CD52 Antigen May Be a Therapeutic Target for Eosinophilic Rhinosinusitis

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Abstract. Allergic rhinosinusitis involves several types of inflammatory cells. The dominant inflammatory cells include mast cells, eosinophils, lymphocytes, and monocytes/macrophages. Since eosinophils are one type of inflammatory cell that is often related to allergy, we investigated in this study whether the eosinophils present in rhinosinusitis may be potential targets for CD52 antibody treatment. First, we found that circulating eosinophils in renal recipients were almost completely depleted after iv bolus of treatment with Campath-1H, a humanized antibody against CD52 antigen. Second, we showed morphologically that eosinophils, lymphocytes, and monocytes gave positive staining reactions for CD52. Third, using an automated clinical imaging system, we found that tissue sections of sinus contents with prominent eosinophils (eosinophilic rhinosinusitis) yielded significantly higher CD52 staining scores than those with lymphocytes as the dominant component (lymphocytic rhinosinusitis). These findings indirectly support the hypothesis that CD52 may be a target for treating eosinophilic rhinosinusitis with Campath 1H.

Keywords: CD52, rhinosinusitis, eosinophilia, tissue microarray, Campath-1H, Alemtuzumab

Introduction

Allergic rhinosinusitis is a common disease that affects approximately one fourth of the U.S. population [1,2]. Although allergic antigens may be different depending on the geographic location and season, the major sequence of the allergic process includes release of histamine by sensitized mast cells and the recruitment of lymphocytes, monocytes, and eosinophils for inflammatory reactions [3,4]. Currently, the main therapeutic strategies are to inhibit histamine release by mast cells, and to block histamine effects and reduce inflammatory reactions by administration of steroids [5,6]. Extensive research is aimed at finding new targets to treat allergic rhinosinusitis.

CD52 is a small molecule that is located on the cell surface of some inflammatory cells (mainly lymphocytes, natural killer cells, and monocytes), but not neutrophils [7,8]. Binding to the CD52 antigen of inflammatory cells causes complement-mediated cell lysis [7,8]. There is only one study that documents the expression of CD52 antigen on human eosinophils [9]. Campath 1-H, a humanized antibody against CD52, is used to deplete circulating inflammatory cells as an induction method in patients undergoing bone marrow or solid organ transplantation [10-13]. In these studies, it is unclear whether eosinophils are depleted by Campath-1H [10-13], possibly because eosinophils represent a relatively small percentage of circulating inflammatory cells (~3%). Eosinophils are not a major type of inflammatory cell that is involved in acute cellular rejection in transplant patients. Thus the changes in eosinophils after Campath-1H treatment have hitherto been essentially ignored.
In the first part of the study, we investigated whether circulating eosinophils are depleted after Campath-1H treatment in renal transplant recipients. Then, using a ChromaVision system, we evaluated whether the expression of CD52 is greater in eosinophilic rhinosinusitis compared to lymphocytic rhinosinusitis.

**Materials and Methods**

**Renal recipients with Campath-1H treatment.** The study protocol was approved by the Institutional Research Review Board of Geisinger Health System. In this study, 17 renal recipients received intra-operative Campath-1H (Alemtuzumab, Berlex Inc., Seattle, WA) induction during 2004 - 2005. The pretreatment regimen included 1 g of iv methylprednisolone, 650 mg of oral acetaminophen, 50 mg of iv diphenhydramine, and 30 mg of iv Campath-1H over 2 hr. Post-operative immunosuppression included either FK506 (target level of 10 ng/ml from day 1) or mycophenolate mofetil, one g bid from day 1. Data for the peripheral blood component of inflammatory cells in these patients before and 2 days after Campath-1H treatment were obtained from the Department of Transplantation. Data for the peripheral blood component of inflammatory cells in 12 randomly selected patients with normal blood counts from the Hematology Laboratory were taken as non-Campath controls.

