後天性肺泡蛋白沉著症病人GM-CSF與GM-CSF受體基因之表現

Expression of GM-CSF and GM-CSF Receptor Gene in Patients with Acquired Pulmonary Alveolar Proteinosis

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一、中文摘要

肺泡蛋白沉著症是一種罕見的疾病，其特徵是病人肺泡內充滿表面張力素和磷脂，引起呼吸困難，甚至衰竭。此症之致病機轉和病因均不明。1994年，Stanley等人報告缺乏GM-CSF基因的小白鼠，其肺部會表現出肺泡蛋白沉著症之變化，此後GM-CSF與肺表面張力素清除之間的關係漸漸引起注意。亦即當GM-CSF對肺泡巨噬之細胞之調控異常時，可能最終會造成肺泡蛋白沉著症。此調控異常可能發生在GM-CSF基因，或GM-CSF之受體基因。

本計劃係以RT-PCR研究肺泡蛋白沉著症病人其肺泡灌洗細胞與周邊血液單核細胞之GM-CSF基因與GM-CSF受體基因之mRNA之表現。結果顯示，在臨床上較嚴重之2例肺泡蛋白沉著症病人，其中1人之肺泡灌洗液細胞與周邊血液單核細胞均無GM-CSF基因之表現，另一人之周邊血液單核細胞無GM-CSF基因表現，而肺泡灌洗液細胞則有正常之表現。至於GM-CSF受體基因之表現，在此兩例病人，其中一人的肺泡灌洗液細胞完全沒有α或β受體之表現，另一人之肺泡灌洗液細胞則缺乏α受體之表現，β受體則正常。至於周邊血液單核細胞之GM-CSF受體基因之表現則二人均正常。至於臨床上較輕微之2例，其周邊血液之GM-CSF基因與GM-CSF受體基因之表現均正常。結論：在臨床上較嚴重之PAP病人，GM-CSF基因與GM-CSF受體基因之異常表現可能與致病機轉有關。

關鍵詞：肺泡蛋白沉著症，GM-CSF基因，GM-CSF受體基因，肺泡灌洗。

Abstract

Pulmonary alveolar proteinosis (PAP) is a rare disease characterized by excessive accumulation of surfactant proteins and phospholipids in the alveolar space, leading to gas exchange insufficiency and even respiratory failure. The etiology of PAP is unknown. Recently, experiments on gene-targeted mice and human patients with PAP suggest that granulocyte-macrophage colony-stimulating factor (GM-CSF) gene or GM-CSF receptor genes may play a role in the development of PAP. In order to test this hypothesis, we studied the mRNA expression of the GM-CSF and GM-CSF receptor genes in the bronchoalveolar lavage (BAL) cells and peripheral blood monocytic cells (PBMCs) from patients with PAP and control subjects by RT-PCR. We found that of the two patients with clinically severe PAP whose BAL cells were studied, one of them showed lack of GM-CSF gene expression in BAL cells, and both of them showed no expression of GM-CSF gene in their PBMCs. As for the mRNA expression of GM-CSF receptor genes, we found that of the two patients with clinically severe PAP, one showed lack of expression of both the α- and β-subunit of GM-CSF receptor genes in BAL cells; the other showed lack of expression of the α-subunit GM-CSF receptor gene. But the expressions of GM-CSF receptor genes in their PBMCs were normal. The two patients with milder clinical disease and the three control subjects all showed normal mRNA expression of GM-CSF and GM-CSF receptor genes in PBMCs. In conclusion, our study suggested that at least in PAP patients with severe clinical disease abnormal expression of the GM-CSF and GM-CSF receptor genes may contribute to the development of PAP.

