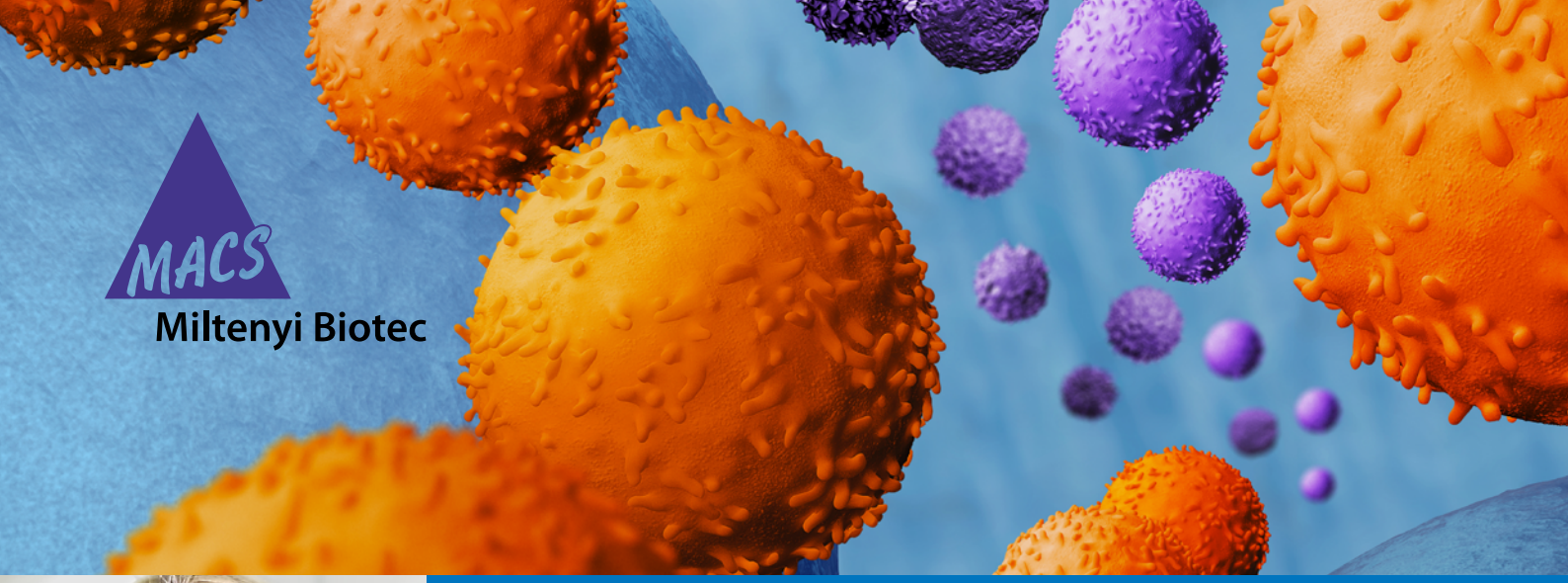


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CD56$^+$ cell depletion from human PBMCs applied to validate an HIV-specific ADCC mechanism

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Ariola Bardhi is a senior Ph.D. student in the Laboratory of Dr. Harris Goldstein at Albert Einstein College of Medicine (AECOM), New York City, USA. Her scientific expertise lies in the field of HIV immunology, particularly antibody-based therapeutic candidates and HIV mouse models. She works in two inter-institutional collaboration projects and a third project in collaboration with a biotech company. During her studies at AECOM, Ariola co-founded the organization INet NYC for the professional development of foreign scientists.

"As a laboratory working on HIV-1 immunology and studying different ways to enhance effector cell activity against HIV-infected cells, we work with a lot of primary human PBMC and mouse models. Our studies often require separation of different cell populations, such as CD4$^+$ T cells for infection or CD8$^+$ T cells and NK cells to study different anti-viral effector functions, etc. As such, we need the ability to separate large numbers of cells at high purity and good cell viability. We have been relying on the MACS® MicroBeads and the autoMACS® Pro Separator, which simplify the system and ensure great quality of work. Because we deal with large cell numbers, the autoMACS Pro Separator ensures that all cells are run through the columns and washed identically in an automated process. This increases the ability to multitask and maintains a great cell viability, which is very important to us."



