Original Article

Eotaxin contributes to renal interstitial eosinophilia

Takashi Wada¹, Kengo Furuichi¹, Norihiko Sakai¹, Miho Shimizu¹, Chikako Segawa¹, Ken-ichi Kobayashi¹, Naofumi Mukaida², Tadashi Kasahara³, Kouji Matsushima⁴ and Hitoshi Yokoyama¹

¹First Department of Internal Medicine and Division of Blood Purification, School of Medicine, ²Department of Molecular Pharmacology, Cancer Research Institute, Kanazawa University, Kanazawa, ³Division of Biochemistry, Kyoritsu College of Pharmacy, and ⁴Department of Molecular Preventive Medicine, University of Tokyo, Tokyo, Japan

Abstract

Background. A potent eosinophil chemotactic cytokine, human eotaxin, is directly chemotactic for eosinophils. Therefore, the specific expression of eotaxin in tissue might play a crucial role in tissue eosinophilia. However, the precise molecular mechanism of the recruitment and activation of eosinophils in human renal diseases remains to be investigated. We evaluated the role of eotaxin in the pathogenesis of human diffuse interstitial nephritis with marked infiltration of eosinophils.

Methods. In this study, we examined 20 healthy volunteers, 56 patients with primary or secondary glomerular diseases and two hypereosinophilic syndrome patients without renal involvement. Urinary and serum eotaxin levels were determined by an enzyme-linked immunosorbent assay. We also detected the presence of eotaxin protein immunohistochemically.

Results. On the one hand, urinary levels of eotaxin were significantly higher before the initiation of glucocorticoid administration in the patient with interstitial nephritis with marked infiltration of eosinophils. On the other hand, urinary eotaxin levels were not detected in any patients with nephrotic syndrome, interstitial nephritis without eosinophils, hypereosinophilic syndrome without renal involvement or other renal diseases. Serum eotaxin levels were not detected in any of the patients. Therefore, the detection of eotaxin in the urine was specific for renal interstitial eosinophilia.

Moreover, endothelial cells, infiltrating mononuclear cells and renal epithelial cells in the tubulointerstitial lesions were immunostained with specific anti-eotaxin antibodies. Furthermore, the elevated urinary levels of eotaxin decreased dramatically during glucocorticoid-induced convalescence.

Hypothesis. We hypothesize that in situ expression of eotaxin may provide a new mechanism to explain the renal interstitial eosinophil infiltration.

Key words: chemokine; eosinophilia; eotaxin; interstitium; renal disease

Introduction

Eosinophils play an important role in various types of human disease including allergic, inflammatory, malignant and cardiovascular disorders [1]. In contrast, aberrantly activated eosinophils release toxic cationic proteins to induce tissue destruction [1]. Eosinophils produce cytokines [interleukin (IL)-3, IL-5 and granulocyte-macrophage colony stimulating factor (GM-CSF)] and lipid mediators (platelet-activating factor, leukotrien B4), which contribute to the inflammatory processes [1]. Therefore, an imbalance towards excess activation may lead to tissue destruction. In addition, since eosinophils are circulating leukocytes, some mediators would be necessary to regulate eosinophil—endothelial cell interaction, resulting in the infiltration and activation of eosinophils in the tissue. However, the precise molecular mechanism of the recruitment and activation of tissue eosinophils remains to be investigated.

A chemokine, human eotaxin, has been reported to be an early response gene of cytokine-stimulated epithelial and endothelial cells, and is expressed in leukocytes including eosinophils [2,3]. Eotaxin is directly chemotactic for eosinophils, but not mononuclear cells or neutrophils. Thus, the specific expression of eotaxin in the tissue might play a crucial role in tissue eosinophilia accompanied by the activation of adhesion molecules. Recent studies indicate that eotaxin is expressed in some organs including kidneys [2]. However, the role of eotaxin in the pathogenesis of human renal diseases has not yet been investigated.

In order to examine whether locally produced eotaxin participates in the pathophysiology of human renal diseases by recruiting and activating eosinophils, we determined both urinary and serum levels of eotaxin in patients with various renal diseases. We also investi-
gated the relationship between eotaxin levels and disease activity.

**Subjects and methods**

In this study, we evaluated 20 healthy volunteers, 56 patients with primary or secondary glomerular diseases and two hypereosinophilic syndrome patients without renal involvement (Table 1). The clinical profiles of normal volunteers and patients are summarized in Table 1. Patients with minimal change nephrotic syndrome showed massive urinary protein excretion (>3.5 g/day). The patients in this study were chosen randomly. There were 39 males and 43 females with a median age of 50.1 years (range 16–82 years). All patients with renal diseases had the diagnosis verified by renal biopsy. Whenever possible, patients did not receive any immunosuppressive agents before the sample were collected. Patients in a clinically active state were treated with glucocorticoids including methylprednisolone pulse therapy (500–1000 mg/day, 3 days) during this study. All renal biopsies were performed with the consent of the patients.

**Pathological studies**

Fifty-six kidney specimens were obtained by renal biopsy. Two observers, without knowledge of the clinical course, examined the renal tissue under a light microscope to establish the diagnosis using standard pathological methods.