Keywords: *Pulmonary alveolar proteinosis, GM-CSF, GM-CSF receptor, bronchoalveolar lavage*

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare disease in which the pulmonary alveoli are filled with phospholipid-rich proteinaceous material, a substance biochemically nearly identical to surfactant (1,2). Most adults with PAP have an idiopathic form of the disease (3,4). Although the pathogenesis of idiopathic PAP is

unknown, recent observations on mice deficient in the gene for granulocyte-macrophage colony stimulating factor (GM-CSF) found that such mice develop an alveolar process histologically identical to PAP (5,6). The murine disease can be reversed by insertion of the GM-CSF gene into respiratory epithelium (7). Mice deficient in the β-subunit of the GM-CSF receptor also develop an alveolar-proteinosis-like disease (8). These observations have led to speculation that either absolute GM-CSF deficiency or poor response of alveolar cells to GM-CSF is etiologic to the human PAP (5).

GM-CSF is a small glycoprotein produced by monocytes/macrophages, endothelial cells and epithelial cells (9,10). Binding of the macrophage receptor by GM-CSF results in enhanced production of immune mediators, including tumor necrosis factor(TNF)-α (11). We studied the expression of GM-CSF, α- and β-subunit of the GM-CSF receptor in bronchoalveolar lavage (BAL) cells and peripheral blood monocytic cells (PBMCs) of patients with PAP and PPBMCs of normal adults, to test the hypothesis that these patients have a defect in GM-CSF or its receptor gene.

MATERIALS AND METHODS

Four patients with open-lung-biopsy-proved pulmonary alveolar proteinosis and three control subjects were included in the study. Two of the patients underwent therapeutic whole-lung lavage and their BAL fluids were included in the study. The other two patients underwent bronchofiberscopic lavage; their BAL fluids were not studied. Whole-lung lavage was performed under general anesthesia, using a double-lumen endotracheal tube. While the left lung was ventilated with 100% O2, the right lung was filled with warm 0.9% saline at 37°C. After gravity drainage of 500 ml of lavage fluid, serial exchange of 500 ml each were made, with vigorous percussion on chest wall. This was continued until the returned fluid was clear, which usually occurred after drainage of 13 liters of fluid. Samples of BAL fluid for the study were obtained from the first 1.0 l of fluid, and 15 ml of blood was also collected.

Bronchofiberscopic lavage was done for two patients with mild disease. It was performed under local anesthesia. For each cycle of lavage about 2 to 3 liters of warm saline were instilled and about 50% were suctioned out.

Lavage fluid from PAP patients were centrifuged on the day of collection at 1000x g to sediment the cells. Patients' and controls' PBMCs were separated on the day of collection by Ficoll-Hypaque density gradient and washed twice with PBS. From the cell pellets of BAL fluid and Ficoll-Hypaque-separated PBMCs, total cellular RNA was isolated by guanidinium isothiocyanate method, using Trisol LS reagent (Life Technologies, Bethesda, MD, USA). Five micrograms of RNA were reverse transcribed with 70 pmol oligodT primer and 200 U superscript II (Life Technologies) in a 40-ul volume for 1 hr at 42°C. Two microliters of cDNA was then amplified with primers.

GM-CSF sense primer (12):

5'-ATG TGG CTG CAG AGC CTG CTG C-3'

GM-CSF antisense primer (12):

5'-TCC AGC CTC ATC GGC CGG T-3'

The PCR protocol consisted of 40 cycles of 94°C for 30s, 58°C for 60s, and 72°C for 60

s.

GM-CSF receptor α-subunit (13):

5'-AGC CCG AGC AAA ACA CA (position 1009-1026)

3'- CCA TGC CAT TCC TAC ACC CT (position 1360-1379)

GM-CSF receptor β-subunit (13):

5'-CTA CAA GCC CAG GCC AGA TGC (position 859-879)

3'-ACC CGT AGA TGC CAC AGA AGC (position 1390-1410)

The PCR conditions were 35 cycles of 94°C for 1 min and 65°C for 2 min.