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CD56 MicroBeads, human

Positive selection or depletion of natural killer (NK) cells from human PBMCs.

Background

According to the WHO there were 36.7 million individuals living with HIV worldwide at the end of 2016[1]. Even though our knowledge of the virus and disease progression increased a lot during the last 3 decades, currently, there is no effective vaccine or cure for HIV. One limitation to study the course of infection and therapeutic candidates was the lack of suitable small animal models, as HIV infects human cells only. The Goldstein Lab constructed the hu-spl-PBMC-NSG model by co-injecting HIV and activated human PBMCs isolated from healthy donors into the spleens of NOD SCID Gamma$^{-/-}$ (NSG) mice[2]. An HIV replication competent molecular clone that expresses the *Renilla* luciferase reporter gene was used for infection, as the reporter allows for the quantification of virus levels via luciferase activity measurement[3]. We used this mouse model to test the efficacy of broadly neutralizing antibodies (bNAb) against HIV, and specifically to study bNAb antibody-dependent cellular cytotoxicity (ADCC) activity.

Experimental setup

In vivo model to test bNAb ADCC activity

Hu-spl-PBMC-NSG mice were generated[2] and HIV-infection of target CD4$^+$ cells within the human PBMC population in the spleen was demonstrated via IVIS *in vivo* imaging five days after HIV-injection[2]. The mice were then treated with a novel engineered bispecific hexavalent defucosylated antibody, which shows enhanced ADCC activity due to its enhanced binding ability to FcγR IIIa (CD16) on the NK cell surface. One day later, the luciferase activity in the harvested spleens of treated versus untreated animals was compared in order to detect virus levels. As the HIV life cycle takes at least 24 hours, detection of HIV one day after treatment indicated antibody effector functions like ADCC rather than neutralizing antibody activity[4]. Additionally, as a negative control, a group of the hu-spl-PBMC-NSG mice received CD56-depleted PMBCs to validate the underlying ADCC mechanism.

CD56$^+$ cell depletion from human PBMCs

Human PBMCs were isolated from healthy donors via density gradient centrifugation. The NK cell fraction was depleted from PBMCs by incubation with MACS® CD56 MicroBeads, human (#130-050-401) and subsequent separation using MACS LS Columns (#130-042-401) for manual separation following the manufacturer's instructions. The CD56$^-$ fraction was collected and stained with anti-human CD3-FITC and anti-human CD56-PE antibodies for 30 minutes at 4 °C. Samples were acquired using the BD™ LSR II Analyzer and data was analyzed with the FlowJo® Software.

Results and conclusion

An *in vivo* model to study the ADCC activity of antibodies to clear HIV-infected cells was generated. In order to proof ADCC-dependent clearance, CD56$^+$ NK cells – the cell population important for ADCC activity – were depleted from human PBMCs using CD56 MicroBeads, human. The immunomagnetic separation resulted in high quality depletion as confirmed by flow cytometric analysis after CD3 and CD56 immunofluorescence staining (fig. 1). Furthermore, cell viability of the CD56-depleted PBMC fraction was not affected by the separation process and the cells were further used for *in vivo* experiments. The high purity of the target cell population as well as the high target cell viability indicate that the same depletion strategy based on surface marker–specific MicroBeads can be used to investigate the role of other effector cell populations, even in an *in vivo* setting.

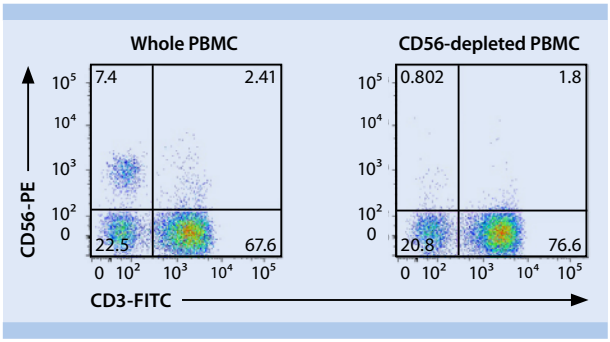


Figure 1: Flow cytometric analysis to evaluate CD56$^+$ cell depletion in human PBMC.

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