Flow Cytometry and Imaging Technologies

Expansive immunophenotyping of natural killer cell populations with the Invitrogen™ Attune™ Xenith™ Flow Cytometer

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Abstract

As the field of flow cytometry expands our knowledge of the immune system and increased panel complexity has put a greater demand on instrumentation. Spectral unmixing has advanced panel size and diversity, enabling the study of novel cell populations. Using the new Invitogen™ Attune™ Xenith™ Flow Cytometer, we have developed a 31-color panel to investigate NK cell lineages and associated T cell populations. With the use of acoustic focusing technology, we demonstrate the ability to separate rare cell types, such as invariant NKT cells (iNKTs) and innate lymphoid cell (ILC) subsets at flow rates up to 500µl/min. Additionally, we investigate activation and inhibition markers on various NK cell populations both with and without cytokine stimulation. Using the Attune Xenith Flow Cytometer we are able to incorporate IR excited dyes (such as LIVE/DEAD™ Fixable Near-IR 876) to be able to expand our panel and reduce spectral unmixing burden. The spectral capabilities of the Attune Xenith Flow Cytometer, coupled with the clog-resistant acoustic focusing allow users to run samples at faster speeds, reducing time to results. We hope to expand this panel further in the future to be able to facilitate research into NK, CAR-NK, and other rare cell subset investigations.

Introduction

With the development of new expansive panels, spectral cytometry is continuing to push the limits of information that users can garner from individual samples. As immunologists dive deeper into cellular populations, we have gained a greater awareness of various rare cell populations important to immune cell development and response. Using the Attune Xenith Flow Cytometer we developed a 31-color panel to investigate expression of many natural killer (NK) cell markers in various stages of their maturation. NK cell surveillance and activation are critical in the immune response to not just infection but also cancer and autoimmunity. Interest in NK cell use as an immunotherapeutic target has also placed an emphasis on understanding how NK cells develop and maintain their various levels of activity. As potent cytotoxic innate lymphoid cells, they not only have the potential to eliminate infected and cancerous cells but also act as a bridge to the adaptive immune response by producing inflammatory cytokines and chemokines at areas of infection. To investigate the NK population, we utilized human peripheral blood mononuclear cells (PBMCs) and analyzed expression levels of NK maturation and activation markers in immature, mature, and terminal NK cell populations. Using the Attune Xenith Flow Cytometer, we were able to run this panel at exceptionally high speeds (500µl/min) to quickly collect 1x10^6 for analysis.

In addition to analysis of NK cells we also analyzed related NK T cells (NKTs) as well as innate lymphoid cell populations. These cells are found at a low frequency in PBMCs but represent important components of the innate immune response in both infection and cancer. Using the Attune Xenith Flow Cytometer we are able to analyze these different populations in the NK lineage. As both NKTs and ILCs are known to produce cytokines in response to activation/differentiation, future expansion of the panel will be focused on detecting these markers within NKT, ILC, and NK populations.

Materials and methods

List of fluorophores used in this panel: NKG2D BUV395 (BD Biosciences), CD19 BUV496 (ThermoFisher), CD56 BUV563 (BD Biosciences), CD161 BUV615 (BD Biosciences), CD127 BUV661 (BD Biosciences), NKp30 BUV737 (BD Biosciences), CD16 BUV805 (ThermoFisher, CB16), NKG2C BV421 (BD Biosciences), CD57 eFluor 450 (ThermoFisher), KLRG1 BV480 (ThermoFisher), CD3 BV510 (Biolegend), CD8 BV570 (Biolegend), CD244 Super Bright 600 (ThermoFisher), CD38 BV650 (Biolegend), cKit Super Bright 702 (ThermoFisher), CD69 BV750 (BD Biosciences), CD2 Super Bright 780 (ThermoFisher), TCR Vα7.2 FITC (Biolegend), CD45 Alexa Fluor 532 (ThermoFisher), TCRγδ PerCP-eFluor 710 (ThermoFisher), HLA-DR NovaFluor Blue 610-30S (ThermoFisher), NKp46 Real Blue 786 (BD Biosciences), CD158a PE (Biolegend), NKG2A PE Dazzle 594 (Biolegend), CRTH2 PE-Vio 670 (Miltenyi Biotec), CD25 PE-Cy5.5 (ThermoFisher), TCR Vβ11 (Miltenyi Biotec), TCR Vα24Jα18 APC (ThermoFisher), CD4 NovaFluor Red 700 (ThermoFisher), CD34 APC-eFluor 780 (ThermoFisher)

