

Short communications

Title;

Defective Efferocytosis by Alveolar Macrophages in IPF patients

Key words: idiopathic pulmonary fibrosis, efferocytosis, BAL, idiopathic interstitial pneumonia

Konosuke Morimoto¹, William J. Janssen², Mayumi Terada¹

¹Department of Clinical Medicine, Institute of Tropical Medicine, Nagasaki University, Japan.

²Division of Pulmonary Medicine, Department of Medicine, National Jewish Health, Denver, Colorado, USA

Address Correspondence to :

Konosuke Morimoto, M.D.

Department of Clinical Medicine, Institute of Tropical Medicine, Nagasaki University
1-12-4, Sakamoto, Nagasaki, 852-8523, Japan.

Email: komorimo@nagasaki-u.ac.jp

Fax: +81-95-819-7843 Phone: +81-95-819-7842

Word count: 994

Abstract;

Rationale: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive fibrosing interstitial pneumonia. The pathogenicity of IPF has been widely investigated but still remains to be clarified. Efferocytosis, the specialized recognition and ingestion of apoptotic cells by phagocytes, is essential for the resolution of inflammation in the lungs and repair of injured tissues. Impaired efferocytosis contributes to the pathogenesis of chronic lung diseases such as emphysema and cystic fibrosis. We hypothesized that efferocytosis would also be reduced in alveolar macrophages isolated from subjects with IPF.

Methods: Efferocytosis, was evaluated using Wright-Giemsa stained cell preparations isolated from the bronchoalveolar lavage (BAL) fluid of patients with IPF (n=5), nonspecific interstitial pneumonitis (n=6), cryptogenic organizing pneumonia (n=4) and eosinophilic pneumonia (EP) (n=5).

Results: Uningested apoptotic cells were significantly higher in BAL fluid from patients with IPF compared to other forms of interstitial lung disease. Macrophages isolated from patients with eosinophilic pneumonia had significantly fewer phagocytic ingestions than macrophages from the other three groups.

Conclusion: Efferocytosis by alveolar macrophages was significantly lower in subjects with IPF compared to subjects with other interstitial pneumonia. Dysregulated efferocytosis may contribute to the pathogenesis of IPF.

Introduction

Efferocytosis, the specialized recognition and ingestion of apoptotic cells by phagocytes, has been shown to down-regulate inflammation by decreasing inflammatory mediator production via production of TGF- β 1, PGE2 and IL-10(1). At the same time, efferocytosis drives tissue repair by upregulating cellular production of growth factors such as hepatocyte growth factor (HGF) (2). Impaired efferocytosis has been documented in a number of chronic respiratory diseases (e.g. COPD, cystic fibrosis and asthma) and contributes directly to their pathogenesis by enhancing inflammation and impairing epithelial repair. (3, 4). Abnormal accumulation of inflammatory cells and dysregulated epithelial repair have been suggested to contribute to the development of fibrotic lung diseases. Accordingly, we sought to determine if efferocytosis was impaired in patients with idiopathic pulmonary fibrosis (IPF) and less-fibrotic idiopathic interstitial pneumonias (IIPs) such as nonspecific interstitial pneumonia (NSIP) and cryptogenic organizing pneumonia (COP) (5).

Methods

Efferocytosis, was evaluated using Dif-Quik™ stained cell preparations isolated from the bronchoalveolar lavage (BAL) fluid of patients with IPF (n=5), NSIP (n=6), COP (n=4) and eosinophilic pneumonia (EP) (n=5). We analyzed BAL from EP because it is rapidly reversible non-fibrotic lung disease. All BAL were performed at the time of diagnosis of IIP or EP. IPF was diagnosed according to ATS/ERS criteria(5) or UIP pattern by surgical biopsy. NSIP and COP were diagnosed by surgical biopsy or transbronchial lung biopsy. EP was diagnosed by

clinical findings including BAL and TBLB. We excluded cases in which collagen vascular diseases, infection or pneumoconiosis were present. Patient characteristics are available as online supplementary data. Macrophages containing apoptotic bodies and apoptotic cells with condensed nuclei (3) were identified as shown in Figure 1a. Efferocytosis was quantified using the following formulas:

$$\text{Phagocytic index (PI)} = \text{number of phagocytosed cells} / \text{number of AMs} \times 100$$

$$\text{Apoptotic cells(\%)} = \text{number of apoptotic cells} / (\text{total cells} - \text{number of AMs}) \times 100$$

$$\text{Defective index (DI)} = \text{apoptotic cells(\%)} / \text{PI}$$

Results

Phagocytic indexes were significantly lower in BAL obtained from patients with IIPs compared to EP (figure 1b). In addition, higher percentages of apoptotic cells were present in BAL from IPF patients (figure 1c) with corresponding elevation of the defective index (figure 1d), suggesting that clearance of apoptotic cells by AMs may be defective in IPF compared to other forms of IIP that have better clinical outcomes.

