Development of Binding and Functional Assays for the Detection of Anti-GM-CSF Autoantibodies

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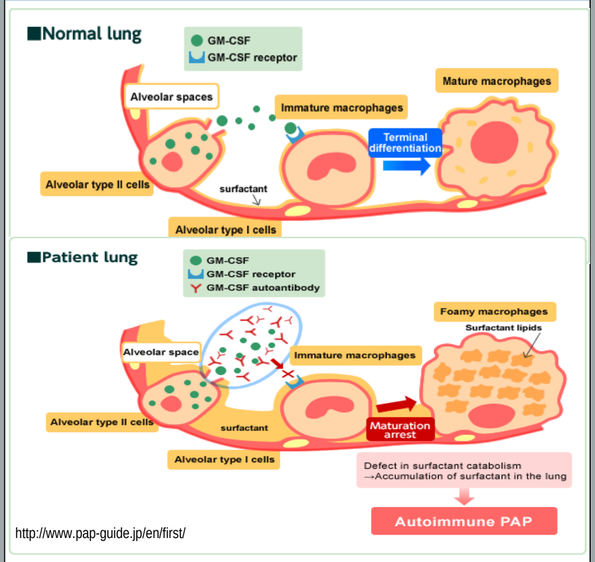
Background

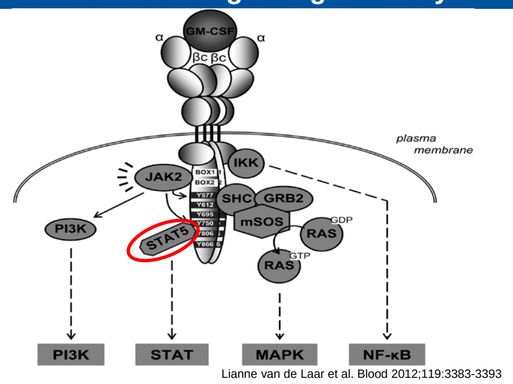
Background: Anti-cytokine autoantibodies are increasingly being recognized as causes of immune deficiency or dysregulation. Autoantibodies to Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) have been noted in patients with pulmonary alveolar proteinosis (PAP) and contribute to compromised alveolar macrophage development, thereby leading to pathological accumulation of pulmonary surfactant. Anti-GM-CSF autoantibodies have also been identified in patients with chronic, treatment refractory infections such as cryptococcal meningitis. Given the importance of GM-CSF in macrophage development and function, these autoantibodies may play a pathological role in the setting of chronic infection. Identification of these antibodies is of importance since it may direct therapeutic intervention.

Role of GM-CSF in the Lung



GM-CSF is responsible for alveolar macrophage development, thus helping to maintain healthy lungs. The presence of GM-CSF autoantibodies can result in PAP.

The GM-CSF Signaling Pathway



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GM-CSF signaling pathway: in our assay, we measured the phosphorylation of STAT5 as our functional readout.

Objectives

Objective: To develop validated binding and neutralization assays for the detection of anti-GM-CSF autoantibodies for diagnostic use.