Results

NK Cell Activation

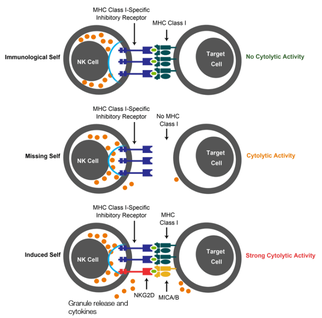

Figure 1: NK Cell Activation. Engagement of a number of receptors can lead to activation or inhibition of NK cells leading to lysis of a target cell. Various conditions such as infection or stress may can lead to a lack of MHC Class I receptors on target cells, which would normally engage various inhibitory receptors on NK cells (i.e. KIRDL1/2/3). Upon downregulation of these MHC Class I receptors on target cells, NK cells can increase their lytic activities by release of proteins such as granzyme B and perforin. NK cells can also increase their cytolytic activity by engagement of several activation receptors (i.e. NKG2A, NKG2C). The balance between activation and inhibition is critical to NK lytic activity and cytokine production. Due to the ability of NK cells to inspect cells for abnormalities they represent an important arm of the innate immune cell pool and a bridge to adaptive immunity.

Initial Scatter and Fluorescent Gating Scheme

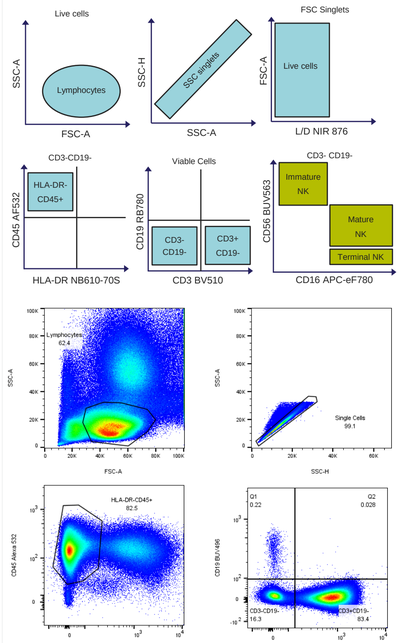

Figure 2: Initial scatter and viability gating. Data here showing the initial scatter, singlet, and viability gating to begin an in-depth analysis of different cells within the lymphocyte population. The final graph here shows the various NK cells populations (immature, mature, and terminal), that will be analyzed in later figures.

Analysis of NK Activation/Maturation Markers

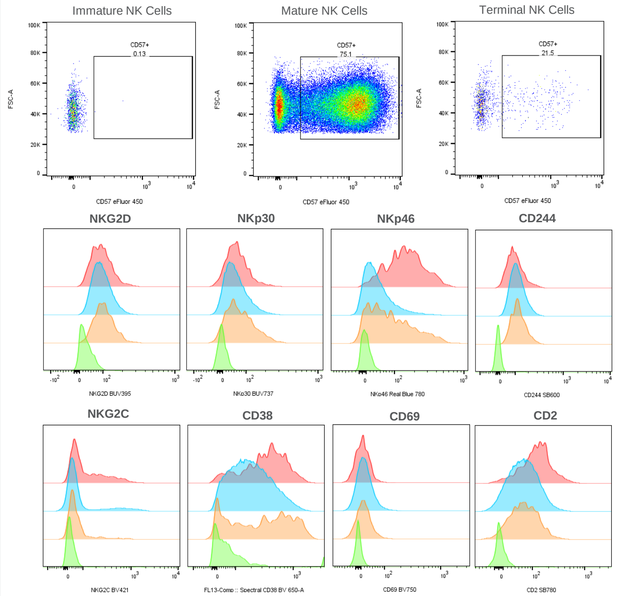

Figure 3: Expression of markers on various NK population in human PBMCs. A number of different markers were used to evaluate maturation, activation, and inhibition in immature (red), mature (blue), and terminal (orange) NK populations in human PBMCs. Expression of these markers in B cells (green) were used as a control. Expression of several inhibitory receptors, including KLRG1, KIR2DL1, and NKG2A, were also analyzed on the NK populations, not shown here.

