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Pulmonary dendritic cell accumulation in usual interstitial pneumonia and nonspecific interstitial pneumonia

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Abstract. *Background*: Pulmonary dendritic cells (DCs) are key regulators of immune responses. An increased accumulation of DCs was reported in the lungs of patients with idiopathic interstitial pneumonia (IIP). *Objective:* This study aimed to investigate the number of pulmonary DCs in patients with collagen vascular disease associated interstitial lung diseases (CVD-ILDs). *Design:* Lung tissue samples obtained from 27 patients with IIP and 39 patients with CVD-ILD were detected using monoclonal antibodies against CD1a, CD1c, CD83, Langerin and DC-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN). *Results:* No significant differences in the number or distribution of DCs were observed between patients with IIP and CVD-ILDs. When DC marker expression was analyzed according to pathological subgroup, patients with idiopathic usual interstitial pneumonia (UIP) showed increased DC-SIGN staining when compared with CVD-UIP ($p<0.05$). *Conclusion:* Both mature and immature DCs accumulate in CVD-ILDs. The number of DCs expressing DC-SIGN in CVD-UIP was decreased compared with that in idiopathic UIP. The variation in accumulated DC-SIGN-positive cells might help to explain the differences in the development and maintenance of lung inflammation between idiopathic UIP and CVD-UIP. *(Sarcoidosis Vasc Diffuse Lung Dis 2012; 29: 69-73)*

Key words: pulmonary dendritic cells, interstitial pneumonia, collagen vascular disease

Introduction

Idiopathic interstitial pneumonia (IIP) is a category of interstitial lung diseases (ILDs) of unknown etiology (1). Usual interstitial pneumonia (UIP) and nonspecific interstitial pneumonia (NSIP) are the most frequently occurring and clinically important IIP. Collagen vascular diseases (CVDs) are a heterogeneous group of autoimmune diseases and cause various pulmonary complications, such as airway, pleural, vascular and parenchymal diseases. CVD-associated ILDs (CVD-ILDs) are frequent and clinically significant complications and contain various histological patterns. Some CVD-ILDs correspond to IIP in their clinical, pathological and radiological properties, and are histologically classified into UIP and NSIP using the same criteria as IIP.

Dendritic cells (DCs) are antigen-presenting cells with a central role in the regulation of immune responses (2) and an important function in various respiratory diseases, such as asthma and COPD (3-7). Marchal-Sommé and coworkers have reported an accumulation of DCs in IIP (8,9) that is caused by chemokines locally produced by epithelial cells and fibroblasts, allowing DCs to cause persistent chron-

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ic inflammation (9). Because DCs are also considered to be involved in the pathogenesis of autoimmune disorders (10-12), we assumed that DCs could be accumulated in CVD-ILDs. Thus, the present study aimed to compare the presence of pulmonary DCs in lung biopsy specimens of patients with CVD-ILD with that in patients with IIP.

Methods

Patients

Lung tissue samples were obtained from 27 patients with IIP and 39 patients with CVD-ILD by surgical lung biopsy. The diagnosis of IIP and CVD-ILD was based on clinical, radiographic and pulmonary physiological features, and histological classification was performed according to the American Thoracic Society/European Respiratory Society consensus classification (1). Patients with histological diagnostic UIP or NSIP patterns were included. No patients were under systemic corticosteroid or immunosuppressive therapy at the time of surgical biopsy. The study protocol was approved by our institutional ethical review board. Each patient gave informed consent.

The characteristics of the patients are shown in Table 1. According to the pathological findings, 17 of the IIP patients were diagnosed with UIP (idiopathic UIP) and 10 patients with NSIP fibrosis patterns (idiopathic NSIP). Using the same criteria, 22 of the CVD-ILD patients were categorized as having UIP (CVD-UIP) and 17 as having fibrosing NSIP (CVD-NSIP). Idiopathic UIP patients were predominantly males ($p<0.05$), while patients with CVD-NSIP were significantly younger and smoked less than those in the other groups ($p<0.05$). The underlying diseases of CVD-ILD were shown in Table 1, all of which fulfilled the diagnostic criteria.