**Histologic staining and evaluation.** Thirty surgical pathologic specimens of sinus contents from November 2003 to May 2004 were identified using our computer system. These cases were divided into eosinophilic or lymphocytic rhinosinusitis groups, depending on whether the dominant inflammatory cells were eosinophils or lymphocytes (more or less than 2 eosinophils per high power field, respectively). The sinus contents were fixed in formalin and embedded in paraffin. Paraffin blocks were cut into 5 µm sections, which were dewaxed in xylene, rehydrated with graded ethanol to water, and processed for routine hematoxylin-eosin staining. To identify the mononuclear cell components, tissue microarray sections were stained for CD117 (for mast cells), CD20 (for B lymphocytes), CD4/CD8 (for T lymphocytes), and CD68 (for monocytes/macrophages). Although myeloperoxidase can stain eosinophils and neutrophils, no significant number of neutrophils was present in any of these cases, based on light microscopy of H&E stained sections. Therefore, myeloperoxidase staining was selected to represent eosinophils. CD117, CD20, CD4, CD8, and myeloperoxidase antibodies were purchased from Dako Cytomation (Redwood City, CA). All cases were also stained for CD52 using Campath-1H as the primary antibody (Genzyme Corp., Cambridge, MA). Slides were stained immunohistochemically using a Dako Autostainer (Model E172566) (Dako Cytomation). Positively stained cells at x100 magnification were counted using an automated clinical imaging system (ACIS, ChromaVision, San Juan Capistrano, CA) and arbitrary scores of positivity at each selected area were automatically generated by the system. The data were exported using GML-PORT (Geisinger Medical Laboratories Pathology Online Research Tool), which linked the data files to the case list on a Microsoft Excel worksheet.

**Statistics.** The results were expressed as mean ± SD. The unpaired t test was used to compare values from 2 groups and p values <0.05 were considered statistically significant.
Results

Role of CD52 antibody in circulating eosinophils.
Before Campath-1H treatment, the renal transplant recipients had similar levels of circulating white blood cells, monocytes, and eosinophils when compared to non-Campath controls, although the neutrophil and lymphocyte counts showed a small but significant difference between the 2 groups (Table 1). After the Campath-1H treatment, the lymphocytes and monocytes were significantly depleted, whereas the neutrophils were reactively increased as expected when compared to prior Campath-1H treatment values and healthy controls. Circulating eosinophils were almost entirely depleted after Campath-1H treatment (Table 1).

Eosinophilic rhinosinusitis shows a high level of CD52 positive cells. Females comprised approximately 1/3 of patients in both the eosinophilic rhinosinusitis and the lymphocytic rhinosinusitis groups; the patients in both groups had similar ages (Table 2). Eosinophils in sinus contents show strong expression of CD52 antigen (Fig. 1). Whereas lymphocytes and monocytes/macrophages also express CD52, mast cells do not express CD52. In patients with eosinophilic rhinosinusitis or lymphocytic rhinosinusitis, the cell markers for mast cells, lymphocytes, and monocytes were similar in both groups (Table 2). The myeloperoxidase staining score was significantly higher in the eosinophilic rhinosinusitis than in the lymphocytic rhinosinusitis group, reflecting the relative abundance of eosinophils. In addition, the CD52 staining score was much higher in the eosinophilic rhinosinusitis group in comparison to the lymphocytic rhinosinusitis group (Table 2).

Discussion

In this study, the most striking finding was that Campath-1H treatment almost entirely depleted circulating eosinophils in renal transplant patients when compared to pretreatment values. Many

Table 1. Effects of Campath-1H on circulating inflammatory cells.

<table>
<thead>
<tr>
<th>Blood leukocytes</th>
<th>Non-Campath controls (N = 12)</th>
<th>Pre-Campath-1H patients (N = 17)</th>
<th>Post-Campath-1H patients (N = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total white blood cells (x 1000/mm³)</td>
<td>5.96 ± 0.46</td>
<td>7.82 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Neutrophils (%)</td>
<td>56.08 ± 1.95</td>
<td>64.59 ± 2.74*</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes (%)</td>
<td>30.43 ± 1.42</td>
<td>22.24 ± 2.32*</td>
</tr>
<tr>
<td></td>
<td>Monocytes (%)</td>
<td>8.96 ± 0.48</td>
<td>8.94 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>Eosinophils (%)</td>
<td>3.91 ± 0.65</td>
<td>3.77 ± 0.41</td>
</tr>
</tbody>
</table>

*p<0.05, statistically significant vs non-Campath controls and #p<0.05, statistically significant vs pre-Campath-1H group.