**Eotaxin measurements**

Spontaneously voided midstream urines were collected. In all cases, urines were collected in the morning of renal biopsy. Ten milliliters of each urine was spun at 200 \( \times \) g for 5 min at room temperature to remove cells and precipitates. Sera were obtained simultaneously from patients. The urinary supernatants and sera were kept frozen at \(-70^\circ\)C until measurement. Urinary and serum eotaxin levels were determined by enzyme-linked immunosorbent assay (ELISA), using a specific murine monoclonal anti-human eotaxin antibody (clone H-I) as a capture and a rabbit polyclonal anti-eotaxin antibody as the second antibody as described previously [4]. This system is highly specific for eotaxin, since there were no cross-reactivities with other chemokines including monocyte chemotactic and activating factor (MCAF)/monocyte chemotactic protein (MCP)-1, IL-8, platelet basic protein, platelet factor 4, regulated upon activation, normal T-cell expression and secreted (RANTES). The recovery rate was confirmed to be >95% up to 3 ng/ml in these ELISA systems. All assays were performed in duplicate. The detection limit of this ELISA system was 100 pg/ml for human eotaxin. Urinary eotaxin levels were standardized by the amount of creatinine in the urine.

**Immunohistochemical studies**

The presence of eotaxin protein was demonstrated immunohistochemically on frozen tissue specimens by the indirect avidin-biotinylated alkaline phosphatase complex method with a specific murine monoclonal anti-human eotaxin antibody (H-I) as described previously [4]. A normal mouse IgG, which had been absorbed with both human liver extracts and immunoglobulin, was used as a negative control. In addition, the absorption test was performed using a monoclonal anti-human eotaxin antibody with an excess amount of recombinant eotaxin.

**Results**

The detection of urinary eotaxin is highly specific for the renal interstitial eosinophilia

The urinary eotaxin levels were detected in only one patient with renal interstitial eosinophilia with membranous nephropathy (Table 1), while other renal disease patients and healthy volunteers did not show detectable levels of urinary eotaxin. In addition, patients with hypereosinophilic syndrome without renal involvement, interstitial nephritis alone and membranous nephropathy with/without interstitial nephritis did not show detectable levels of urinary eotaxin. Moreover, serum eotaxin levels from normal volunteers and all patients, including those with hypereosinophilic syndrome, were below the detection limit of ELISA.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients (male: female)</th>
<th>Age (mean, years)</th>
<th>Urinary eotaxin levels (ng/ing creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>20 (10:10)</td>
<td>16–82 (57.1)</td>
<td>0</td>
</tr>
<tr>
<td>Renal diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with renal interstitial eosinophilia</td>
<td>1 (1:0)</td>
<td>48</td>
<td>17.0</td>
</tr>
<tr>
<td>Membranous nephropathy alone</td>
<td>12 (10:2)</td>
<td>45–67 (58.6)</td>
<td>0</td>
</tr>
<tr>
<td>Membranous nephropathy with interstitial nephritis</td>
<td>1 (1:0)</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>2 (1:1)</td>
<td>17–45 (31.0)</td>
<td>0</td>
</tr>
<tr>
<td>Minimal change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nephrotic syndrome</td>
<td>6 (3:3)</td>
<td>17–60 (30.0)</td>
<td>0</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>16 (10:5)</td>
<td>16–72 (42.9)</td>
<td>0</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>18 (3:15)</td>
<td>22–59 (38.5)</td>
<td>0</td>
</tr>
<tr>
<td>Hypereosinophilic syndrome without renal involve ment</td>
<td>2 (02)</td>
<td>55–68 (61.5)</td>
<td>0</td>
</tr>
</tbody>
</table>
Clinical profile of the renal interstitial eosinophilia patient

A 48-year-old man, suffering from nephrotic syndrome with eosinophilia and had a past history of bronchial asthma and atopic dermatitis in childhood, but denied receiving any recent treatment. Urine analysis showed proteinuria of 4.2 g/day and microscopic hematuria with granular casts. Hematological tests demonstrated leukocytosis (10 100/mm³) associated with marked eosinophilia (3030/mm³). There was moderately deteriorated renal function as indicated by a decreased creatinine clearance (Ccr, 33 ml/min). Remarkable hypergammaglobulinemia was observed with a marked elevation of immunoglobulin E (12 010 unit/ml). Renal biopsy revealed diffuse interstitial nephritis with a marked infiltration of eosinophils as well as mononuclear cells and membranous nephropathy associated with segmental sclerotic lesions (Figure 1).

Immunohistochemical detection of eotaxin protein in renal tissue

We evaluated the presence of antigenic eotaxin protein in renal tissue. We found that eotaxin-positive cells were detected mainly in endothelial cells, infiltrating mononuclear cells in the tubulointerstitial lesions and in a small number of renal epithelial cells in immunohistochemical analysis in the patient with renal interstitial eosinophilia with membranous nephropathy. However, there was no specific staining in glomeruli (Figure 2). In contrast, eotaxin was hardly detected in the kidneys of the other 55 biopsy-proven renal diseases examined, as similarly observed in the normal part of resected kidneys of reninoma (data not shown). In addition, normal mouse IgG and the absorption test showed no staining in the case with positive staining for eotaxin. Thus, in this case, the staining for eotaxin may be specific and renal interstitial infiltration of eosinophils may have been caused by the local production of eotaxin. As positive controls, we used resected tonsils derived from the patient with allergic rhinoritis and tonsillitis, since few reports reveal the detection of in situ expression of eotaxin in human organs.

