ImmunoTools IT-Box-139 Award 2013

Dilip Shrestha

PhD Supervisor: Prof. Dr. János Szöllősi

Department of Biophysics and CellBiology
University of Debrecen, Medical and Health Science Center
Life Sciences Building, Nagyerdei Krt. 98
Debrecen H-4012, Hungary

Phenotypic and Functional Characterization of the Different Subsets of iNKT Cells

A conventional theory on antigen presentation views only peptide as an antigenic element. However, now it is proven that certain groups of T cells, apart from the vast majority of peptide restricted T cells, can distinctly recognize lipid-based antigens displayed on cluster of differentiation (CD) 1 receptors. These T cells are called as Natural Killer (NK) T cells. It expresses receptors common to both T cells and NK cells. Among these T cells, a subgroup termed invariant NK T (iNKT) cells is only activated by lipid bound CD1d receptors and expresses the non-variable set of α- and β- T cell receptors (TCRs). In humans, such TCRs are always formed by pairing of Vα24- with Vβ11- subunits in these T cells. iNKT cells have important functions in autoimmune diseases, cancer, infection and inflammation. Like innate cells, these cells rapidly activate in response to infection and produce both T helper (TH)1 and TH2 cytokines and chemokines that can tailor the ensuing immune response. Studies have also demonstrated that iNKT cells interact with and can activate/recruit many immune cells such as NK cells, macrophages, DCs, B cells and cytotoxic T cells. Thus, it combines the features of both classically innate and classically adaptive immune system and function as an innate-adaptive hybrid unit. In human peripheral blood, iNKT cells constitute only a small population (0.01-0.1 %) of the whole lymphocytes. Because of the limited amount of iNKT cells that could be obtained from the few hundred milliliters of peripheral blood, functional assays related to iNKT cells are generally performed after expanding these cells in vitro. Using the panel of ImmunoTools *IT-box-139* antibodies, we plan to sort and expand the subsets of iNKT cells ($CD4^+$, $CD8^+$, $CD4^-CD8^-$ and $CD4^+CD8^+$). In addition, we would also like to explore the spatiotemporal organization of the various cell-surface proteins in these cells. Mainly, we would like to follow the reorganization dynamics of CD3, CD4, CD8, CD28, CD45 etc. proteins in the immune synapse by fluorescence methods, including fluorescence resonance energy transfer (FRET). For this purpose, various antigen presenting cells (APC) will be used from B cells, macrophages, dendritic cells to monocytes. The antibodies against CD14 and CD19 proteins will be used to isolate monocytes and B cells where as many other sets of antibodies, including against CD14, CD11b, HLA-ABC, HLA DR and CD1a proteins will be used to monitor the maturation state of the above cells. Besides, Annexin V would also be useful to examine the apoptotic status of both iNKT cells and APCs. On a long term, we would like to demonstrate the differences in immune synaptic behavior exhibited by various APCs. This would help us to answer some of the questions pertaining to the superiority of dendritic cells in antigen presentation activity and would also give important insights into the

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relatively unknown field of lipid antigen presentation. Lastly, the other sets of dye-antibody conjugates in ImmunoTools *IT-box-139* would be very handy for pilot experiments, which would help us develop our future projects as well.

ImmunoTools *IT-Box-139.3* for **Dilip Shrestha** includes 100 antibodies

FITC - conjugated anti-human CD1a, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD11a, CD11b, CD14, CD15, CD16, CD18, CD19, CD21, CD25, CD29, CD36, CD41a, CD43, CD45, CD45RA, CD46, CD52, CD53, CD54, CD58, CD62p, CD63, CD69, CD71, CD80, CD86, CD95, CD235a, HLA-ABC, HLA-DR, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE - conjugated anti-human CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD18, CD19, CD20, CD21, CD22, CD27, CD33, CD34, CD37, CD38, CD40, CD42b, CD45, CD45RB, CD50, CD72, CD95, CD105, CD147, CD177, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE/Dy647 -tandem conjugated anti-human CD45

APC -conjugated anti-human CD3, CD4, CD7, CD8, CD10, CD11c, CD14, CD16, CD19, CD27, CD37, CD40, CD44, CD56, CD59, CD61, CD62L, CD62P, CD69, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V DETAILS