The Significance of CD13/Aminopeptidase N in Interstitial Lung Diseases

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CD13/aminopeptidase N (E.C.3.4.11.2) is an ectoenzyme located in the outer cell membrane in a variety of cells. Since CD13/aminopeptidase N was shown to induce in vitro chemotactic migration of human lymphocytes, we examined here the significance of CD13/aminopeptidase N in pulmonary sarcoidosis and radiation pneumonitis caused by a single-dose thoracic irradiation (15 Gy) in a rat model. The activity of aminopeptidase in bronchoalveolar lavage fluid (BALF) was significantly higher in sarcoidosis patients than in normal volunteers (NV) and control patients (CP). CD13/aminopeptidase N protein was detectable in alveolar macrophages (AM) from sarcoidosis patients at higher levels than in those from NV. Higher chemotactic activity for lymphocytes was detected in the BALF from sarcoidosis patients that in that from NV, and the activity was significantly decreased by the treatment with bestatin, an specific inhibitor for aminopeptidase N. Significantly increased CD13/aminopeptidase N activity and expression were also detected in BALF and AM obtained from irradiated rats at 4 weeks after irradiation compared with the activity in unirradiated rats. Chemotactic activity for normal rat lymphocytes was detected in BALF from irradiated rats at 4 weeks, and approximately 60% of the activity was inhibited by pretreatment of BALF with bestatin. This study suggests that CD13/aminopeptidase N may play an important role as a lymphocyte chemoattractant in lymphocyte-mediated alveolitis in interstitial lung diseases.

Key words: CD13, Aminopeptidase N, Sarcoidosis, Lymphocytes, Radiation pneumonitis

Introduction

The cell-surface glycoprotein CD13 in leukocytes of the myeloid series is identical to aminopeptidase N (EC 3.4.11.2)1). CD13/aminopeptidase N is widely distributed in various mammalian cells, including monocytes/macrophages, fibroblasts, neutrophils, endothelial cells and epithelial cells, and play an important role in the activation, proliferation and communication in hemopoietic cells2). We recently found that CD13/aminopeptidase N has the chemotactic activity for T lymphocytes by its enzymatic activity3). Accordingly, CD13/aminopeptidase N may play a role in regulating lymphocyte-mediated inflammatory and immunologic responses

In this study, we find increased activity of aminopeptidase in bronchoalveolar lavage fluid (BALF) and increased expression of CD13/aminopeptidase N protein in alveolar macrophages (AM) from sarcoidosis patients and rats with experimental radiation pneumonitis which correlates with the activity of alveolitis in this disorder. Moreover, we demonstrate here that CD13/aminopeptidase N may have a significant role in the pathogenesis of sarcoidosis and radiation pneumonitis as a lymphocyte chemoattractant.

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Methods
CD13/aminopeptidase N (E.C. 3.4.11.2; specific activity, 34 U/mg protein) and bestatin were purchased from Sigma Chemical Co. (St. Louis, MO). L-leucine-7-amino-4-methyl-coumarine (L-leucine-AMC) was purchased from Peptide Institute (Osaka, Japan). Monoclonal antibody against human CD13 was purchased from Becton Dickinson (San Jose, CA). Studies were made on 30 patients with sarcoidosis. Twelve normal volunteers (NV) and 10 control patients (CP) were used as controls. BAL was performed as described previously. Protease activity of aminopeptidase was assayed fluorometrically as described previously. Lymphocytes were separated from peripheral blood of healthy donors by centrifugal elutriation in a Beckman JE-5.0 elutriation system (Beckman Instruments Fullerton, CA). Lymphocyte migration was assessed by a 48-well microchemotaxis chamber technique as previously described. Thoracic irradiation (15 Gy) was performed using male Wistar rats as described previously. All results are expressed as mean ± SEM. Statistical analysis was performed using the Student’s two-tailed unpaired t test for comparisons between two groups.

Results
Aminopeptidase activity in the BALF from NV, CP, and patients with sarcoidosis is shown in Fig. 1. The mean value of aminopeptidase activity in the BALF from patients with sarcoidosis was significantly higher (49.9 ± 10.8 nmol/h) than that of NV and CP, but individual values were widely ranged from 6 to 221 nmol/h. The aminopeptidase activity in the BALF showed significantly positive correlations with lymphocyte percentages and the ratio of CD4+ to CD8+ T lymphocytes. Sarcoidosis patients were classified into two groups by chest radiographs: Group I without parenchymal involvement (stage I), and group II with parenchymal involvement (stage II/III). By this classification, 15 patients belonged to group I, and 15 to group II (Table 1). There was no significant difference in the mean ages between

![Fig. 1 Aminopeptidase activity in the BALF from NV, CP, and patients with sarcoidosis.](Image)

Table 1 Comparison of clinical and laboratory data in groups I and II of sarcoidosis

<table>
<thead>
<tr>
<th>Group*</th>
<th>Age (yr)</th>
<th>Number</th>
<th>Serum ACE (U/ml)</th>
<th>Bronchoalveolar lavage</th>
<th>aminopeptidase (nmol/h)</th>
<th>%Ly</th>
<th>CD4+/CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>47.5 ± 3.5**</td>
<td>15</td>
<td>25.7 ± 1.8</td>
<td>16.3 ± 3.7</td>
<td>21.2 ± 4.3</td>
<td>4.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>50.2 ± 3.6</td>
<td>15</td>
<td>31.9 ± 3.9</td>
<td>83.6 ± 17.6</td>
<td>44.6 ± 4.3</td>
<td>9.6 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>N.S.</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.S.: not significant.