Discussion

Efferocytosis of dying cells is critical for the resolution of inflammation and repair of injured tissues. Efficient removal of apoptotic cells prevents the release of potentially toxic intracellular contents from the dying cells and enhances the production of growth factors and cytokines by the phagocyte, including TGF- β and HGF. The anti-inflammatory effects of efferocytosis (i.e.

through TGF- β production) may be considered of paramount importance in resolving inflammation in lung diseases where leukocyte infiltration plays a role, such as EP, COP and NSIP. On the other hand, sustained production of TGF- β may lead to fibrosis. In comparison, HGF enhances epithelial cell repair and may counteract the pro-fibrotic effects of TGF- β . Thus in non-inflammatory injury processes (such as IPF) the production of HGF may be of greater importance in driving epithelial repair. In other words, the normal resolution of inflammation and the initiation of lung repair almost certainly require a delicate balance between the production of anti-inflammatory factors (TGF- β) and molecules that stimulate repair (HGF). In this context, both the nature of the underlying disease and the timing of apoptotic cell clearance may be critical, such that impaired efferocytosis during early lung injury may delay the resolution of inflammation whereas during later phases, failure to clear apoptotic cells may result in disordered repair.

Alveolar macrophages are the most abundant leukocytes in the airspaces and are the major cell responsible for efferocytosis. Their programming (or activation) is highly dependent on the environment in which they reside. Accordingly, macrophages that exist in a Th1 environment are generally pro-inflammatory and “classically activated.” Conversely, a Th2 environment promotes the development of “alternatively activated” macrophages that produce molecules implicated in mesenchymal repair and fibrosis. It is therefore not surprising that AMs taken from patients with IPF exhibit features of alternative activation (6). As such, it has been suggested that they may contribute to fibrosis by producing TGF- β and CCL18 (6). Indeed,

intentional depletion of alveolar macrophages in animal models of IPF abrogates the development of fibrosis (7). Similar results have been achieved by inhibiting alternative macrophage activation (8). Interestingly, debris from dead or dying cells that have not been effectively cleared may promote alternative activation of macrophages (9). This raises the intriguing possibility that the presence of apoptotic cells drives alternative activation of macrophages, which in turn enhances fibroproliferation.

Nuclear condensation, cytoplasmic membrane blebbing, and reduced cell volume are morphologic hallmarks of apoptosis. Unfortunately, these changes make it difficult to distinguish the source (or origin) of apoptotic bodies. Therefore we cannot be sure whether the apoptotic cells identified in our study are effete leukocytes or sloughed epithelial cells. Since neutrophils and macrophages isolated from BAL of patients with IPF are resistant to apoptosis (10, 11) we favor the latter, especially since lung tissue from patients with IPF contains high numbers of apoptotic epithelial cells (12).

Increased apoptotic cell numbers may result from accelerated cell death or defective apoptotic cell removal. Since under most circumstances, clearance of apoptotic cells from injured tissues is incredibly efficient, we suggest that our results reflect the latter. For example, during bacterial pneumonia there are over 10^6 neutrophils per mm^3 of lung tissue, yet clearance of dying neutrophils is so efficient that less than 0.3% are apoptotic(13). On the other hand, impaired clearance of apoptotic cells has been suggested as a mechanism to explain the high levels of

apoptotic cells noted in the parenchyma and airspaces in patients with COPD(4). While our study suggests that efferocytosis may be impaired in IPF, prospective research using primary cells taken from patients with IIPs is required to clarify this finding and determine the mechanisms that underlie this defect.

Acknowledgement: We thank Dr. Masahiko Mori at Nagasaki University for data analysis.