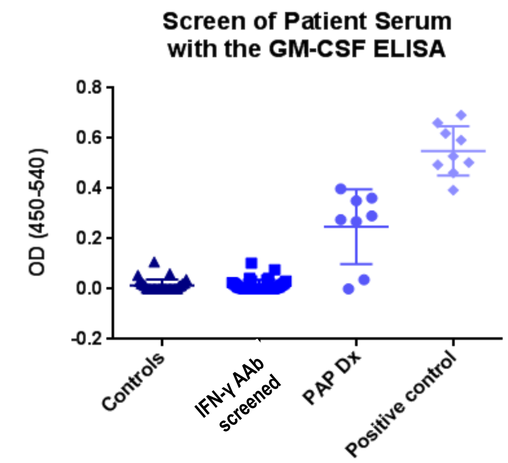
Materials and Methods

Methods: An ELISA was developed for the detection of anti-GM-CSF antibodies. ELISA optimization was performed utilizing a commercially-available rabbit anti-human-GM-CSF polyclonal antibody. The performance of the optimized ELISA was then evaluated using patient serum known to be positive for anti-GM-CSF autoantibodies by the National Institutes of Heath (NIH). Functionality of the anti-GM-CSF antibodies was assessed by Phosphoflow, making use of the GM-CSF signaling pathway. In brief, peripheral blood mononuclear cells were stimulated with GM-CSF to induce STAT5 phosphorylation in monocytes that was detected by intracellular staining followed by flow cytometry. The ability of known positive patient serum to abrogate phosphorylation of STAT5 was assessed. Serum from healthy controls, patients with known chronic intracellular infections and patients with a diagnosis of PAP (obtained from NJH biorepository: protocol #HB0028) were then tested with the optimized ELISA and the Phosphoflow assay to determine clinically useful cut-off parameters.

Results

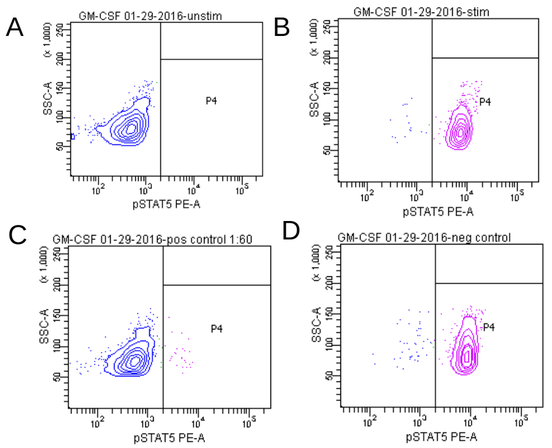
Results: Both the ELISA and the functional flow cytometry assay successfully demonstrated the presence of anti-GM-CSF autoantibodies in a known positive serum sample. We also identified anti-GM-CSF autoantibodies in 6 of 8 PAP patient samples. We had 100% correlation between the ELISA and functional assay.

Detection of GM-CSF Autoantibodies by ELISA



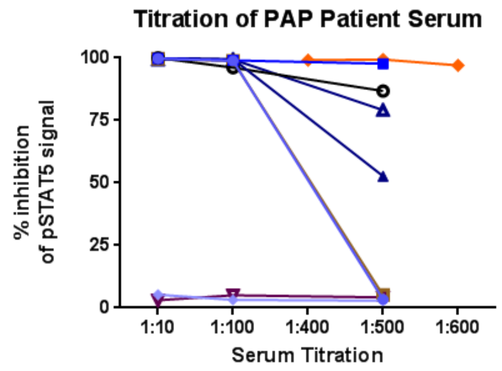
Anti-GM-CSF autoantibodies were detected by ELISA in the serum of PAP patients and positive control serum from the NIH. These antibodies were not detected in healthy control serum or patient serum previously screened for the presence of IFN-γ autoantibodies. All serum samples were run at a 1:100 dilution.

Functional Inhibition of GM-CSF



Measurement of pSTAT5 by flow cytometry in CD14+ monocytes. A) cells alone, B) cells stimulated with GM-CSF, C) cells stimulated with GM-CSF in the presence of positive control serum, and D) cells stimulated with GM-CSF in the presence of negative control serum.

Functional Analysis of Serum Samples from PAP Patients



Eight PAP patient serum samples were assayed at different concentrations (dark colored curves) and exhibited varying titers of inhibition. The positive control serum sample (orange curve) exhibited complete inhibition at all concentrations tested.

Conclusions

Conclusions: There are currently few well-validated assays for the assessment of anti-GM-CSF autoantibodies for diagnostic use. Here, we describe the development of assays for both binding and function and their ability to detect anti-GM-CSF autoantibodies in human serum samples. We expect the assays to have utility in the evaluation, diagnosis and management of patients with PAP or chronic, intracellular infections.

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