)</td>
<td>0.012</td>
</tr>
<tr>
<td>Stroma COX-2 IDV</td>
<td>16.0, 16.5 (0.5-42.5)</td>
<td>18.4, 18.7 (15.4-46.1)</td>
<td>0.37</td>
</tr>
<tr>
<td>% Ki67⁺ cells</td>
<td>39.4, 50.3 (15.4-87.4)</td>
<td>36.5, 42.7 (15.5-83.8)</td>
<td>0.032</td>
</tr>
<tr>
<td>MVD</td>
<td>24.8, 31.1 (5.0-55.6)</td>
<td>21.0, 23.2 (10.0-49.5)</td>
<td>0.037</td>
</tr>
</tbody>
</table>

*COX-2 expressed as IDV. Ki67 expressed as the percentage of tumor cells with nuclear staining. MVD expressed as the percentage of CD31 positivity per stromal cells. See also Patients and Methods.

Calculated by Wilcoxon’s signed rank sum test for paired samples.

### Table 4. Circulating levels of cytokines and angiogenesis-regulating factors in patients with cervical cancer

<table>
<thead>
<tr>
<th>Marker*</th>
<th>Pre-celecoxib</th>
<th>Post-celecoxib</th>
<th>P ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, median (range)</td>
<td>Mean, median (range)</td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.9, 0.6 (0.5-0.9)</td>
<td>1.5, 1.0 (0.5-6.3)</td>
<td>not significant</td>
</tr>
<tr>
<td>IL-10</td>
<td>21.3, 25.0 (0.4-82.0)</td>
<td>20.9, 22.8 (0.4-78.0)</td>
<td>not significant</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>14.0, 12.6 (3.6-42.0)</td>
<td>181.141 (3.8-109.0)</td>
<td>not significant</td>
</tr>
<tr>
<td>IL-6</td>
<td>15.5, 7.5 (0.260.0)</td>
<td>19.4, 9.0 (1.5-1420)</td>
<td>not significant</td>
</tr>
<tr>
<td>IL-12</td>
<td>1.9, 1.5 (0-10.9)</td>
<td>1.5, 1.1 (0-32.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>VEGF</td>
<td>656, 622 (126-4,954)</td>
<td>526, 586 (136-1,226)</td>
<td>0.068</td>
</tr>
<tr>
<td>Endostatin</td>
<td>43.5, 46.5 (17.0-85.0)</td>
<td>48.4, 57.0 (25.0-95.0)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

*All markers expressed as pg/mL with the exception of endostatin expressed as ng/mL.

Calculated by Wilcoxon’s signed rank sum test for paired samples.
In conclusion, we reported the first evidence in humans that celexib, besides the direct effects on tumor proliferative potential and neoangiogenesis, restores $\xi$ expression by TIL in primary cervical tumors, suggesting that a positive modulation of immune function may serve as an additional mechanism supporting the antitumor effect of this class of drugs.

These data are especially relevant in the context of the efforts to correct by biological response modifiers the dysfunction of T-cell receptor–associated signaling pathway (30), and to provide the biological basis for designing specific clinical trials aimed at improving antitumor response through the restoration of TIL normal function.

References