Flow cytometry assay and cell staining protocol

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Abstract

This protocol describes antigen stimulation of human peripheral blood mononuclear cells (PBMC), staining for various surface and intracellular molecular markers, followed by flow cytometry measurements. PBMC contain various T and NK cell populations, which produce cytokines upon specific stimulation with antigens. T (and NK) cells can be stimulated in various ways: using whole virus, bacteria (or membrane fractions of bacterial lysates), antigenic peptides etc. The stimulation guide provided with this protocol refers to stimulation with antigenic peptides, influenza virus and a standard cell mitogen (Concanavalin A, ConA), though it can be adapted to incorporate any other cell stimulant.

This method is used to stimulate influenza specific cytokine production. After PBMC stimulation, cells are stained to detect cytokine producing T (and/or NK) cells by flow cytometry. Antigens are presented by antigen presenting cells (APC), which are also present among PBMC. In this procedure the Golgi apparatus is blocked by Brefeldin A and Monensin in order to keep all the cytokines produced after stimulation within the cell. The number of influenza specific cytokine producing T (and/or NK) cells can be determined by staining cells with fluorophore conjugated antibodies specific for the cytokine(s) of interest and counted on a flow cytometer.

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Protocol

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