Epigenetic Immune Cell Quantification For Diagnosis and Monitoring of Patients with Inborn Errors of Immunity, Primary Immune Deficiencies, and Immune Regulatory Disorders

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Background

Inborn Errors of Immunity (IEI)

are caused by monogenic germline mutations resulting in loss or gain of function of the encoded protein. They can be dominant or recessive, autosomal or X-linked, and with complete or incomplete penetrance. They manifest as increased susceptibility to a broad or narrow spectrum of infectious diseases, as well as a growing diversity of autoimmune, autoinflammatory, allergic, and/or malignant phenotypes. They now comprise 406 distinct disorders with 430 different gene defects listed in the 2019 International Union of Immunological Societies (IUIS) classical classification

Primary Immune Deficiencies (PID)

caused by an inherited flaw in the immune system that increases the susceptibility to infections. Primary immunodeficiency diseases are unlike secondary or acquired immune deficiency diseases, which are caused by infectious, chemical or radiological agents.

Primary Immune Regulatory Disorders (PIRD)

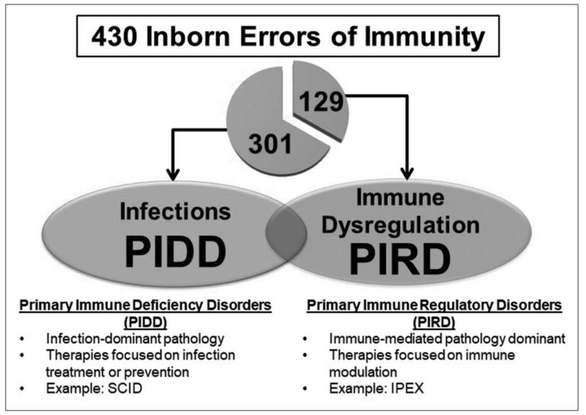
are a group of immune diseases characterized predominantly by immune dysregulation leading to organ-specific autoimmunity, excessive inflammation, and non-malignant lymphoproliferation. Unlike classical primary immunodeficiencies, susceptibility to infections is typically less prominent in these disorders.

Most widespread analysis approach and limitations

Currently, immune cell quantification is mostly performed by flow cytometry, allowing flexible analysis of the cell types of interest and accuracy. Over the last decades, the usage of flow cytometry has been contently optimized and the analyzers have been further developed. Thus, the major limitation of the method is the demand of intact cells, which can be unavailable in some circumstances.

Objective

Quantitative immune cell enumeration is important in all inborn errors of immunity, and all primary and secondary immune deficiencies. Within this study, the application of a new *in vitro* diagnostic test for the epigenetic quantification of CD3+, CD4+, and CD8+ T cells, B cells and NK cells from as little as 40 µl of fresh or frozen whole blood as well as dried blood spots is described and correlated to flow cytometry data.



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Methods

i.Mune™ TBNK [CE]

i.Mune™ TBNK is a quantitative in vitro test to determine the percentages and absolute counts of human lymphocyte subsets in liquid venous whole blood and to determine the percentages of human lymphocyte subsets in capillary whole blood specimens dried on filter paper (Dried Blood Spot; DBS).

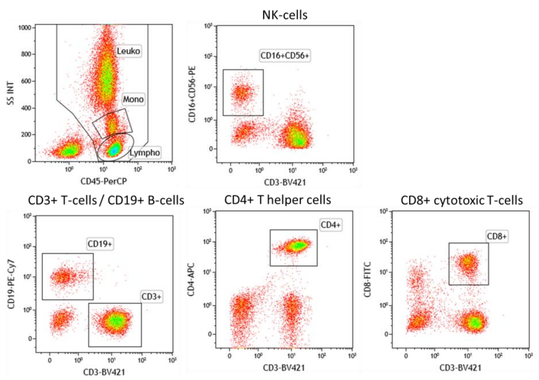
Flow cytometry

Flow cytometry is a sophisticated instrument measuring multiple physical characteristics of a single cell such as size and granularity simultaneously as the cell flows in suspension through a measuring device. Extracellular molecules located on the surface or intracellular molecules inside the cell can be determined.

Cohort and lymphocyte gating strategy

247 whole blood samples of different patients with Primary Immune Deficiencies (PID) or Inborn Errors of Immunity (IEI) were analyzed.

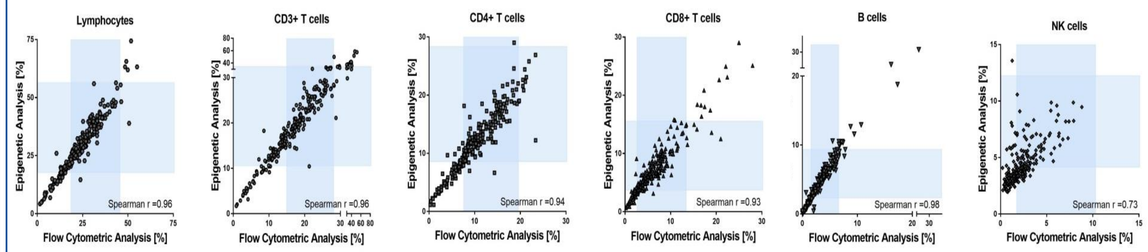
IUIS	Diagnosis	# of patients
IA) Immunodeficiencies affecting cellular and humoral immunity	ADA deficiency	1
IB) Immunodeficiencies affecting cellular and humoral immunity	ICOS deficiency	2
	FCHO1 deficiency	1
IIA) Combined immunodeficiencies with associated or syndromic features		3
IIB) Combined immunodeficiencies with associated or syndromic features	Hyper IgE syndromes (HIES)	6
IIIA) Predominantly antibody deficiencies	CVID / hypogammaglobulinemia / agammaglobulinemia	146
IIIB) Predominantly antibody deficiencies	selective IgA deficiency	3
	IgG subclass deficiency	25
IVA) Diseases of immune dysregulation	XIAP deficiency	3
IVB) Diseases of immune dysregulation	FAS ALPS	1
	STAT3 GOF	3
	CTLA4 deficiency	16



Patient grouped according to IUIS classification (n=249), female / male: 134 / 115, age: mean 46.7 yrs (19 – 80 yrs).

Results

Spearman correlation of > 0.9 was observed for all immune cell types analyzed except for NK cells. With regard to NK cells, the epigenetic analysis shows an overestimation of the cell percentages, but the correlation of 0.73 was still robust. Relative immune cell counts are shown as percentage of total leukocytes.



Conclusion

This study underscores the suitability of epigenetic immune cell quantification for the accurate measure of multiple immune cell types from PID and IEI patients. We propose this method as uniquely suitable for novel molecular diagnostic applications in settings with limited fresh blood sample or limited cell number, at the point of care, as well as for newborn screening.

Outlook

Development of quantification kits for regulatory T cells, primary immunodeficiencies and new born screenings to facilitate future diagnosis of IEI, PID and PIRD.

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