Detection and Functional Assay of Anti-IFN-γ Autoantibody in Patients with Adult-Onset Immunodeficiency

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ABSTRACT

Interferon (IFN)-γ confers crucial immune surveillance positively for immunomodulation (such as macrophage activation, antigen presentation, and T cell differentiation), antimicrobial (such as antiviral replication, microbial killing, and Major Histocompatibility Complex (MHC) induction), and anticancer activity (such as growth inhibition, cytotoxicity, and immune priming). Patients with adult-onset immunodeficiency, negative in Human Immunodeficiency Virus (HIV) infection, show defects in IFN-γ signaling and immune surveillance against mycobacterial infection. In addition to genetic defects, the presence of neutralizing anti-IFN-γ autoantibody (autoAb) is speculated. In the first part, detection of anti-IFN-γ autoAb and characterization of its neutralizing activity were carried out. First, Enzyme-linked Immunosorbent Assay (ELISA)-based colorimetric assays and immunoblotting was utilized for detecting autoAbs. Antibody-antigen reactivity and epitope clarification showed different patterns among these patients. Results showed the blockade of IFN-γ-activated Signal Transducer and activator of transcription (STAT)-1 activation and Interferon Regulatory Factor (IRF1) transactivation by patient serum containing autoAbs. Furthermore, IFN-γ-regulated inflammation, chemokine production, and cytokine production after T cell activation were also blocked. These results provide potential methods for detecting anti-IFN-γ autoAb and for characterizing the blockade effects of autoAbs on IFN-γ signaling and bioactivity. For the second part, the blockade effect of that antibody on IFN-γ-regulated antimicrobial activity will be detected. We will perform a model of monocyte-derived type 1 macrophage by using Phorbol-12-myristate-13-acetate (PMA) and IFN-γ induction. Furthermore, blockade effect of type 1 macrophage differentiation and bacterial phagocytosis/killing by anti-IFN-γ autoAbs will be performed by detection of inhibition on cell marker expression, attachment/engulfment phase, and Reactive Oxygen Species (ROS)/ Nitric oxide (NO) production. Those results will provide evidence of blockade effects of IFN-γ against antimicrobial activity by anti-IFN-γ autoAbs.

Keywords: Adult-onset immunodeficiency, Anti-IFN-γ autoAbs, Signaling, Mycobacteria, Macrophage, Phagocytosis