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OMIP-012: Phenotypic and Numeric Determination of Human Leukocyte Reconstitution in Humanized Mice

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

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• **Key terms**
cytometry; humanized mice; HIV; phenotyping; immune activation

Purpose and Appropriate Sample Types

This panel was developed to determine both the frequency and absolute number of human leukocytes and leukocyte subsets present in the peripheral blood of humanized mice. The panel also provides information concerning the activation state of peripheral leukocytes by cell surface staining for HLA-DR and CD38, relevant to studies of HIV disease pathogenesis (1–3). This panel has been used with EDTA anticoagulated whole blood in conjunction with bead-based enumeration for quantitative assessment of human cell chimerism. This panel also works well for staining of peripheral blood mononuclear cells (PBMCs) prepared by density gradient centrifugation and for dispersed splenocytes. The multicolor panel described here has been used in studies of humanized mouse reconstitution (4) and longitudinal studies of HIV pathogenesis in NSG-BLT mice (5).

Background

The development and utilization of humanized mice has accelerated greatly over the past decade as the importance of small animal models for human infectious disease research has become more recognized (6). The need is most apparent in HIV research where there is a strong push to evaluate current and prospective antiretroviral agents for their prophylactic potential to prevent HIV transmission (7,8). Here, the inability of HIV to infect mouse $CD4^+$ T cells is circumvented by the implantation of human thymus and liver tissue under the kidney capsule of immunodeficient strains of mice, which develops into a Thy/Liv organoid replete with human T cell targets for HIV. The recognition that particular mutations specific to the NOD strain of mice (9), plus knockout of the cytokine common gamma chain (10,11), allows for peripheral dissemination of human leukocytes throughout the mouse periphery has opened the door to studying the natural routes of HIV transmission in these mice. Unfortunately, varying levels of mouse hematopoietic recovery following irradiative preconditioning makes for an unreliable denominator when ascertaining the percentage of human leukocyte chimerism in mouse peripheral blood. This necessitates the need for quantitative enumeration of human cells on a per unit volume basis for reliable assessment of human cell engraftment. To that end, we have employed a bead enumeration technique using BD Trucount tubes in which the number of events within each cell gate is related to a known number of fluorescent beads to calculate the absolute number of cells per unit volume of whole blood.

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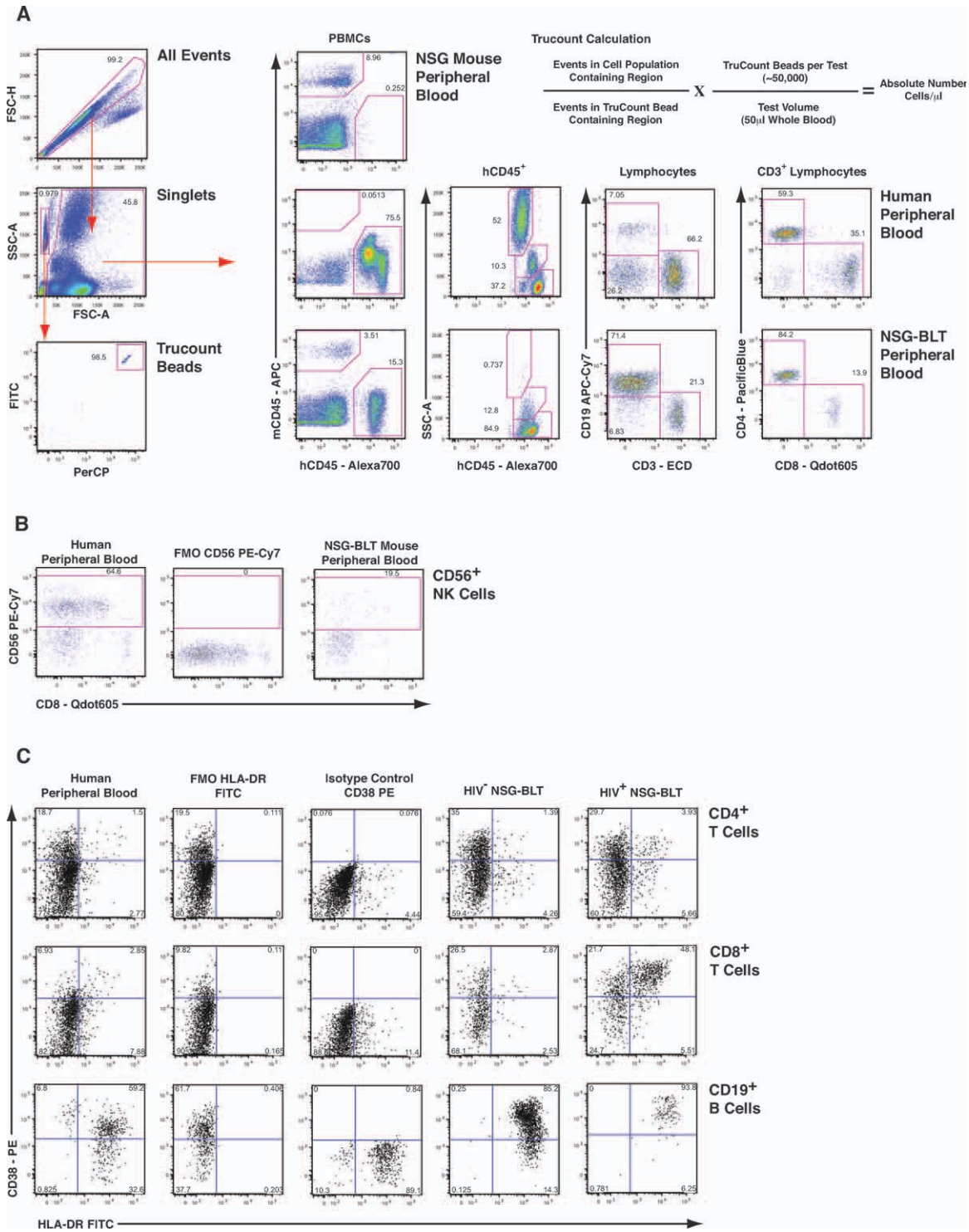