Immunohistochemistry

Lung tissue samples were immediately frozen and stored at –80°C. Acetone-fixed serial 5-μm-thick cryostat sections were stained using the Histofine SAB-PO and the AEC substrate kits (Nichirei, Tokyo, Japan). The following mouse monoclonal antibodies were used: CD1a, CD1c, CD83, CD207/Langerin and CD209/DC-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) from Immunotech (Marseille, France). A CD68 monoclonal antibody (Immunotech) was used to distinguish macrophages (5). Antibodies were diluted 1:100 in phosphate-buffered saline, except for CD1a, which was used undiluted. To confirm immunostaining specificity, antibodies were omitted or replaced by an isotype-matched control antibody. Images of tissue sections were recorded with a computerized image analyzer and positively stained cells with a characteristic dendritic morphology were counted by two independent observers (M.K. and N.E.) in 10 random microscopic fields.

Table 1. Patients characteristics according to ILDs pathological subgroup

	Idiopathic UIP	Idiopathic NSIP	CVD-UIP	CVD-NSIP
No.	17	10	22	17
Sex, male/female	16/1*	4/6	13/9	8/9
Age (range), years	61 (50-79)	65 (38-72)	66 (42-81)	55 (40-68)#
Smokers	13	6	10	6‡
Underlying CVDs				
Polymyositis/dermatomyositis			0	10
Rheumatoid arthritis			6	3
Sjögren's syndrome			5	1
Systemic sclerosis			2	2
Systemic lupus erythematosus			1	0
Mixed connective tissue disease			0	1
Undifferentiated connective tissue disease			8	0

Values are expressed as number or median unless otherwise indicated
* $p<0.05$ compared with idiopathic NSIP, CVD-UIP and CVD-NSIP
# $p<0.05$ compared with idiopathic UIP and CVD-UIP
‡ $p<0.05$ compared with idiopathic UIP
ILD: interstitial lung disease; UIP: usual interstitial pneumonia; NSIP: nonspecific interstitial pneumonia; and CVD: collagen vascular disease

The total area occupied by lung tissue (the airspace-subtracted area) was measured with ImageJ software (National Institutes of Health, Bethesda, MD, USA). The results for each field of view were expressed as the number of positive cells per square millimeter of measured lung tissue area.

Statistical analysis

All values were analyzed using JMP version 5.0.1J software (SAS Institute Japan, Tokyo, Japan). Pearson's chi-square test, Wilcoxon test and analysis of variance were used for the statistical analyses. Values of $p<0.05$ were considered to indicate significant differences. All data are expressed as the median (interquartile range).

Results

DCs were identified based on the characteristic dendritic morphology and classified according to their surface marker expression into immature DCs (CD1a-, CD1c-, Langerin- and DC-SIGN-positive cells) and mature DCs (CD83-positive cells). There were no significant differences in the expression of any of these markers between patients with IIP and CVD-ILDs (Figure 1). There was also no difference