References

1. Lauber K, Blumenthal S, Waibel M, Wesselborg S. Clearance of apoptotic cells: getting rid of the corpses. *Mol Cell*. 2004;14:277-87.
2. Morimoto K, Amano H, Sonoda F, Baba M, Senba M, Yoshimine H, et al. Alveolar macrophages that phagocytose apoptotic neutrophils produce hepatocyte growth factor during bacterial pneumonia in mice. *American Journal of Respiratory Cell and Molecular Biology*. 2001;24:608-15.
3. Vandivier RW, Fadok VA, Hoffmann PR, Bratton DL, Penvari C, Brown KK, et al. Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. *J Clin Invest*. 2002;109:661-70.
4. Vandivier RW, Henson PM, Douglas IS. Burying the dead: the impact of failed apoptotic cell removal (efferocytosis) on chronic inflammatory lung disease. *Chest*. 2006;129:1673-82.
5. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med*. 2002;165:277-304.
6. Prasse A. A Vicious Circle of Alveolar Macrophages and Fibroblasts Perpetuates Pulmonary Fibrosis via CCL18. *American Journal of Respiratory and Critical Care Medicine*. 2006;173:781-92.
7. Murray LA, Argentieri RL, Farrell FX, Bracht M, Sheng H, Whitaker B, et al. Hyper-responsiveness of IPF/UIP fibroblasts: interplay between TGFbeta1, IL-13 and CCL2. *International Journal of Biochemistry and Cell Biology*. 2008;40:2174-82.
8. Murray LA, Rosada R, Moreira AP, Joshi A, Kramer MS, Hesson DP, et al. Serum amyloid P therapeutically attenuates murine bleomycin-induced pulmonary fibrosis via its effects on macrophages. *PLoS ONE*. 2010;5:e9683.
9. Mares CA, Sharma J, Li Q, Rangel EL, Morris EG, Enriquez MI, et al. Defect in efferocytosis leads to alternative activation of macrophages in *Francisella* infections. *Immunology and Cell Biology*. 2010;89:167-72.
10. Mermigkis CM, Tsakanika K, Polychronopoulos V, Karagianidis N, Mermigkis D, Bouros D. Expression of bcl-2 protein in bronchoalveolar lavage cell populations from patients

with idiopathic pulmonary fibrosis. *Acta cytologica*. 2001;45:914-8.

11. Drakopanagiotakis F, Xifteri A, Tsiambas E, Karameris A, Tsakanika K, Karagiannidis N, et al. Decreased Apoptotic Rate of Alveolar Macrophages of Patients with Idiopathic Pulmonary Fibrosis. *Pulmonary medicine*. 2012;2012:981730.