Figure 1. Classification of human leukocyte subsets in NOD.Cg-*Prkdc*^{scid}*Il2r*γ^{tm1Wjl}/SzJ (NSG)-BLT mice and evaluation of lymphocyte activation in HIV-infected mice. **A**: Left column; samples were first gated for singlet events using a FSC-A by FSC-H plot. Singlet events were then gated for Trucount beads and PBMCs. Trucount tubes were used according to manufacturer's instructions. Briefly, 50 μl of antibody master mix was added to each tube followed by the addition of 50 μl EDTA anticoagulated whole blood and briefly vortexed. After 15 min incubation, 450 μl of BD FACS-Lyse solution was added. Samples were then stored at 4°C until acquisition (within 6 h). The number of Trucount beads is used in conjunction with the number of events within each cell gate to calculate the absolute number of cells per unit volume of whole blood using the formula shown. At right, PBMC were separated by species of origin based on CD45 expression using mouse and human specific anti-CD45 antibodies. Examples are shown for an unmanipulated NSG control mouse (top), a human peripheral blood control (middle), and a humanized NSG-BLT mouse (bottom). Human CD45$^+$ leukocytes were further typed into neutrophils (SSC-A high), monocytes (SSC-A intermediate), and lymphocytes (SSC-A low). Lymphocytes were separated into CD19$^+$ B cells, CD3$^+$ T cells, and a double-negative population. CD3$^+$ T cells were separated into CD4$^+$ and CD8$^+$ T cells. We typically observe most human cells falling in the lymphocyte gate but have also noted that there is an increased frequency and a lower MFI for CD19$^+$ B cells in NSG-BLT mice. **B**: CD56$^+$ NK cells are enumerated from the CD3$^-$, CD19$^-$ (double negative) lymphocyte gate in ''A.'' A fluorescence minus one (FMO) gating control was used to set the NK cell gate (middle). **C**: CD4$^+$ T cells (top), CD8$^+$ T cells (middle), and CD19$^+$ B cells (bottom) were evaluated for the activation markers HLA-DR and CD38. Human EDTA anti-coagulated peripheral blood was used as a gating control and for both the FMO (HLA-DR FITC) and isotype (CD38-PE) control. In our experience, an isotype control has been more useful that an FMO for PE staining of whole blood, as there appears to be some nonspecific background binding of PE. (This is not observed when using density gradient separated PBMC or splenocytes). Examples are shown for an NSG-BLT mouse at the time of inoculation (intravaginal with 220,000 $TCID_{50}$ JR-CSF) when it was HIV-1 negative, and the same mouse 8 weeks later with HIV viremia (HIV RNA 3.86 $\log_{10}$ copies/100 μl plasma), displaying increased expression of HLA-DR and/or CD38 on each of the lymphocyte populations.

Table 1. Summary Table for OMIP-012

Purpose	Human leukocyte reconstitution and phenotype
Species	Humanized mouse
Cell type	Whole blood, Ficoll-separated PBMC, splenocytes
Cross reference	None to date

Table 2. Reagents used for OMIP-012

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
Mouse CD45	30-F11	APC	Exclusion of mouse leukocytes
Human CD45	HI30	Alexa 700	Human leukocyte detection
Human CD3	UCHT1	ECD	Human T Cell detection
Human CD19	HIB19	APC-Cy7	Human B-cell detection
Human CD56	HCD56	PE-Cy7	Human NK cell detection
Human CD4	RPA-T4	Pacific Blue	Human CD4+ T cell subset
Human CD8	3B5	Qdot-605	Human CD8+ T cell subset
Human HLA-DR	L243	FITC	Activation
Human CD38	HIT2	PE	Activation
Isotype Control	MOPC-21	PE	Set gate for CD38 expression[a]

[a] We detect a significant level of nonspecific PE binding in whole blood samples that necessitates the use of an isotype control rather than an FMO control.

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