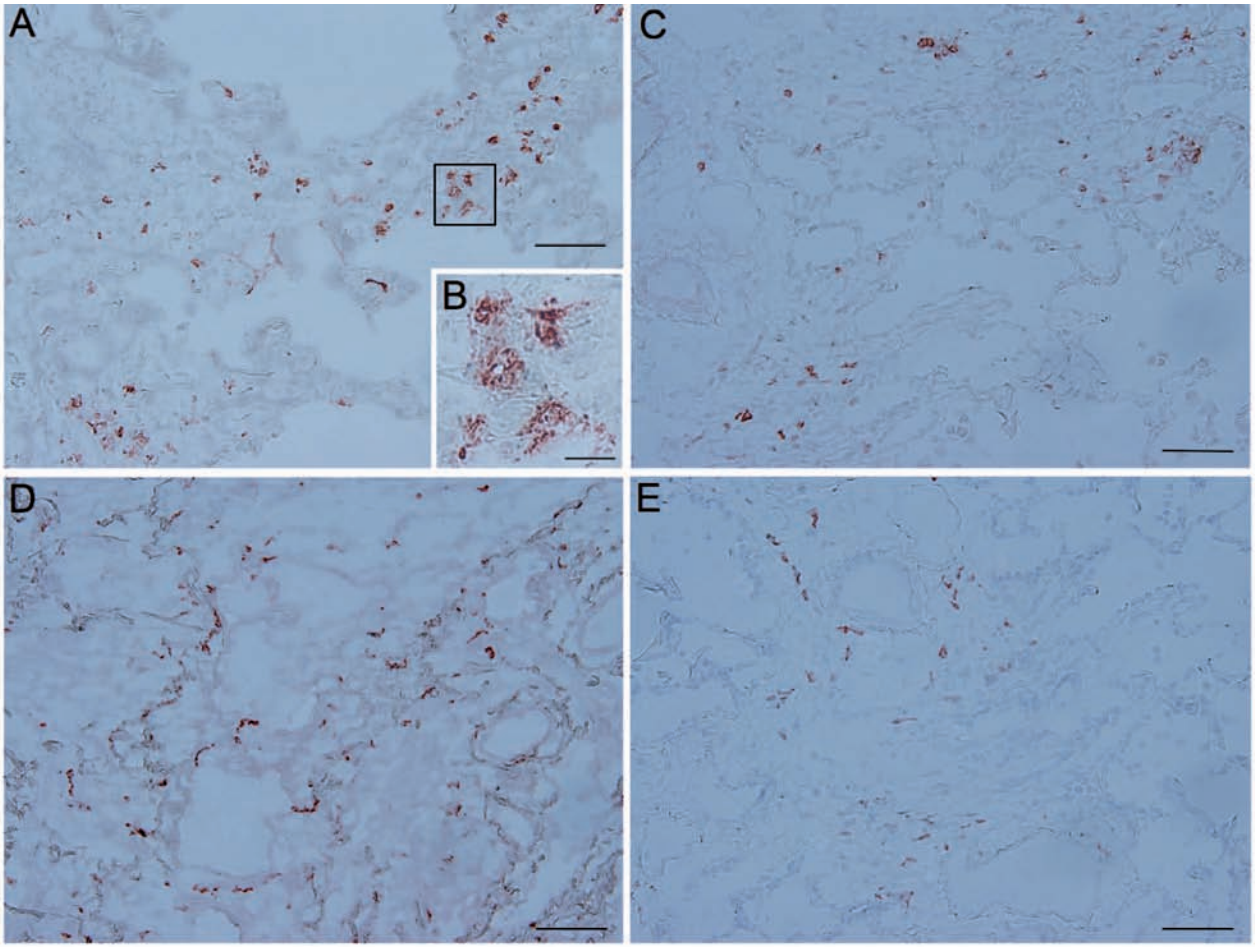

Fig. 1. Dendritic cell (DC) immunostaining in the lung tissues of patients with idiopathic interstitial pneumonia and collagen vascular disease (CVD)-associated interstitial lung disease (ILD). (A) DCs that express CD1c are distributed in areas of alveolitis and fibrosis in the lung tissue of a patient with idiopathic usual interstitial pneumonia (UIP). (B) DCs with CD1c positive staining in the framed region in (A) are shown at higher magnification. (C) DCs that express CD1c in the lung tissue of a patient with CVD-UIP. (D) DCs expressing DC-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) in the lung tissue of a patient with idiopathic UIP. (E) DCs expressing DC-SIGN in the lung tissue of a patient with CVD-UIP. The number of DC-SIGN-positive cells in idiopathic UIP was increased compared to that in CVD-UIP. Scale bar in (A, C, D, E) represents 100 μm, scale bar in (B) represents 20 μm

Table 2. Pulmonary DC marker expression according to ILDs pathological subgroup

	Idiopathic UIP	Idiopathic NSIP	CVD-UIP	CVD-NSIP
CD1a	14.8 (7.3-24.5)	13.4 (8.1-15.4)	13.4 (7.8-18.8)	11.4 (6.9-23.2)
CD1c	20.4 (7.9-35.4)	17.6 (12.3-31.2)	15.4 (6.2-23.6)	16.4 (10.2-23.2)
DC-SIGN	19.5 (11.2-52.0) §	20.4 (5.1-31.7)	12.0 (7.2-19.1)	15.5 (10.9-30.0)
Langerin	9.8 (1.9-17.9)	7.5 (4.2-16.0)	5.8 (4.0-16.0)	9.8 (4.1-16.1)
CD83	14.1 (6.8-26.3)	10.9 (7.0-27.3)	11.0 (7.2-17.6)	13.6 (7.6-22.7)