Results

The demographic data for the PAP patients are shown in Table 1, and were similar to those reported previously (3,4). The median age of the patients were 48 years. There were three male and one female patient, a ratio similar to the reported male-to-female ratio of 4:1 (1,3). Two of our patients were cement workers, and the female patient had had close contact with wooden furniture for 5 years. The control subjects were two healthy females (age 49 and 25 years, respectively) and one healthy male (age 19). All the controls were nonsmokers.

mRNA expression of GM-CSF and that of the α- and β-subunit of GM-CSF receptors are shown in Table 2 and 3. Of the two PAP patients whose BAL cells were studied, one (FT) showed no expression of GM-CSF mRNA in BAL cells and PBMCs. The mRNA expression of the α- and β-subunit of GM-CSF receptors in his BAL cells was also absent. The other patient (CY) showed normal expression of GM-CSF mRNA in BAL cells, but not PBMCs. The expression of the α-subunit of GM-CSF receptor in BAL cells was also absent, but that of the β-subunit was normal.

The other two patients (FL and NJ) and the three control subjects only had PBMCs studied. The mRNA expression of GM-CSF gene, and that of the α- and β-subunit of the GM-CSF receptor genes of these two patients and the controls were all normal.

Table 1 Clinical Data from Patients with PAP

Pt.No.	Age	Sex	Smoking history	Occupation	No. of lavage
1(CY)	54	M	30 p-y	taxi driver	4
2 (FT)	68	M	50 p-y	cement worker	2
3 (FL)	36	M	20 p-y	cement worker	2
4 (NJ)	42	F	none	furniture store owner	4

Table 2 mRNA Expression of GM-CSF Gene in BAL Cells and PBMCs from Patients with PAP and Control Subjects

P't No.	mRNA expression of GM-CSF gene	
	BAL cells	PBMCs
1(CY)	+	-
2(FT)	-	-
3(FL)	ND	+
4(NJ)	ND	+
Control		
1(LL)	ND	+
2(SC)	ND	+
3(SJ)	ND	+

Table 3 mRNA Expression of GM-CSF Receptor Gene in BAL Cells and PBMCs from PAP Patients and Control Subjects

Patient No.	Expression of GM-CSF receptor			
	BAL cells		PBMCs	
	α	β	α	β
1(CY)	-	+	+	+
2(FT)	-	-	+	+
3(FL)	ND	ND	+	+
4(NJ)	ND	ND	+	+
Control				
1(LL)	ND	ND	+	+

2(SC)	ND	ND	+	+
3(SJ)	ND	ND	+	+

DISCUSSION

In our four patients with active PAP, at least two of them showed abnormal expression of GM-CSF gene. They were also the clinically more severe cases, requiring 13 liters of saline during each cycle of whole lung lavage to remove proteinaceous material from each lung. One of them showed no expression of the GM-CSF gene either in BAL cells or PBMCs. The other showed normal GM-CSF expression in BAL cells but no expression in PBMCs. The other two patients with clinically mild disease who needed only 2 to 3 liters of saline during each cycle of bronchofiberscopic lavage to remove proteinaceous substance showed normal expression of GM-CSF gene in their PBMCs. The results suggested that at least more severe cases of PAP were associated with defective expression of the GM-CSF gene.

As for the mRNA expression of the GM-CSF receptor gene, one patient of severe PAP (FT) showed no expression of the α- and β-subunit in his BAL cells. Patient CY showed no expression of the α-subunit but normal expression of the β-subunit in his BAL cells. The mRNA expression of the α- and that of the β-subunit in PBMCs of every PAP patient were normal. Thus at least more severe cases of PAP were also associated with lack of expression of either α- or β-subunit of the GM-CSF receptor gene, or both of them.

Our study suggested that the expression of the GM-CSF gene and that of the GM-CSF receptor α/β subunit in BAL cells could all be absent in patients with severe PAP. Moreover, the expression of GM-CSF gene in PBMCs in such patients could also be absent, suggesting that the lack of expression of GM-CSF gene was not due to alveolar macrophage defect secondary to the presence of excessive surfactant, but a more generalized gene defect involving alveolar macrophages as well as PBMCs.

CONCLUSION

Patients with PAP may show lack of expression of GM-CSF gene in BAL cells or PBMCs, or both of them. They may also show lack of expression of the α-subunit, or α- and β-subunit, of the GM-CSF receptor gene in BAL cells. The findings suggest that abnormal expression of the GM-CSF and its receptor genes may be involved in the development of PAP.

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