Correlation of Tissue and Plasma RANTES Levels with Disease Course in Patients with Breast or Cervical Cancer

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INTRODUCTION

RANTES (regulated upon activation, normal T-cell-expressed and secreted), which is one of the β (C-C) chemokines (1–12) that are chemoattractant for a variety of cells, particularly mononuclear cells, basophils, and eosinophils, is thought to be released by activated T lymphocytes and monocytes/macrophages (12), epithelial cells (7), bronchial epithelium (8, 9), and dermal fibroblast (10) and renal tubular epithelium (11). RANTES is thought to play an important role in a variety of disease states, including allergic inflammatory processes such as asthma, allergic rhinitis, and atopic dermatitis (13–17). We have found that plasma RANTES levels are significantly increased in the patients with severe, treatment-resistant atopic dermatitis (18).

Recently, it has been reported that platelets are also an important source of RANTES, and that platelet-released RANTES may play a role in allergic reaction (1–6). In this study, the levels of RANTES and another platelet-produced chemokine, β-TG, and the levels of IFN-γ, IL-2, -4, -5, and -10 which are known to be produced by Th1 and Th2 T lymphocytes and increased in RANTES-relating allergic inflammatory process (5, 6, 19–27) were assessed in the patients with various malignancies. The levels of (IL-4-stimulated) IgE and blood (IL-5-stimulated) eosinophils, both of which increase with the elevation in RANTES in allergic reactions, were also assessed. RANTES and β-TG content of platelets was also assayed, and RANTES was also measured in primary and metastatic tumors, and in clinically uninvolved tissue taken peri- or postoperatively. The results indicate a strong correlation between elevated tissue RANTES levels and progressive malignancy.

PATIENTS AND METHODS

Forty-three patients with breast cancer and 23 cases of cervical cancer (all females, ages 23–56 years) were studied. The patients were classified into four stages according to 1997 TNM classification. The diagnosis of each cancer was made by tumor biopsy, imaging studies (computed tomography scan, magnetic resonance imaging) and corresponding tumor markers. “Uncontrolled or progressive stage” was diagnosed by the appearance of relapse, metastasis, or other worsening clinical findings, including an increase in tumor size on imaging studies and rise in plasma and tumor markers. Patients with inflamma-
RANTES Levels in Cancer Patients

Table 1  The levels of RANTES and β-TG in plasma and platelets in patients with breast and cervical cancers according to clinical stage and in progression or in remission

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Stage</th>
<th>Plasma RANTES pg/ml mean ± SD</th>
<th>No. of cases with RANTES levels</th>
<th>Platelet RANTES pg/10⁶ platelets mean ± SD</th>
<th>Platelet β-TG IU/ml mean ± SD</th>
<th>Plasma β-TG IU/10⁶ platelets mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer patients</td>
<td>I (5)</td>
<td>2,781 ± 315</td>
<td>0/4</td>
<td>1,706 ± 183</td>
<td>1,196 ± 104</td>
<td>136 ± 12</td>
</tr>
<tr>
<td></td>
<td>II (11)</td>
<td>4,263 ± 604b</td>
<td>1/5</td>
<td>1,498 ± 124b</td>
<td>1,251 ± 106</td>
<td>124 ± 11</td>
</tr>
<tr>
<td></td>
<td>III (12)</td>
<td>7,489 ± 1,234c</td>
<td>2/5</td>
<td>1,409 ± 128b</td>
<td>1,395 ± 120b</td>
<td>108 ± 12b</td>
</tr>
<tr>
<td></td>
<td>IV (15)</td>
<td>8,769 ± 1,527c</td>
<td>4/8</td>
<td>1,112 ± 126b</td>
<td>1,496 ± 138b</td>
<td>98 ± 11b</td>
</tr>
<tr>
<td>Cervical cancer patients</td>
<td>I (3)</td>
<td>2,743 ± 306</td>
<td>0/2</td>
<td>1,698 ± 176</td>
<td>1,203 ± 116</td>
<td>137 ± 10</td>
</tr>
<tr>
<td></td>
<td>II (4)</td>
<td>3,929 ± 346</td>
<td>2/2</td>
<td>1,550 ± 162</td>
<td>1,229 ± 121</td>
<td>126 ± 13</td>
</tr>
<tr>
<td></td>
<td>III (6)</td>
<td>7,046 ± 1,149e</td>
<td>2/3</td>
<td>1,369 ± 115b</td>
<td>1,346 ± 113b</td>
<td>114 ± 13b</td>
</tr>
<tr>
<td></td>
<td>IV (10)</td>
<td>8,272 ± 1,564c</td>
<td>3/5</td>
<td>1,204 ± 141b</td>
<td>1,428 ± 145b</td>
<td>108 ± 11b</td>
</tr>
<tr>
<td>Patients with breast and</td>
<td>Progressive (45)</td>
<td>8,472 ± 1,432c</td>
<td>12/22</td>
<td>1,125 ± 138b</td>
<td>1,462 ± 130b</td>
<td>103 ± 12b</td>
</tr>
<tr>
<td>cervical cancers (66)</td>
<td>In remission (21)</td>
<td>4,123 ± 623c</td>
<td>2/12</td>
<td>1,684 ± 181b</td>
<td>1,114 ± 112</td>
<td>128 ± 12</td>
</tr>
<tr>
<td>Healthy volunteers (12)</td>
<td></td>
<td>2,916 ± 348</td>
<td>2/8</td>
<td>1,866 ± 175</td>
<td>909 ± 82</td>
<td>140 ± 12</td>
</tr>
</tbody>
</table>

