



2011 Research Grant Program Winning Abstract

Functional Immunophenotyping for Primary Immunodeficiencies

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Genetically determined disorders of the protective immune function, commonly referred to as primary immunodeficiency diseases (PID), were recognised clinically 60 years ago with the identification of X-linked agammaglobulinemia. Today, the group of PID involves over 280 distinct entities which are scattered to present as phagocytic disorders, complement deficiencies, T-cell deficiencies, and—predominantly—antibody disorders. PID are not rare diseases, having a summarized prevalence ranging from 1 in 250 to 1 in 500. However, they remain undetected in many cases because of their subtle clinical image and their prolonged course of disease, which often worsens the outcome. Albeit PID's increasing appreciation as a major health problem, there is a lack of research efforts to provide diagnostic procedures and screening profiles that would enable a better understanding of the immune function in PID patients, ultimately leading to earlier diagnoses.

To improve this situation, we intend to develop a functional immunophenotyping strategy for peripheral blood cells that will serve (i) as an initial research platform for individuals who attend medical examination on the suspicion of PID (~600 patients per year in Sweden), and (ii) as a confirmatory platform to study the characteristics of PID patients revealed by nationwide neonatal screening efforts in 2012, which will allow identification of newborns featuring a T- or B-lymphopenia (~100,000 newborns per year tested in Sweden). Such functional immunophenotyping strategies require methods for high-throughput in-depth analysis (HTIDA) of precious samples, which is ensured on the basis of well established instrumentation at our institution, including BD™ LSR and BD FACSAArray™ cell analyzers. To allow the translation of our clinical research into routine diagnostics in the future, multicolor flow cytometry protocols will also be evaluated on the BD FACSCanto™ II cell analyzer at our site.

The HTIDA approach makes high demands on polychromatic flow cytometry in terms of sample preparation for both surface and intracellular marker detection, creation of reliable antibody “backbone” clusters for cell lineage discrimination, and functional determination of cell cycling, proliferation, apoptosis, and the phosphorylation status of pathway transducers. We thus intend to employ proprietary buffer solutions, such as BD Pharm Lyse™ buffer and BD Cytotfix™ solution, to prepare comprehensive whole blood “lyse/wash” specimens, containing an unbiased composition of peripheral blood leukocytes. The phenotypic flow cytometric workup of lymphocyte subsets' configuration and their differentiation route is a powerful strategy to classify characteristic changes due to the etiopathology of various PID. To do so, “backbone” clusters of at least eight fluorochrome-conjugated antibodies per panel will be set up for (i) a general evaluation of lymphocyte subpopulations, (ii) T-cell subsets (including Th1-like, Th2-like, Th17/Th22-like, and regulatory T cells), (iii) early immature B and late memory B-cell subsets (naïve B, transitional B, marginal-zone like B, follicular B, tissue-homing B, and regulatory B cells), (iv) plasma blasts and plasma cells (bone marrow emigrant plasma cells, MALT-homing plasma cells, circulating plasma cells), (v) the expression of surface



and intracellular immunoglobulins and Ig receptors on B- and plasma cell subsets (IgM, IgG, IgA, IgE, kappa/lambda chains, FcRgamma, FcRN), and (vi) the activation status of T, B, and plasma cells (antigen receptor induced upregulation of co-stimulatory molecules). In contrast to cell-stage based analyses in commonplace hematological approaches, we will interpret the full spectrum of lineage differentiation and subset constitution by automatic population separation and multidimensional pathway retrieval (Infinicyt™ software).

In addition to phenotypic HTIDA, we intend to establish an intracellular protein detection workflow for flow cytometry. The lack, reduction, or structural abnormality of disease-associated proteins in PID is generally well defined. In disease entities that are caused by genetic alterations in bulky genes (eg, in ataxia telangiectasia), the detection of protein intermediates as a first-tier test might thus agree better with our HTIDA approach than genomic sequencing strategies. To reliably identify intracellular protein expression in primary lymphocyte subsets, short-term cell culture protocols consisting of stimulatory cocktails (eg, BD recombinant cytokines and purified agonistic antibodies), hosted in BD synthetic PureCoat™ plates, will be evaluated. Using the BD IntraSure™ kit or BD Cytofix/Cytoperm™ Plus kit, the expression and structural configuration of targeted proteins can be analyzed using specific anti-human antibodies from BD. Moreover, the use of 96-well format culture plates readily conforms with BD™ Cytometric Bead Arrays, eg, for the functional analysis of B-cell subsets as to the extent of in vitro production of protective immunoglobulins, which is a helpful technique to assess individual humoral immune responses during treatment of PID patients with IgG substitution solutions.

Several primary immunodeficiencies that severely affect the function of T and/or B lymphocytes are characterized by defects in antigen receptor activation and downstream signal transducer recruitment. To investigate and reveal such diseases, calcium mobility shift assays (eg, BD™ Ratiometric Calcium Assay Kits) are likely to indicate the antigen receptor malfunction as exemplified by diseases due to mutations in the ORAI1, STIM1, or Coronin-1A genes. Other defects affecting the function of signal transducers will be tackled with aid of the BD Phosflow™ technology. In diseases such as the Hyper-IgE-syndrome (STAT3), some chronic mucocutaneous candidiasis syndromes (STAT1), and in STAT5b deficiency, the capability of phosphoprotein signaling is reduced and will be analyzed at the single-cell level.

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