Label-free assessment of B cell differentiation by Ghost Cytometry

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Yoko Kawamura[1], Asako Tsobushi[1], Juliet Packiasamy[2], Kazuki Teranishi[2], Ryo Tamato[1], Janette Phi[2], Sadao Ota[1,3]

[1]ThinkCyte, KK, Tokyo, Japan | [2]ThinkCyte, Inc., San Carlos, CA | [3]University of Tokyo, Tokyo, Japan

Abstract

Identification and sorting of distinct B cell subsets is critical for modern antibody screening and drug development programs. Here, we used a morphological profiling approach together with artificial intelligence (AI) to identify B cell phenotypes. This approach described here can be used to isolate B cells without the use of external labels or markers for therapeutic development programs.

Introduction

B cells are critical for a functioning immune system. Differentiated plasma B cells help the body defend against foreign entities through the production of antibodies. This antigen-specific antibody production by B-cells has also been exploited for the development of targeted therapeutics for cancer and immune disorders. In addition to playing a protective role in the adaptive immune system, dysfunctional B cells can also lead to life-threatening immunological disorders. Due to the importance of B cells for human health, studying the process of B-cell activation and differentiation into plasma cells is an important part of drug screening. However, highly specific surface markers for identifying plasma B cells are lacking and impose a challenge for researchers who want to isolate, characterize, and use them for drug development campaigns. In this proof-of-concept study, we used the VisionSort platform employing Ghost Cytometry technology to generate label-free markers of B cell differentiation into plasma B cells.

Methods

Human B cells were cultured for 6 days under conditions that promoted either B cell activation (i.e., IgM and CD40 stimulation) or plasma cell differentiation (i.e., IgM, CD40, IL021, CpG ODN stimulation). IgD, CD38, and CD27 were used as 'ground truth' markers to define populations of B cells (defined as CD38(-)) and plasma cells (defined as IgD(-) / CD38high / CD27high). Cells were the analyzed on the VisionSort platform to generate ghost motion image (GMI) signals for each population. Using the GMI signals from the gated populations, a classifier was built using supervised machine learning. GMI signals were also analyzed by unsupervised learning algorithms embedded in the instrument to identify discrete populations of B-cells.

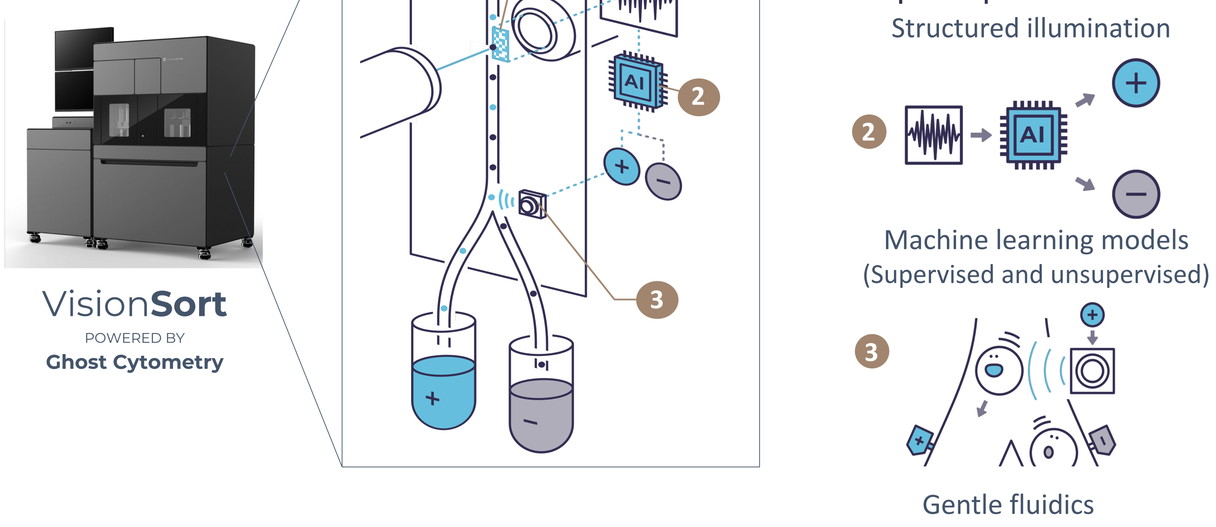

Figure 1. Principles of the VisionSort platform.

