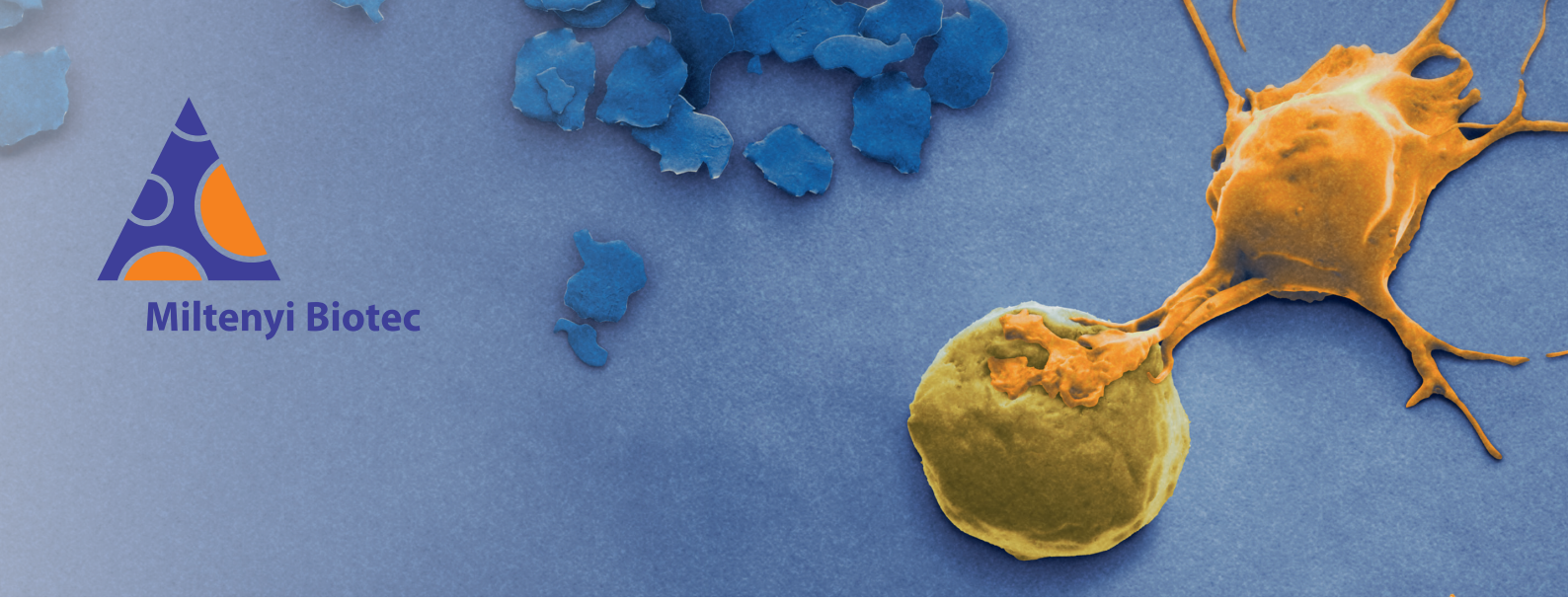


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Comprehensive flow cytometry analysis of chemokine receptors expressed on human natural killer cells

Mieszko Lachota
Medical University of Warsaw

Introduction

Natural killer (NK) cells are effector cells of the innate immune system, defined as $CD56^+$ and $CD3^-$ lymphocytes, capable of exerting natural cytotoxicity and antibody-dependent cytotoxicity (ADCC). These cells play an important role in cancer surveillance due to their unique ability of spontaneous cytotoxicity against cancer cells, which also makes them an attractive tool for immunotherapy. They can be divided into functionally different subsets, depending on their differentiation and education status, based on the expression of CD56, CD16, CD57, NKG2A, KIRs, and other markers. To exert their function, NK cells migrate along the gradient of chemotactic factors secreted in different tissues[1,2].