Values are expressed as median (interquartile range). §p =0.04 compared with CVD-UIP
DC: dendritic cell; ILD: interstitial lung disease; UIP: usual interstitial pneumonia; NSIP: nonspecific interstitial pneumonia; CVD: collagen vascular disease; and DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin

in the expression of DC markers among the underlying CVDs (data not shown). DCs were similarly distributed in IIP and CVD-ILD, with immature DCs mainly detected in areas of alveolar hyperplasia and fibrosis, and mature DCs in lymphoid follicles, while no DCs were observed in the area of fibroblastic foci. We investigated the expression of DCs according to the pathological subgroups in ILD and CVD-ILD (Table 2). The only significant difference in expression was observed in patients with idiopathic UIP, who expressed increased levels of DC-SIGN when compared with CVD-UIP patients (Figure 1, p<0.05).

Discussion

We initially investigated the number of DCs in the lungs in patients with CVD-ILD. The accumulation of both immature and mature DCs was detected in CVD-ILDs, with a similar quantity and distribution as in IIP. Pulmonary DCs in CVD-ILD may participate in maintaining chronic inflammation as is suggested in IIP (9). We also investigated DC surface marker expression in terms of the pathological patterns. Patients with idiopathic UIP and NSIP showed similar immature DC marker expression, which confirmed the previous report (9). Likewise, there was no difference in DC marker expression between patients with CVD-UIP and CVD-NSIP.

Meanwhile, the number of DC-SIGN-positive cells, which were always CD68-negative, in idiopathic UIP were significantly increased compared with CVD-UIP. DC-SIGN binds to the intercellular adhesion molecule (ICAM) and T cells, where it plays an important role in their activation and DC migration (13). Additionally, it is reported that DC-SIGN is present at the periphery of lung tumors and is involved in tolerance (14).

What are the pathogenic implications of DC-SIGN-expression in ILDs? ILD is characterized by persistent inflammation and an excessive repair response, and DCs are assumed to be involved, either by inducing immune responses or maintaining tolerance. Marchal-Sommé and co-workers showed that immature DCs expressing CD1a, CD1c and DC-SIGN infiltrate in alveolar hyperplastic and fibrotic areas, while mature DCs infiltrate within lymphoid follicles (9). They emphasized that DC-SIGN-positive DCs are specifically distributed in areas of fibrosis and around blood vessels, and are the most important population of DCs infiltrating the fibrotic lung. Additionally, they showed that DCs expressing DC-SIGN were present at the periphery of lymphoid follicles associated with fibrotic lesions and suggested that interaction of DC-SIGN with its ligand ICAM-2 helps DC trafficking. In the present study, the number of DC-SIGN-positive cells in CVD-UIP was decreased compared with idiopathic UIP and immature DCs, including DC-SIGN-positive cells, were mainly detected in areas of alveolar hyperplasia and fibrosis. Although accumulated DCs in ILDs had the potential to sustain chronic inflammation, the precise role of DCs according to their maturation state or distribution in the pathogenesis of fibrotic lung disease remains to be elucidated.

There is a notion that altered cytokine expression is involved in the process of pulmonary infiltration and fibrosis in ILDs. For example, interferon-suppresses fibroblast proliferation and collagen production. Conversely, type 2 cytokines such as interleukin-4 (IL-4) and IL-13 potentiate fibroproliferative responses and extracellular matrix deposition (15). DC-SIGN expression is regulated by numerous cytokines and growth factors; in particular IL-4

potentiates DC-SIGN expression and interferon-inhibits its expression (16). Although not all the cytokine profiles in the pathogenesis of ILDs have been elucidated, variations in cytokine levels regulate DC-SIGN expression, which may lead to a difference in the development and maintenance of lung inflammation between idiopathic UIP and CVD-UIP. Further studies, including the measurement of DC-attracting chemokines, are needed to define the precise role of pulmonary DCs in ILDs.

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