Clinical significance of anti-GM-CSF antibodies in idiopathic pulmonary alveolar proteinosis

Fang-Chi Lin, Geen-Dong Chang, Ming-Sheng Chern, Yi-Chu Chen, Shi-Chuan Chang

Online only and supplemental materials

Detection of anti-granulocyte-macrophage colony-stimulating factor antibodies (anti-GM-CSF antibodies) in blood and bronchoalveolar lavage fluid (BALF) by in-house enzyme-linked immunosorbent assay (ELISA)

In-house ELISA kit was performed as described by Schoch *et al* with some modification.[1] Using Vivapure Protein A Mini Spin Columns (Vivascience AG, a member of Sartorius AG, Germany), antibodies serving as the standard were purified from a patient with high concentration of anti-GM-CSF antibodies in the blood. Fifty μL standard solution in serial dilutions and blood from 30 healthy medical personnel serving as normal controls were transferred to the plate coated with 2♣μg/mL of human recombinant GM-CSF (rh-GM-CSF, Calbiochem, Affiliate of Merck KGaA, Darmstadt, Germany) and blocked with 1% bovine serum albumin (BSA)/phosphate buffered saline (PBS) containing 0.05% Tween 20 and 1% polyvinyl-pyrolidone-25 (PTP) for 90♣minutes at room temperature. After three washes with PBS containing 0.1% Tween 20 (PBST), 50♣μL of 10♣mM ammonium acetate buffer (pH♣5.0) was transferred into each well and kept 15♣minutes at room temperature. The antibodies

captured by rhGM-CSF were detected by peroxidase labeled anti-human IgG antibodies (Jackson Immuno Research Lab Inc, Pa, USA). After washing, color was developed using tetramethylbenzidine and absorbance was measured at 450♣nm. The dilution at which the absorbance value is equal to the mean absorbance of the normal controls is given as one ELISA unit arbitrarily.[2] A standard curve was generated by plotting the optical density (OD) reading versus the ELISA units of various dilutions of the standard solution. Anti-GM-CSF antibodies were measured in paired specimens of blood and BALF samples obtained from 13 idiopathic pulmonary alveolar proteinosis (iPAP) patients, 3 patients with secondary PAP, 35 patients with other pulmonary diseases (including collagen vascular diseases in 12, cytomegalovirus pneumonitis in 12, idiopathic pulmonary fibrosis in 6, and sarcoidosis in 5 patients), and 10 patients without pulmonary lesions (lung controls).

Results of anti-GM-CSF antibodies in blood and BALF measured by in-house ELISA

Significantly higher blood and BALF levels of anti-GM-CSF antibodies were found in 12 of 13 iPAP patients, as compared with various control groups. Lowest level of anti-GM-CSF antibodies was found in the iPAP patient who had undetectable blood and BALF anti-GM-CSF antibodies measured by immunoblotting. The blood levels of anti-GM-CSF antibodies determined by ELISA were comparable among 3 patients with secondary PAP, 35 patients with other pulmonary diseases, 10 lung controls and 30 normal controls (fig S1A). There was no

significant difference in the BALF levels of anti-GM-CSF antibodies among patients with secondary PAP, patients with other pulmonary diseases and lung controls (fig S1B).

In iPAP patients, BALF levels of anti-GM-CSF antibodies were highly correlated with the values of $AaPO_2$ (r=0.58, p=0.037). Nevertheless, the blood anti-GM-CSF antibodies measured by ELISA did not significantly correlate with the severity indicators evaluated.

Figure legend

Figure S1 Box and whisker plots for the levels of anti-GM-CSF antibodies measured by ELISA in blood (A) and BALF (B) are given. The box represents the interquartile range that contains 50% of values. The whiskers are lines that extent from the box to the highest and lowest values. Anti-GM-CSF antibodies: anti-granulocyte-macrophage colony-stimulating factor antibodies; BALF: bronchoalveolar lavage fluid; iPAP: idiopathic pulmonary alveolar proteinosis; IPF: idiopathic pulmonary fibrosis; CVD: collagen vascular disease; CMV: cytomegalovirus pneumonitis; NC: normal controls.

References

1. Schoch OD, Schanz U, Koller M, *et al*. BAL findings in a patient with pulmonary alveolar proteinosis successfully treated with GM-CSF. *Thorax* 2002;**57**:277–80.

2. Carpenter AB. Enzyme-linked immunoassays. In: Rose NR, de Macario EC, Folds JD, eds. *Manual of clinical laboratory immunology*. Washington, DC: American Society for Microbiology, 1997:20–9.