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

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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 2µg/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), 50µL of 10mM 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.


**References**