*Sarcoidosis patients were classified into two groups by chest radiographs; group I without parenchymal involvement, and group II with parenchymal involvement.

**Results are expressed as mean ± SEM.
Fig. 2 Western blot analysis of CD13/aminopeptidase N protein expression in AM. Fifty μg of AM lysate protein were separated on SDS polyacrylamide gel and blotted. CD13/aminopeptidase N was detected by a monoclonal anti-human CD13 antibody. Lanes 1–3 contain lysates of AM from NV, and lanes 4–10 contain those from sarcoidosis patients.

Fig. 3 Aminopeptidase activity in BALF from unirradiated and irradiated rats at 2, 4, 8 and 18 weeks. Aminopeptidase activity was assayed fluorometrically with L-leucine-AMC as a substrate as described in Materials and Methods. Aminopeptidase activity was expressed as the ratio of irradiated rats to unirradiated rats. Results are expressed as mean ± SEM, with n=5 in each group. *indicates statistically significant (p<0.05) differences from the value of unirradiated rats.

Fig. 4 Effect of bestatin on aminopeptidase activity and chemotactic activity for lymphocytes in BALF. Effect of bestatin on chemotactic activity for lymphocytes in BALF. The BALF from unirradiated rats and irradiated rats at 4 weeks was preincubated with an equal volume of bestatin solution (500 μg/ml) or medium alone at 37°C for 60 min. The solution was then tested for chemotactic activity for lymphocytes as described in Materials and Methods. Experiments were repeated three times with cells from different rats (n=3). Results are expressed as mean ± SEM. *indicates statistically significant (p<0.05) differences from the value of untreated samples.

The level of aminopeptidase activity in the BALF was significantly higher in group II than in group I. The percentage of lymphocytes and the ratio of CD4+ to CD8+ T lymphocytes were also higher in group II than in group I. The AM lysate protein from six of seven sarcoidosis patients contain higher amount of CD13 in Western blot analysis (Fig. 2). Chemotactic activity for lymphocytes was detected in the BALF from sarcoidosis patients but not in that from NV, and the treatment of the BALF from sarcoidosis patients with bestatin, an specific inhibitor for aminopeptidase N, resulted in inhibition of the chemotactic activity in the BALF (43 to 74% inhibition).

In rat model of radiation pneumonitis, significantly increased aminopeptidase activity was detected in BALF from irradiated rats at 4 weeks after a single irradiation (Fig. 3). The
activity decreased to the level of unirradiated rats at 8 and 18 weeks. Western blot showed that higher amounts of CD13 protein were observed in AM from irradiated rats. Considerable chemotactic activity was detected in the BALF from irradiated rats. Treatment of BALF from irradiated rats with bestatin partially inhibited the chemotactic activity of the BALF (57.4% inhibition) (Fig. 4).

Discussion

We showed here significantly higher aminopeptidase activity in the BALF from sarcoidosis patients and rats with radiation pneumonitis. The treatment of the BALF with bestatin, an specific inhibitor for aminopeptidase N, partially decreased its chemotactic activity. These results suggest that enzymatically active CD13/aminopeptidase N may have a significant role in lymphocyte involvement as a chemoattractant in interstitial lung diseases.

Activated macrophages have an important role in initiating and amplifying immunologic and inflammatory responses in various inflammatory lung diseases. In interstitial pneumonitis, T lymphocytes and macrophages play a pivotal role in orchestrating the inflammatory process. CD13/aminopeptidase N expressed in macrophages was shown to be an ectoenzyme marker that increases as macrophages mature or becomes activated. This study indicates that AM may be responsible, at least in part, for the aminopeptidase activity detected in the BALF from sarcoidosis patients.

The mechanism for increased expression of CD13/aminopeptidase N in radiation pneumonitis is still uncertain. T cell-derived cytokines such as interferon-γ and IL-4 have been shown to up-regulate the expression of CD13/aminopeptidase N mRNA and protein in fibroblasts and monocytes/macrophages. IL-4 is produced by macrophages during radiation-induced pneumonitis and pulmonary fibrosis. Accordingly, certain cytokines released in injured lungs may have a role in increased CD13/aminopeptidase N expression in sarcoidosis and radiation pneumonitis.

The data presented here raise the possibility that CD13/aminopeptidase N activity may serve as a marker of the activity of alveolitis in sarcoidosis and radiation pneumonitis, and suggest that CD13/aminopeptidase N may participate in the mechanism of T cell involvement in these disorders. Increased BALF aminopeptidase activity was found not only in sarcoidosis and radiation pneumonitis but also in those with other interstitial lung diseases. Therefore, it is possible that CD13/aminopeptidase N plays a role in recruiting lymphocytes to disease sites in various interstitial lung diseases.

REFERENCES