Analysis of rare NK lineage cells

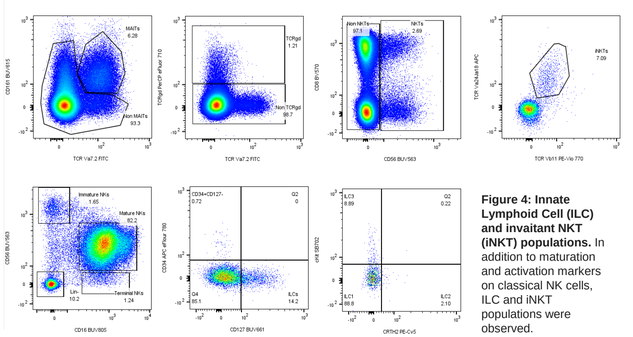

Figure 4: Innate Lymphoid Cell (ILC) and invaitant NKT (iNKT) populations. In addition to maturation and activation markers on classical NK cells, ILC and iNKT populations were observed.

Flow Rate Comparison

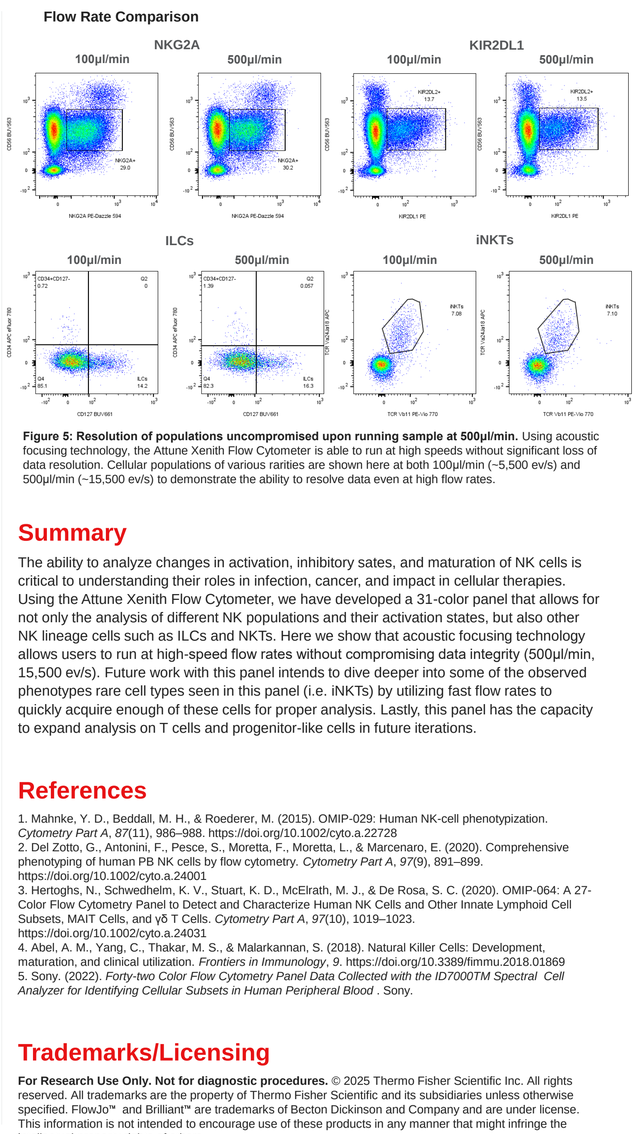

Figure 5: Resolution of populations uncompromised upon running sample at 500µl/min. Using acoustic focusing technology, the Attune Xenith Flow Cytometer is able to run at high speeds without significant loss of data resolution. Cellular populations of various rarities are shown here at both 100µl/min (~5,500 ev/s) and 500µl/min (~15,500 ev/s) to demonstrate the ability to resolve data even at high flow rates.

Summary

The ability to analyze changes in activation, inhibitory sates, and maturation of NK cells is critical to understanding their roles in infection, cancer, and impact in cellular therapies. Using the Attune Xenith Flow Cytometer, we have developed a 31-color panel that allows for not only the analysis of different NK populations and their activation states, but also other NK lineage cells such as ILCs and NKTs. Here we show that acoustic focusing technology allows users to run at high-speed flow rates without compromising data integrity (500µl/min, 15,500 ev/s). Future work with this panel intends to dive deeper into some of the observed phenotypes rare cell types seen in this panel (i.e. iNKTs) by utilizing fast flow rates to quickly acquire enough of these cells for proper analysis. Lastly, this panel has the capacity to expand analysis on T cells and progenitor-like cells in future iterations.

References

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