Table 2. Marker expression of the various inflammatory cells in eosinophilic versus lymphocytic rhinosinusitis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eosinophilic rhinosinusitis patients (N = 15)</th>
<th>Lymphocytic rhinosinusitis patients (N = 15)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>5 F/10 M</td>
<td>4 F/11 M</td>
<td>-</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>43.2 ± 4.4</td>
<td>42.7 ± 6.5</td>
<td>0.960</td>
</tr>
<tr>
<td>CD52 (AU)</td>
<td>3639 ± 565</td>
<td>1806 ± 364</td>
<td>0.010*</td>
</tr>
<tr>
<td>Myeloperoxidase (AU)</td>
<td>475 ± 110</td>
<td>142 ± 38</td>
<td>0.007*</td>
</tr>
<tr>
<td>CD117 (AU)</td>
<td>291 ± 37</td>
<td>265 ± 24</td>
<td>0.563</td>
</tr>
<tr>
<td>CD20 (AU)</td>
<td>783 ± 218</td>
<td>1265 ± 486</td>
<td>0.385</td>
</tr>
<tr>
<td>CD4 (AU)</td>
<td>251 ± 46</td>
<td>382 ± 119</td>
<td>0.326</td>
</tr>
<tr>
<td>CD8 (AU)</td>
<td>1446 ± 333</td>
<td>1612 ± 274</td>
<td>0.701</td>
</tr>
<tr>
<td>CD68 (AU)</td>
<td>430 ± 71</td>
<td>458 ± 77</td>
<td>0.790</td>
</tr>
</tbody>
</table>

AU = arbitrary units. * p <0.05
CD52 in rhinosinusitis

studies have shown that Campath-1H profoundly depletes circulating lymphocytes and monocytes whereas neutrophils do not carry CD52 and therefore do not respond to Campath-1H [10-13]. Eosinophils, as a small component of circulating inflammatory cells, have not previously been evaluated before and after administration of Campath-1H. To our knowledge, only one previous study indicates that human eosinophils express both CD52 mRNA and protein [9]. These data, reported by Elsner et al [9], suggest that CD52 on eosinophils is anchored to the membrane through a glycosylphosphatidylinositol moiety. Cross-linking of CD52 by its antibody results in inhibition of reactive oxygen species production by eosinophils after stimulation with multiple factors. Elsner et al [9] point out that the CD52 antigen on human eosinophils may have clinical relevance, because cross-linking of this molecule might block the destructive power of human eosinophils in inflammatory tissue [9]. Our study shows that eosinophils show diffuse expression of cytoplasmic CD52 antigen, suggesting that CD52 might be a therapeutic target in rhinosinusitis, particularly eosinophilic rhinosinusitis.

Basophils represent a very small portion of circulating white blood cells (approximately 1%), and no studies have been able to document a depletion of basophils in response to Campath-1H treatment. Mast cells are another important type of inflammatory cell that mediates allergic rhinosinusitis. Mast cells are characterized by expression of CD117 [14,15], but they do not express CD52. Targeting CD52 for treating rhinosinusitis might represent a new departure from the current commonly used medications, which inhibit histamine release of mast cells [5,6].

In conclusion, eosinophils are sensitive to Campath-1H depletion and they stain positively for CD52, similar to lymphocytes and monocytes. Eosinophilic rhinosinusitis shows significantly higher expression of CD52 than lymphocytic rhinosinusitis. Since CD52 is extensively present in the cytoplasm of the inflammatory cells involved in rhinosinusitis, we suggest that CD52 may be a therapeutic target for allergic rhinosinusitis, especially eosinophilic rhinosinusitis.

References