AAI Congress 2018: Detection and Functional Assay of Anti-IFN-γ Autoantibody in Patients with Adult-Onset Immunodeficiency - DyahIkaKrisnawati

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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-γregulated antimicrobial activity will be detected. We will perform a model of monocytederived 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 IFNγ against antimicrobial activity

by anti-IFN-γ autoAbs.Form II interferon (IFN)-ÿ is developed by T cells or natural killer cells as a response to antigen and/or cytokine stimulation. IFN-ÿ facilitates immune monitoring and has immunomodulatory effects:(1) activation of MHC-associated antigen presentation pathways; (2) production of Th1 responses; (3) anti-microbial activity; (4) anti-cancer stimulation; (5) leukocyte traffic control; and (6) facilitation of inflammation;In the case of anti-bacterial effects, IFN-π acts as a macrophage activator and promotes innate defense through receptor-mediated phagocytosis, enhancing microbicidal effects1, in general, through transcriptional induction of gp91phox and p67phox priming for the production of nitric oxide, tryptophan depletion and up-regulation of lysosomal enzymes promoting microbe destruction. Immunity-regulated GTPase (IRG) proteins, recognized as p47 GTPases, are the main mediators of IFN-ÿ immune surveillance against pathogen and mycobacterial intracellular infection. IFN-π increases the expression of IRG proteins to regulate the processing of pathogen-containing vacuoles, particularly in autophagy, which stimulates host cell immune defenses. Host cells are vulnerable to mycobacterial infection without IFN-ÿ and/or IRG proteins.Adult-onset immunodeficiency is currently reported in patients with IFN-π signaling deficiencies that are commonly caused by the generation of anti-IFN-ÿ autoantibodies (autoAbs) and partly due to inherited mutations in the IFN-π signaling-associated factors; the syndrome first appeared in Thailand and Taiwan in 2004. In the Studies belowTuberculosis, non-tuberculous mycobacteria (NTM), cryptococcusneoformans, penicilliummarneffei, and non-typhoidal salmonella spp. Confirming that IFN-π is a potent anti-tuberculosis, antibacterial and immunomodulatory cytokine are frequently identified. This immunosuppressive condition tends to be chronic and non-contagious mostly affecting people under the age of 50 years. The major pathogenic processes causing the disease were explored in limited publications. Genetic factors are suspected in part but the disease

does not seem inheritable.The disease may also be triggered by environmental stimuli including infections and toxins7. Primary epitope area (amino acid residue 121–131 of human IFN-π) is essential for the activation of the IFN receptor (IFN-ÿR) and the epitope is strongly homologous to a stretch of Aspergillusspp's Noc2 protein. Two subunits, IFNGR1 and IFNGR2, are associated with Janus kinases (JAK) 1 and JAK2, respectively, and bind to a distinct IFN-ÿR. JAKs activation results in tyrosine phosphorylation of a signal transducer and transcription activator (STAT1). STAT1 is phosphorylated on tyrosine 701, undergoes dimerisation, translocates to the nucleus and regulates gene expression by binding the promoters of IFN-π-regulated genes with sequence elements that are activated by IFN. Studies in Thailand and Taiwan show that 81% of patients with NTM (52 patients) and 96% of patients with other opportunistic infections have high-titles of anti-IFN-ÿ autoAb7.STAT1 is phosphorylated on tyrosine 701, undergoes dimerisation, translocates to the nucleus and regulates gene expression by binding the promoters of IFN-π-regulated genes with sequence elements that are activated by IFN. Studies in Thailand and Taiwan show that 81% of patients with NTM (52 patients) and 96% of patients with other opportunistic infections have high-titles of anti-IFN-ÿ autoAb7.Anti-IFN-γ autoAbs assay is required to confirm immunodeficiency in adults; however, the methods are not routinely used. ELISA and Luciferase Immunoprecipitation System can detect the generation of anti-IFN--00 autoAbs. A modified colorimetric sandwich ELISA was developed in this study using a conventional and commercially available IFN-ÿ sandwich ELISA kit to measure autoAbs in the serum of NTM infected patients. Tested sera collection and preparation is described in the Materials and Methods.In brief, a total of five patient clinically diagnosed with recurrent opportunistic infection and NTM patients without HIV infection were enrolled for testing the generation and the neutralizing activity of anti-IFN-γ autoAbs.

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

invulnerability is known to have a vital job in controlling disease, malignant growth and immune system issue in the liver. In this article, we will concentrate on hepatic infection contaminations, hepatocellular carcinoma and immune system issue as guides to represent the present comprehension of the commitment of T cells to cell resistance in these diseases. Cell safe concealment is basically answerable for constant viral diseases and malignancy. Be that as it may, an uncontrolled auto-receptive invulnerable reaction represents autoimmunity. Therefore, these safe variations from the norm are attributed to the quantitative and practical changes in versatile insusceptible cells and their subsets, intrinsic immunocytes, chemokines, cytokines and different surface receptors on invulnerable cells. A more noteworthy comprehension of the mind boggling coordination of the hepatic versatile insusceptible controllers during homeostasis and safe fitness are truly necessary to recognize applicable focuses for clinical intercession to treat immunological scatters in the liver.