Chemokines are small, secreted proteins involved in cell trafficking and cell-to-cell interactions. Each chemokine is secreted in a tissue- and cell-specific manner and is responsible for the recruitment of certain cell types, depending on their expression pattern of chemokine receptors. Trafficking of appropriate immune cells to different tissues and inflammation sites enables the development of an immune response tailored to specific pathogens or cancers[3–5].

The profile of chemokines secreted within the tumor determines what subsets of NK cells are capable of migrating towards it. These tumor-infiltrating NK cells can have a different effect on tumor progression, depending on the subset. Infiltration of $CD56^{bright}$ NK cells might promote tumor progression by secreting proangiogenic factors. Alternatively, infiltrating $CD56^{dim}$ NK cells have been shown to be highly cytotoxic against tumor cells and their presence correlates with a better prognosis[6–9].

This application note describes the analysis of the human chemokine receptor repertoire by flow cytometry. We present an antibody panel allowing simultaneous assessment of two chemokine receptors on both $CD56^{bright}$ and $CD56^{dim}$ NK cells that were either rested or stimulated with IL-15.

Materials and methods

PBMC isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats by centrifugation in a Ficoll™ gradient. After isolation, cells were resuspended in MACS® Buffer (PBS supplemented with 1% BSA and 2 mM EDTA) and transferred into cell culture within 1 h.

NK cell culture and stimulation

NK cells were isolated from PBMCs using the NK Cell Isolation Kit, human (Miltenyi Biotec, 130-092-657) in combination with the autoMACS® Pro Separator. Isolated cells were cultured in RPMI-1640 supplemented with 10% fetal calf serum (FCS), and 10 ng/mL IL-15 in selected samples. The cells were incubated for 48 h at 37 °C and 5% CO_2.

Cell staining

PBMCs or NK cells were labeled with fluorochrome-conjugated monoclonal antibodies (table 1 and 2). Firstly, cells were washed, then resuspended in staining buffer with two anti-chemokine receptor antibodies per sample and incubated for 15 min at 37 °C, and then for 15 min at room temperature. Subsequently, cells were spun down and resuspended in staining buffer with the rest of the antibodies listed in table 1 and incubated for 15 min at room temperature. Finally, cells were washed twice and analyzed by flow cytometry.

Antigen	Fluorochrome	Clone	Manufacturer and product number
Viobility™ 405/520 Fixable Dye			Miltenyi Biotec, 130-110-206
CD3	VioGreen™	REA613	Miltenyi Biotec, 130-113-142
CD14	V500		BD, 561391
CD19	V500		BD, 561121
CD56	BV785 (SA)	REA196	Miltenyi Biotec, 130-113-311
Chemokine receptor #1	PE		Miltenyi Biotec
Chemokine receptor #2	APC		Miltenyi Biotec

Table 1: Antibody panel used for cell staining. Samples were stained in two steps: In the first staining step, cells were incubated with two chemokine receptor antibodies from table 2, either coupled to PE or APC. And in the second staining step, cells were incubated with all other antibodies listed in this panel.

Antigen	Clone
CCR1 (CD191)	REA158
CCR2 (CD192)	REA264
CCR3 (CD193)	REA574
CCR4 (CD194)	REA279
CCR5 (CD195)	REA245
CCR6 (CD196)	REA190
CCR7 (CD197)	REA108
CCR9 (CD199)	REA469
CCR10	REA326
CMKLR1 (ChemR23)	REA455
CXCR1 (CD181)	REA958
CXCR2 (CD182)	REA208
CXCR3 (CD183)	REA232
CXCR4 (CD184)	REA649
CXCR5 (CD185)	REA103
CXCR6 (CD186)	REA458
CX3CR1	REA385

Table 2: Chemokine receptor REAfinity™ Antibodies.

Gating strategy

To analyze NK cells, doublets, T cells, B cells, and monocytes were excluded prior to gating on CD56^{+} cells (fig. 1). Based on the CD56 expression, CD56^{bright} and CD56^{dim} NK cells can be distinguished.