Results

Classification of B cells and plasma cells

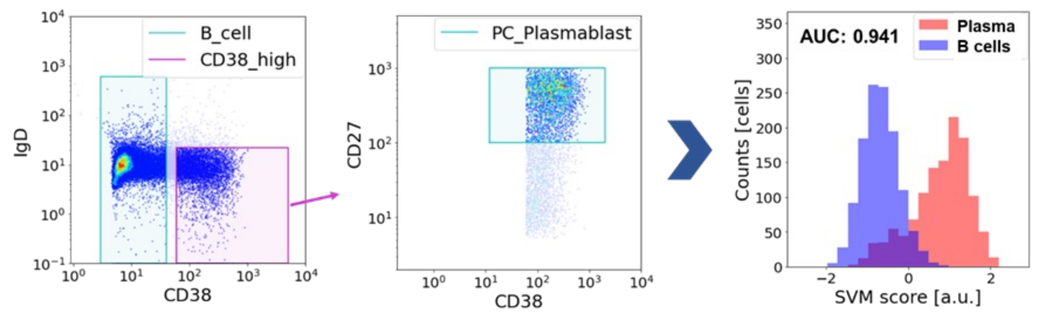

Figure 2. Development and assessment of the GMI-based classifiers for B-cell subtypes. B cells were defined as CD38 (-) and plasma cells were defined as IgD(-) / CD38high / CD27high using ground truth labels. A machine learning classifier was built from the GMI signals from the gated populations using Ghost Cytometry. When the classifier was used on unlabeled cells, it achieved excellent performance in classifying B cells from plasma cells with an AUC score of 0.941.

Microscopic differences in B cell phenotypes

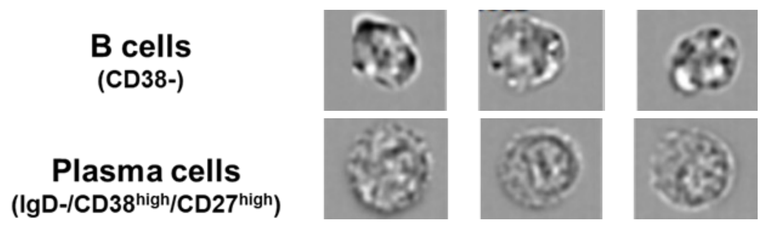

Figure 3. Confirmation of B-cell phenotypes by imaging. Distinct morphological differences, reflective of changes during the activation / plasma cell differentiation process, were observed in microscopic images of the different B-cell populations (taken with an Amnis Flowsight instrument at 60X magnification).

Morphological separation of B cell phenotypes

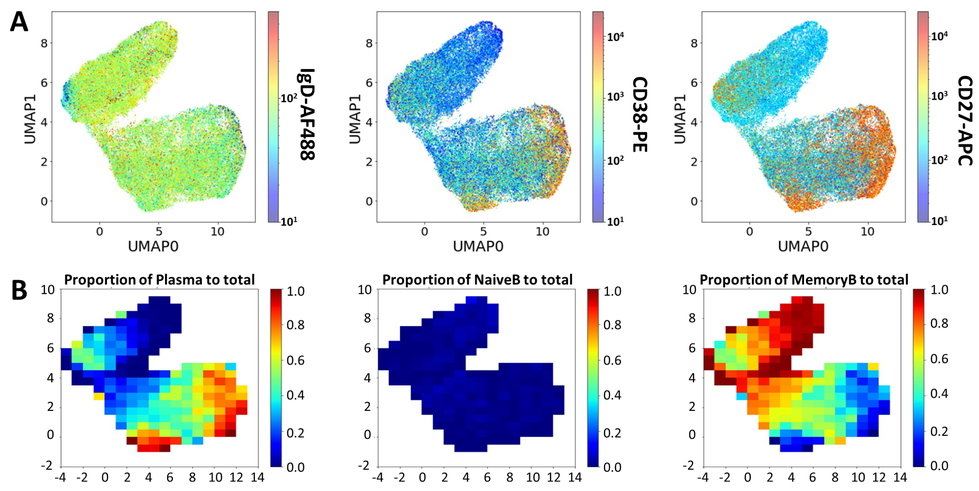

Figure 4. Unsupervised clustering analysis of B-cell subtypes by morphology. Uniform manifold approximation & projection (UMAP) plot of B cells analyzed by GMI signals. Staining intensity of ground truth markers (IgD, CD38 and CD27) are overlayed in A. The proportion of different ground truth marker-defined populations are shown in B.

Conclusions

The VisionSort platform can be used to identify and classify activated B cells and differentiated plasma cells without the use of external molecular markers or labels. This has practical applications for antibody screening programs, cell line development, and B cell therapy programs where isolation of B cell subsets minimal external intervention is needed to preserve cells for further downstream R&D applications.