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OMIP-027: Functional Analysis of Human Natural Killer Cells

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Additional Supporting Information may be found in the online version of this article.

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• **Key terms**
NK cells; function; ICS; lymphocyte; HIV

Purpose and Appropriate Sample Types

The current panel was developed to characterize the function of human natural killer (NK) cells from cryopreserved peripheral blood mononuclear cells (PBMC). The application of this panel is to identify changes in bulk NK cells and NK cell subsets with regard to receptor expression, and function in the setting of acute human immunodeficiency virus (HIV-1) infection. However, this panel may be applied to a wide variety of disease states and normal conditions to characterize human NK cells (Table 1). The performance of this panel was optimized using frozen PBMC from HIV-infected and uninfected individuals. The panel is being used to evaluate NK cell responses in individuals with acute HIV-1 infection as well as normal healthy individuals participating in HIV vaccine clinical trials.

Background

NK cells are large granular lymphocytes within the innate immune system that play a critical role in the control of viral infections (1). Human NK cells have been classically defined as $CD3^-$ lymphocytes expressing the neural cell adhesion molecule (NCAM), $CD56^+$, and/or the FcγIIIa receptor, CD16. NK cells can be further subdivided into $CD56^{bright}$ NK cells, which lack the expression of CD16 and $CD56^{dim}$ NK cells, which express CD16. In addition, a subset of $CD56^-CD16^+$ NK cells appears to be expanded in chronic viral infections and seems to represent an exhausted/anergic subset of NK cells (1,2).

The most prominent function of NK cells is cytolysis, which is mediated by a number of different mechanisms, including exocytosis of cytoplasmic granules containing perforin and granzyme and antibody-dependent cellular cytotoxicity (ADCC) (1). $CD56^{bright}$ NK cells also play an important role as immune modulatory cells, bridging the innate and adaptive immune responses as they produce soluble factors including cytokines and chemokines (1). The exact mechanisms and function of NK cells in response to virally infected targets are complex and still need to be further characterized.

This panel originated from OMIP-007 (3) to include exclusion and lineage markers that identify NK cells and was expanded to add markers to explore functional responses to various stimulation conditions. One of these functions, ADCC, leads to NK cell degranulation, identified by CD107a (4), and the release of cytotoxic granules containing perforin and granzyme B (5). Activation of NK cells by various targets also induce the release of cytokines and chemokines, including interferon gamma (INF-γ) and tumor necrosis factor alpha (TNF-α) (5). Due to differential expression patterns of cytokines, cytolytic proteins, and degranulation markers across stimulation conditions, CD107a, INF-γ, TNF-α, granzyme B, and perforin

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Table 1. Summary table for OMIP-027

Purpose	Characterize the phenotype and function of stimulated NK Cells
Species	Human
Cell Types	Cryopreserved PBMC
Cross References	OMIP-007 and OMIP-025

Table 2. Reagents used in OMIP-027

COMMERCIALLY AVAILABLE REAGENTS			
SPECIFICITY	FLUOROCHROME	CLONE	PURPOSE
Live Dead	Aqua	n/a	Viability
CD3	APC-Cy7	SP34-2	Exclusion
CD4	APC-H7	SK3	
CD14	TRI-COLOR (PE-Cy5)	TuK4	
CD19	TRI-COLOR (PE-Cy5)	SJ25-C1	
CD16	Pacific Blue	3G8	NK subsets
CD56	PE-Cy7	NCAM16.2	
CD107a	FITC	H4A3	Degranulation
IFN-y	BV711	4S.B3	Function
TNF-a	BV650	Mab11	
Perforin	PE	B-D48	
Granzyme B	PE CF594	GB11	
CD8	BV785	RPA-T8	
Eomesodermin	PerCP eFlour 710	Dan11mag	Transcription factor
CD57	APC	HCD57	Differentiation