12. Uhal BD. The role of apoptosis in pulmonary fibrosis. *European Respiratory Review*. 2008;17:138-44.

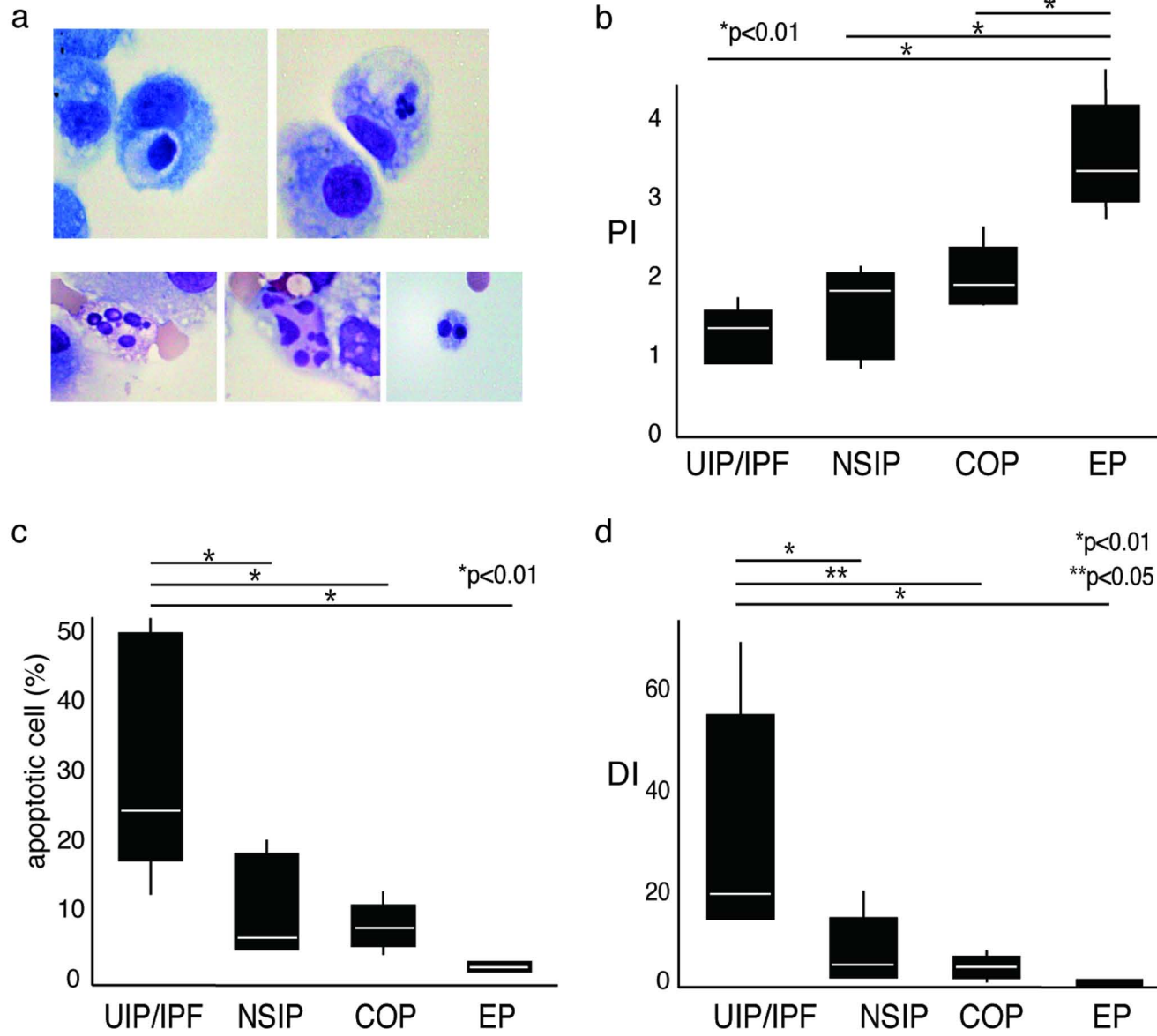
13. Droemann D, Aries SP, Hansen F, Moellers M, Braun J, Katus HA, et al. Decreased apoptosis and increased activation of alveolar neutrophils in bacterial pneumonia. *Chest*. 2000;117:1679-84.

Figure Legends

Figure 1

a); Representative findings of Dif-Quik™ stained cell preparations isolated from the bronchoalveolar lavage (BAL) fluid. b~d); Phagocytic indexes (PI) (b), percentages of apoptotic cells (c) and defective index (DI) (d) in BAL cells from patients with IIPs and EP. Statistical analysis was performed by two-way ANOVA, followed by Student Newman Keuls test for multiple comparisons.

figure 1



Supplementary Table

Patients characteristic

	IPF	NSIP	COP	EP
N	5	6	4	5
Male/Female	5 / 0	3 / 3	3 / 1	3 / 2
age (mean±SD)	62.8±9.7	59.3±5.1	68.75±2.6	60.2±22.1
smoking history	5	1	3	3
BAL total cells [#]	2.6±1.6	4.9±2.3	4.4±1.4	5.0±4.4
BAL macrophages [#]	2.2±1.2	2.8±1.4	2.5±0.5	1.2±0.8
BAL neutrophils [#]	0.07±0.08	0.27±0.43	0.27±0.07	0.14±0.27
BAL lymphocytes [#]	0.2±±0.2	1.8±1.9	1.6±1.0	0.52±0.55
BAL Eosinophils [#]	0.06±0.09	0.050.07	0.091.12	3.0±5.0*
serumKL-6(U/ml)	1175±551.3	1497.8±822.3	726±309.1**	269.3±132.2**

[#]Number of cells in Bronchial alveolar lavage (BAL) fluid (x10⁵/ml); * significantly higher than IPF, NSIP and COP; ** significantly lower than NSIP

Definition of abbreviations: IPF = Idiopathic pulmonary fibrosis; NSIP = nonspecific interstitial pneumonia; COP = cryptogenic organizing pneumonia; EP = eosinophilic pneumonia