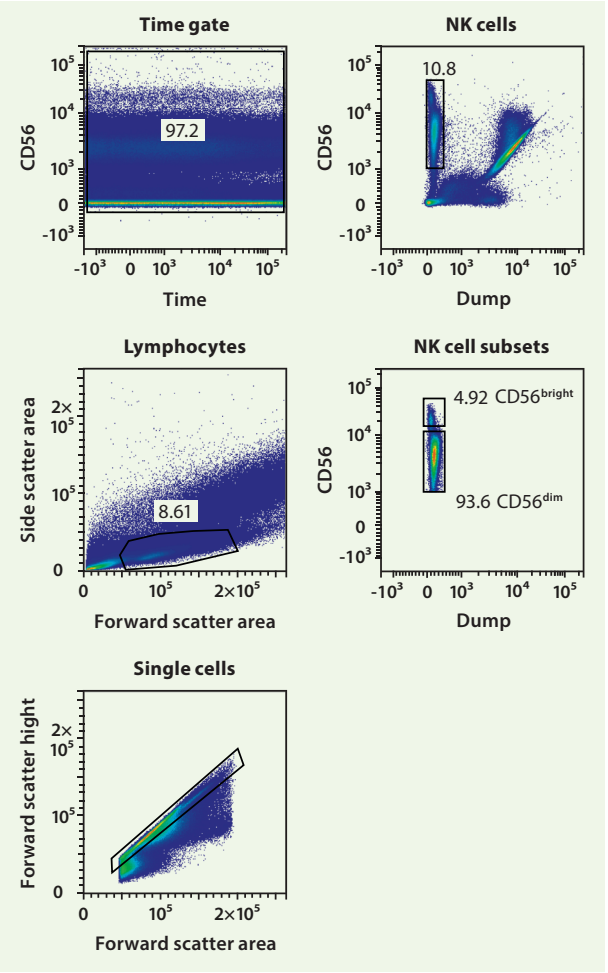


Figure 1: Gating strategy of NK cells. Dump channel contains CD3, CD14, CD19, and dead cells.

Results

Results presented in figure 2 confirm that NK cells express 13 chemokine receptors: CCR1, CCR2, CCR4, CCR5, CCR6, CCR7, CMKLR1, CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, and CX3CR1. The expression level differs between CD56^{bright} and CD56^{dim} NK cells with CCR5, CCR7, and CXCR3 being primarily expressed on CD56^{bright} NK cells, and CCR6, CMKLR1, CXCR1, CXCR2, and CX3CR1 on CD56^{dim} NK cells. As expected, only very small populations of CCR9^{+} and CCR10^{+} NK cells were identified[10]. CCR3 and CXCR5 were not expressed on NK cells (fig. 3).

After stimulation with IL-15, a cytokine that is known to induce proliferation and increase cytotoxic abilities of NK cells, the expression of several receptors was altered (fig. 2).