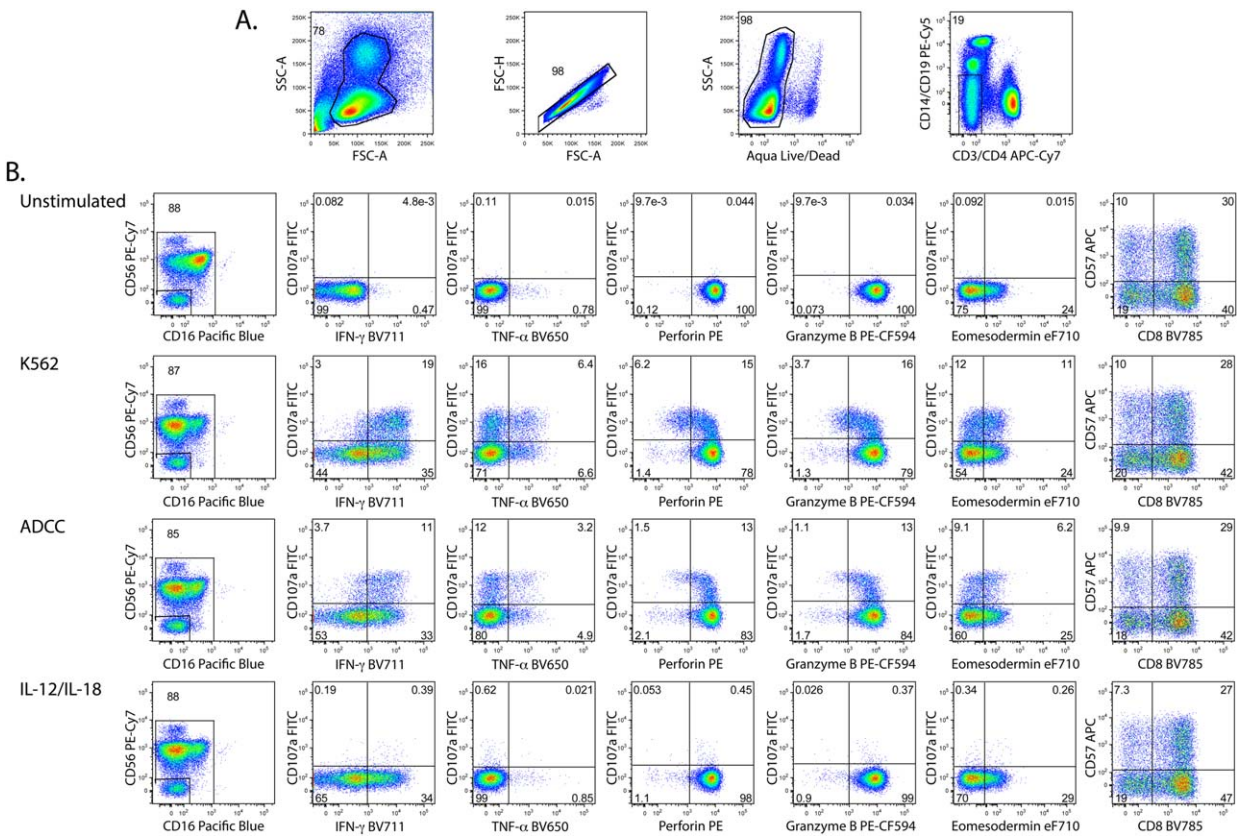

Figure 1. Gating strategy and panel performance for OMIP-027. PBMC were thawed and stained with the human NK cell functional panel as outlined in the Supporting Information. **A**: Overall successive gating strategy demonstrates initial broad gating on forward and side scatter to include large lymphocytes. Forward area and height are used to discriminate single cells followed by identification of viable cells using an amine reactive dye. Monocytes and B cells are excluded using CD14 and CD19 and T cells are excluded using CD3 and CD4. **B**: Functional responses of NK cells after various stimulation conditions are displayed in each row. CD56 and CD16 are used to identify NK cells discriminating between multiple populations on the basis of $CD56^{bright}$, $CD56^{dim}$, and $CD56^{negative}$ expression levels. The relative amounts of CD107a, INF-γ, TNF-α, Granzyme B, Perforin, and Eomesodermin expression from the total NK cell population as well as CD57 and CD8 expression are shown.

were included in this panel. Independent or combinatorial expression of these markers can give insight into the mechanism of how NK cells respond to various stimulation conditions and how this may be affected in disease states. For laboratories that face technical limitations, INF-γ remains the most sensitive functional marker across stimulation conditions using this procedure. The net effect is to kill virally infected target cells and create an antiviral environment (5). CD57, a marker for terminal differentiation of NK cells and eomesodermin, a transcription factor which regulates functional NK cell maturation (6,7) are also included. These markers have been shown to be important in identifying the maturational state of NK cells (8–11). All reagents included in the final panel are listed in Table 2.

The NK functional panel was tested on HIV-infected and -uninfected individuals. Measurable differences in cytokine production and overall total function between healthy individuals and chronically infected patients (Supporting Information Figure 4) were observed, providing evidence that utilizing this panel is capable of identifying differences in NK cell subsets within various disease states.

Details of the optimization of this panel can be found in the Supporting Information. The overall performance of this panel in healthy individuals is displayed in Figure 1.

Similarity to Published OMIPs

This panel is an expansion and modification of OMIP-007, which now includes functional markers, whereas KIR markers, $\alpha 4\beta 7$, CD62L, and HLA-DR, were removed. In addition it represents similarities to OMIP-025, which includes the NK marker CD56 and intracellular cytokines INF-γ and TNF-α. However, our panel includes Fcγ-receptor IIIa (CD16), a proxy marker for cytolytic activity (degranulation marker CD107a), lytic proteins perforin and granzyme B, transcription factor eomesodermin, and differentiation marker CD57, which are not addressed in any other OMIP panels.

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