Effects of glucocorticoid therapy on clinical course and urinary eotaxin levels

In this patient, the elevated urinary eotaxin levels decreased dramatically during glucocorticoid treatment including methylprednisolone pulse therapy (Figure 3). This treatment improved Ccr (46 ml/min) and proteinuria (1.6 g/day) associated with the decrease in the number of eosinophils (<100/mm³) in the circulation. Re-biopsy after the therapy demonstrated a marked improvement of the interstitial nephritis and the disappearance of tissue eosinophils.

Discussion

This study revealed that urinary eotaxin was detected only in the patient with renal interstitial eosinophilia. We did not detect urinary eotaxin in any other patients with hypereosinophilic syndrome without renal involvement, membranous nephropathy with/without interstitial nephritis or interstitial nephritis alone. The most noticeable difference between this patient and the others was the drastic infiltration of renal interstitial eosinophils. In addition, other renal diseases examined

![Fig. 1. Light-microscopic findings in a renal interstitial eosinophilia patient. Tubulointerstitial nephritis associated with the infiltration of eosinophils was detected. (Stain, hematoxylin & eosin; original magnification, ×320).](image-url)
Eotaxin and renal interstitial eosinophilia

Fig. 2. Immunohistochemical examination of eotaxin. Endothelial cells and infiltrating mononuclear cells in the tubulointerstitial lesions were eotaxin-positive in immunohistochemical analysis in this figure. (Original magnification, ×320).

We detected the presence of eotaxin in endothelial cells, renal epithelial cells and infiltrating mononuclear cells in the tubulointerstitial regions in the kidney of renal interstitial eosinophilia. In addition, eotaxin was hardly detected in the other renal diseases and normal kidneys. Even though recent studies indicate that eotaxin is expressed in kidneys [2], the site of production in the kidney remains unclear. In addition, the presence of eotaxin protein in any other organ, including the lung, remains to be investigated, even though increased expression of eotaxin has been detected in bronchoalveolar lavage from asthma patients [6] and eotaxin mRNA has been detected in resected colons from patients with ulcerative colitis and Crohn’s disease [3]. Collectively, the existence of eotaxin in the kidney may have induced tissue eosinophilia in this patient.

Urinary levels of eotaxin decreased to undetectable levels during the convalescence induced by glucocorticoids including methylprednisolone pulse therapy. Human eotaxin has been reported to be an early response gene of cytokine-stimulated epithelial and endothelial cells, and is expressed in leukocytes including eosinophils [3]. Eotaxin is directly chemotactic for eosinophils, but not mononuclear cells or neutrophils. Thus, the specific expression of eotaxin in the tissue may play a crucial role in the tissue eosinophilia with the activation of adhesion molecules. Eotaxin mRNA is up-regulated by cytokines, including IL-1, tumor necrosis factor (TNF)-α and interferon (IFN)-γ [3]. These cytokines are regulated by the activation of nuclear factor (NF)-κB. In addition, glucocorticoids here included lupus nephritis, in which increased production of cytokines has been reported [4]. These data suggest that eotaxin is highly specific for the renal interstitial eosinophilia and that the up-regulation of eotaxin in the kidney may require some specific stimuli. Moreover, patients with minimal change nephrotic syndrome showed undetectable levels of urinary eotaxin. Patients with minimal change nephrotic syndrome often show high levels of IgE, suggesting that an allergic mechanism may be involved in the pathogenesis of minimal change nephrotic syndrome [5]. However, our results suggest that there might be not be a correlation between eotaxin and the pathogenesis of minimal change nephrotic syndrome or proteinuria itself. Thus, the specific regulator(s) of eotaxin in the kidney and the precise mechanism involved in renal interstitial eosinophilia via eotaxin remain to be investigated.

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have been shown to suppress cytokine production at the transcriptional level via the inactivation of NF-κB [7]. Thus, it is tempting to speculate that one of the pharmacological actions of glucocorticoids for eosinophils might be the direct and/or indirect inhibition of eotaxin production through the activated kidney resident cells and infiltrating cells in accordance with the disease activity.

In summary, we hypothesize that eotaxin protein provides a key to explain renal interstitial eosinophil infiltration, and that the measurement of eotaxin reflects the disease activity during glucocorticoid-induced convalescence. Thus, eotaxin may be generally involved in tissue eosinophilia in various eosinophil-associated diseases including kidney diseases.

Acknowledgements. This work was supported by a grant from Japan Research Foundation for Clinical Pharmacology (TW) and a Grant-in-Aid (No.09671157) from the Ministry of Education, Science, Sport and Culture of Japan (HY).

References

Received for publication: 16.6.98
Accepted in revised form: 18.9.98