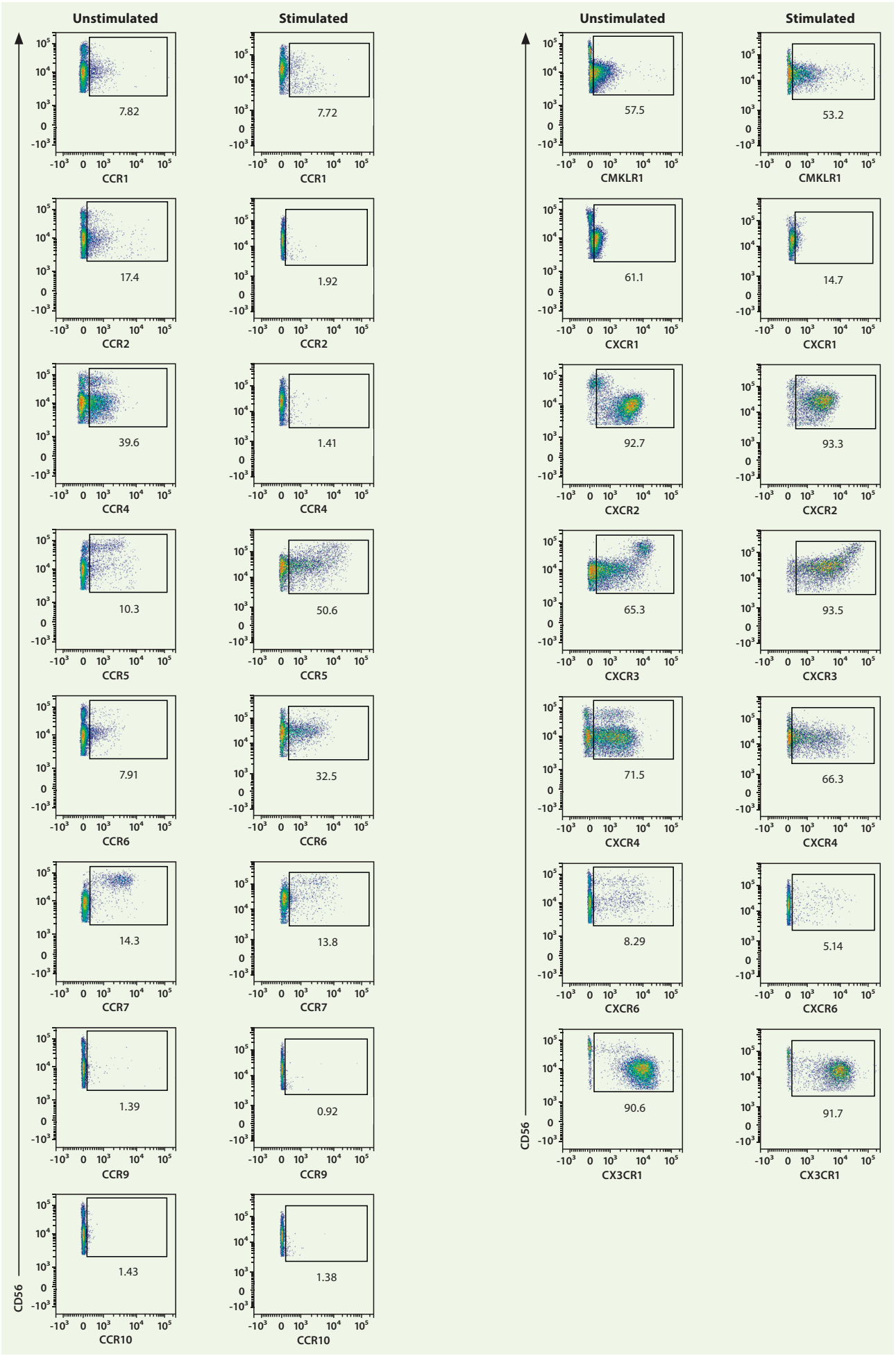


Figure 2: Chemokine receptor expression on CD56^{bright} and CD56^{dim} NK cells. Results are shown for unstimulated NK cells versus NK cells stimulated with IL-15.

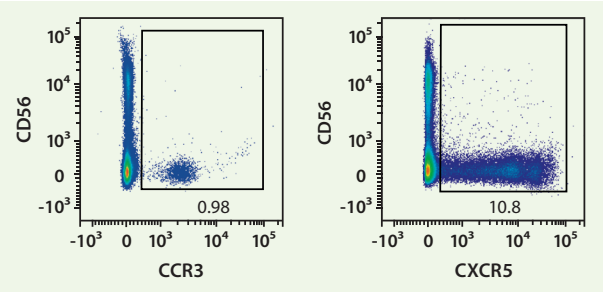


Figure 3: CCR3 and CXCR5 expression on total, unstimulated lymphocytes.

Conclusion

- REAfinity Recombinant Antibodies tested in this study ensure reliable detection of multiple chemokine receptors.
- The knowledge generated by deep and thorough analysis of chemokine receptors on NK cells will be helpful to scientists to assess their role in health and disease.

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