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Comparison of BALF concentrations of ENA-78 and IP10 in patients with idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia

Seiko Nakayama[a], Hiroshi Mukae[a,*], Hiroshi Ishii[a], Tomoyuki Kakugawa[a], Kanako Sugiyama[a], Noriho Sakamoto[a], Takeshi Fujii[a], Jun-ichi Kadota[b], Shigeru Kohno[a]

[a]*Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki*
[b]*Second Department of Internal Medicine, Oita University Faculty of Medicine, Oita, Japan*

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KEYWORDS
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Summary
Background: Epithelial neutrophil-activating peptide 78 (ENA-78) and interferon γ-inducible protein 10 (IP10) belong to the CXC chemokine family and are considered to be important factors in idiopathic pulmonary fibrosis (IPF). Idiopathic nonspecific interstitial pneumonia (NSIP) and IPF are the two largest subsets of idiopathic interstitial pneumonias (IIP). In patients with NSIP, the prognosis is generally good compared with IPF. Therefore, the pathogenesis of NSIP seems to be different from that of IPF, but this remains unclear. The aim of the present study was to evaluate the contribution of ENA-78 and IP10 in the two diseases.
Methods: We measured the levels of ENA-78 and IP10 in serum and bronchoalveolar lavage fluid (BALF) of patients with IPF ($n = 17$), idiopathic NSIP ($n = 10$) and healthy subjects ($n = 12$) by enzyme-linked immunosorbent assays.
Results: The level of ENA-78 in BALF was significantly higher in IPF patients than in NSIP patients and controls. Serum levels of ENA-78 and BALF levels of IP10 in NSIP patients were significantly higher than in patients with IPF and controls. In BALF of patients with NSIP, IP10 level significantly correlated with the absolute number of lymphocytes. In IPF patients, BALF IP10 levels also correlated with the proportion of lymphocytes in BALF.
Conclusion: Our results show distinct profiles of CXC chemokines in IPF and NSIP, and suggest that these chemokines play an important role in inflammatory cell recruitment into the lung in patients with IIP.

*Corresponding author. Tel.: +81 95 849 7273; fax: +81 95 849 7285.
E-mail address: hmukae@net.nagasaki-u.ac.jp (H. Mukae).

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Introduction

The classification of idiopathic interstitial pneumonia (IIP) includes seven clinico-radiologic-pathological entities. Idiopathic pulmonary fibrosis (IPF) and nonspecific interstitial pneumonia (NSIP) are the two largest subsets of IIP.[1–3] NSIP is distinguished from IPF by the temporal uniformity of interstitial inflammation and/or fibrosis on histology.[1–3] The distinction between NSIP and IPF is important for clinical decision-making because the prognosis is generally good and the response to corticosteroids and immunosuppressants is also good in patients with NSIP compared with IPF.[1–3] It is well known that NSIP is associated with relative lymphocytosis with a predominance of CD8+ cells in bronchoalveolar lavage fluid (BALF) compared with IPF.[4–6] Previous studies demonstrated that lymphocytosis correlated with the levels of interleukin (IL)-6 in BALF of patients with NSIP.[5] These results suggest that the pathogenesis of NSIP is different from that of IPF, though their mechanisms remain elusive.

Chemokines are important for leukocyte recruitment to the inflamed tissue. Chemokines are divided into four subfamilies depending on the position of the conserved cysteine residues (CXC, CC, C and CX_3C).[7] It is well known that the CC chemokines such as monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein-1alpha (MIP-1α), and regulated upon activation, normal T cell expressed and secreted (RANTES), are closely related to the expression of adhesion molecules and the migration of inflammatory cells into the lung. There is sufficient evidence for the importance of these chemokines in IPF.[8–12] These CC chemokines are also important in inflammatory cell recruitment to the lung in idiopathic NSIP.[13] The CXC chemokines such as epithelial neutrophil-activating peptide (ENA)-78, interferon γ-inducible protein (IP)10 and IL-8 are also important factors in IPF.[14,15] However, to our knowledge, there are no reports demonstrating the role of the CXC chemokine in idiopathic NSIP. We hypothesized that these CXC chemokines mediate the recruitment of inflammatory cells into the lung in patients with NSIP as well, similar to the behavior of the CC chemokine.

In the present study, we measured the concentrations of ENA-78 and IP-10 in BALF and serum samples obtained from patients with IPF and idiopathic NSIP to evaluate the contribution of these chemokines, and to determine whether there is a distinct profile of these factors in these diseases.

Materials and methods

Study population

The 39 subjects of this study were patients and healthy volunteers enrolled in the Hospitals of Nagasaki University School of Medicine. They included 17 patients with idiopathic IPF (14 males and 3 females, consisting of 5 current smokers, 3 ex-smokers and 9 nonsmokers, age; 61 ± 10 years, mean $\pm$ SD;), 10 with idiopathic NSIP (5 males and 5 females, consisting of one current smoker, 2 ex-smokers and 7 nonsmokers, age; 56 ± 13 years), and 12 healthy volunteers (8 males and 4 females, 12 nonsmokers, age; 24 ± 5 years). None of the enrolled patients had received steroid and other immunosuppressive therapy at the time of clinical sample collection. Patients with cancer in any organ and those suspected to have malignancy were excluded from the study. The diagnosis was pathologically confirmed using surgical lung biopsy specimens obtained in at least two different sites in all patients. The patients with usual interstitial pneumonia (UIP) and NSIP associated with collagen vascular diseases were excluded in this study. In all of 10 NSIP cases, the diagnosis was pathologically confirmed as fibrotic NSIP, consisting of 5 Group II and 5 Group III.[16] In these NSIP patients, the following high-resolution computed tomography (HRCT) criteria were used: (1) predominantly basal/subpleural distribution, (2) a mixture of reticular and ground-glass abnormalities, with traction bronchiectasis when ground-glass attenuation was prominent, (3) the absence of consolidation or nodules. All patients with NSIP fulfilled these criteria. There were no significant differences in the mean %VC, FEV_1%, %DLCO, PaO_2 and $PaCO_2$ between IPF and NSIP. All healthy volunteers had normal chest radiographs, were free of symptoms and not taking any medications. The study protocol was approved by the Human Ethics Review Committees of Nagasaki University School of Medicine and a signed consent form was obtained from each subject.

Bronchoalveolar lavage and blood sampling

With informed consent, BAL was performed as described previously[6,13,17] using a flexible fiberoptic bronchoscope (Olympus, P-20 Olympus, Tokyo, Japan). Briefly, the bronchoscope was wedged into one of the segmental or subsegmental bronchi of the right middle lobe. Then, 50 ml of sterilized saline at body temperature was instilled through the bronchoscope. The fluid was immediately