* Parentheses denotes the number of the cases tested.

*<sup>a</sup> 0.01 < P < 0.05 versus healthy volunteers.

<sup>b</sup> P < 0.01.

ulatory states, e.g., allergy and infections, were excluded from this study; those who had bronchial asthma, rhinitis, and atopic dermatitis (13–18) and those with serum IgE levels above 1000 IU/l and/or a percentage of eosinophils above 10% in peripheral blood leukocytes were also excluded. Also excluded were patients with positive blood cultures, and those who showed both CRP above 3 mg/dl and peripheral leukocyte counts above 10⁶/mm³ were also excluded because of the possibility of bacterial infection.

Because of the potential effect of chemotherapy or radiation on these parameters, only patients who were treated with natural products were studied, and patients with thrombocytopenia were excluded. Patients were tested for plasma levels of RANTES, β-TG (another chemokine produced by platelets), IFN-γ, IL-2, 4, 5, 8, and 10, serum IgE, and blood eosinophils. Platelet RANTES and β-TG content was also measured. RANTES levels were also assayed in tumor tissue. This included primary tumors and tumors metastatic to lymph nodes or skin from 22 breast cancer patients. In an additional 18 breast cancer patients, 8 with progressive disease and 10 in remission, RANTES content was measured in skin biopsies taken near the operative site from 1–3 months after tumor excision (postoperative skin). RANTES content was examined in resected tumor from 17 cervical cancer patients, including 10 with invasive disease and 7 with CIS. In addition, RANTES content was determined in the cervical mucosa near the operative site from six patients in remission and six patients with progressive malignancy, as assessed by rectal and vaginal examination and/or biopsy. For control values, plasma RANTES and skin were measured in 12 healthy controls and in biopsies of normal cervical mucosa from 11 women attending a gynecological clinic for routine pap test. Informed consent was obtained from each subject.

In the plasma RANTES assay, citrated blood was drawn from each subject. RANTES levels were assessed by ELISA (28), as follows. One hundred μl of a 1:10 dilution of plasma were added to microtiter wells coated with a mouse monoclonal antibody to human RANTES. After mixing, the plate was incubated for 2 h at 25°C; the wells were then washed six times with 300 μl of 0.9% NaCl and 0.05% Tween 20, and then 100 μl alkaline phosphatase-labeled RANTES monoclonal antibody were added to each well, and plate was incubated for 1 h at 25°C. After another six washings, 100 μl of paranitrophenyl-phosphate (final concentration, 5.4 mm) were added, and the reaction was terminated after 1 h at 25°C by the addition of 50 μl of EDTA. Absorbance was measured at 450 and 595 nm by spectrophotometer (Hitachi, U-3200, Tokyo, Japan), and concentration was determined from a standard calibration curve.

For tissue RANTES assay, biopsy specimens, lymph node, skin, or mucosa, from the tumor were homogenized in a 10-fold (wt/vol) volume of PBS with 0.1% Triton X-100, and the homogenates were assessed by routine clinical laboratory methods. For statistical analysis, the Student t test was applied for each plasma or blood assay level for comparisons between patients with progressive disease, patients in remission, and healthy volunteers, and for RANTES content between tumor or metastatic lesion and improved or normal skin or mucosa.

RESULTS

RANTES Levels in Plasma. Plasma RANTES levels were found to be statistically significantly elevated in patients with stage II, III or IV breast cancer or stage III or IV cervical cancer (stage IV, P < 0.001; stage III, P < 0.01; stage II, 0.01 < P < 0.05; stage I, P > 0.05; Table 1). Moreover, as

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shown in Table 1, plasma RANTES levels correlated directly with clinical stage for both breast and cervical cancer.

