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.

The GM-CSF Signaling Pathway

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 Health (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 NIH biorepository; protocol #HB0028) were then tested with the optimized ELISA and the Phosphoflow assay to determine clinically useful cut-off parameters.

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

Functional Inhibition of GM-CSF

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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