This correlation is also seen when patients were classified either as having progressive disease or as being in remission. As indicated in Table 1, mean plasma RANTES levels were significantly elevated in the patients with progressive and uncontrolled malignancy, compared with those in remission, and both groups showed elevated levels compared with healthy individuals. Moreover, in contrast to patients in remission and healthy volunteers, the distribution of RANTES levels in cancer patients with progressive disease showed a bimodal distribution. As shown in Fig. 1, the mean RANTES level in the first group was 11,435 pg/ml and that of the second group was 3,864 pg/ml, which is similar to that of the patients in remission. Of the patients with progressive disease, 27% of the patients in progression had a plasma RANTES level of >10,000 pg/ml, compared with none in either the group of cancer patients (the second group of progressive patients and the controlled group) or the healthy control group.

Because RANTES is produced from platelets, we measured levels of another platelet-secreted cytokine, β-TG, as a specificity control. The plasma levels of β-TG showed a modest correlation with cancer stage, but were much less dramatically elevated than those of RANTES (Table 1). The platelet content of both RANTES and β-TG were decreased in the patients with stage III or IV cancer, compared with healthy volunteers (0.01 < P < 0.05; Table 1), but, again, the decrease in RANTES content was more pronounced.

Interestingly, the other parameters that were measured, including IgE, eosinophils, IFN-γ, and IL-2,-4,-5,-8, and -10, were not significantly elevated in the cancer patients in whom plasma RANTES levels were found to be increased, nor in the cancer patients as a whole, compared with the healthy controls (data not shown).

RANTES Content in Tissues. The mean RANTES content of primary or metastatic tumor from patients with breast or cervical cancer was 50-fold greater than that of normal skin of healthy volunteers (breast cancer, P < 0.0001; invasive cervical cancer, P < 0.00001) and at least 5-fold greater than that of postoperative skin (as defined in “Patients and Methods”) or normal skin or mucosa taken at surgery near the site of the original tumor (breast cancer, P < 0.001; invasive cervical cancer, P < 0.01; Table 2). Both invasive cervical cancer and CIS of the cervix showed markedly elevated RANTES levels, although somewhat lower than in primary breast tumors. An increased RANTES content was found in all of the breast and cervical tumors examined, although only one-fourth of these patients showed elevated plasma RANTES levels. The RANTES content in the clinically normal skin or mucosa, taken perioperatively near original tumor or metastatic lesions from the patients with progression, relapse, and/or metastasis, was also markedly (7–10 times) increased compared with those from the biopsy specimens from patients in remission and from healthy controls (breast cancer, P < 0.001; invasive cervical cancer, P < 0.0001; Table 2), whereas there was no significant difference in the RANTES content of specimens from the latter two groups.

DISCUSSION

The β-chemokine RANTES is now known to be chemotactic for monocytes and eosinophils and to play a major role in allergic inflammatory processes (13–17, 19), and investigation on a receptor for IgE on platelet and the correlation of allergic reaction-inducing IgE (5, 6, 13, 14, 32) and eosinophils (2, 3, 33) or basophils (34) to RANTES has been reported. RANTES expression in atopic dermatitis skin, and allergen-induced transcription of mRNA for RANTES and subfamily of RANTES, MCP3, and an increase in plasma RANTES levels have been reported. More recently, we have again observed a marked increase in plasma RANTES levels accompanied by a marked decrease in IL-10 levels in one-third of patients with severe, treatment-resistant atopic dermatitis, as well as a marked increase in the RANTES content of skin lesions and in improved or normal skin from these patients (18). In this study, we have observed that RANTES levels were markedly increased in the plasma of one-fourth of patients with advanced breast or cervical cancer. Moreover, the RANTES content in all of the specimens of tumor, metastases-containing lymph nodes, or tumor-involved skin or mucosa was markedly increased. We also have observed similar patterns of plasma RANTES levels in patients with many other malignancies.3 Markedly elevated levels of plasma RANTES (>10,000 pg/ml) were found only in 27% of patients with progressive cancer, whereas those in remission had normal levels. However, because only 27% of the patients with progressive cancer had elevated levels, the role of RANTES in cancer progression, if any, remains to be determined. It is, of course, possible that RANTES plays a key role in this process in some patients but not in others.

3 Unpublished data.
Although RANTES is known to be released by a variety of cells, including activated T lymphocytes, monocytes, and fibroblasts, there is a suggestion that the elevated plasma RANTES levels seen in some of the patients in this study were derived from platelets. RANTES is known to be produced by platelets, with strong effects on allergic processes (1–6). Moreover, the platelet-secreted chemokine β-TG was also increased in the plasma of the patients with elevated plasma RANTES levels, and these same patients had decreased plasma RANTES content due to platelet secretion. The stimulus for RANTES secretion in association with platelets is a potent attractant for human eosinophils. J. Exp. Med., 176: 445–449, 1992.


### REFERENCES


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