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OMIP-026: Phenotypic Analysis of B and Plasma Cells in Rhesus Macaques

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• **Key terms**
B cells; Rhesus Macaques; African green monkeys; OMIP

Purpose and Appropriate Sample Types

Our purpose was a broad phenotypic analysis of B and plasma cells regarding differentiation status, activation, proliferation, and chemokine receptor expression in rhesus macaques. We developed two staining panels, which were tested on fresh samples of whole blood or peripheral blood mononuclear cells (PBMCs), bone marrow collected from the iliac crest and femur, lymph nodes, spleen, and tonsils (Figure 1, Table 1). A 10-color-panel was developed to mainly focus on B cells, whereas a 11-color panel concentrated on plasmablasts/plasma cells. Both panels are also applicable for whole blood and bone marrow samples from African green monkeys.

Background

B cells play crucial roles in a variety of infectious or autoimmune diseases, but still only few studies focused on B cells in rhesus macaques, which serve as useful models for various human diseases (1,2). To fully characterize rhesus B and plasma cells we sought to analyze a multitude of markers, including CD45 for lymphocyte identification, CD3, CD19, CD20, CD38, and CD138 to identify T, B, and plasma cells, CD10 to distinguish between immature and mature B cells as well as IgD, CD21 and CD27 to discriminate between naïve and memory B cell subsets. Furthermore, activation and proliferation of B cells via analysis of CD69, CD80, CD95, and Ki67 and expression of the chemokine receptors CCR7 (CD197) and CXCR4 (CD184) were included. The markers had to be split into two staining panels, since flow cytometric analysis of different cell types in non-human primates mainly relies on the availability of cross-reactive anti-human monoclonal antibodies (3,4) and is restricted due to the offered fluorochrome variety. Both panels included CD3, CD10, CD20, CD27, and CD45, whereas the 10-color B cell staining panel additionally allowed analysis of CD21, CD69, CD80, CD197, and IgD (Table 2; Supporting Information Table 3). Antibodies against CD19, CD38, CD138 as well as Ki67, CD95, and CD184 were added to the plasma cell staining panel to characterize plasmablast/plasma cells in more detail (Table 2; Supporting Information Table 3). Optimization of both panels included validation of cross-reactive clones and analysis of best antigen–fluorochrome combinations. Our analysis underline the importance of verifying reported cross-reactivity, the applicability of anti-human monoclonal antibodies for analysis of non-human primate cells and use of appropriate fluorochrome conjugates. The CD80 clone 2D10—despite reported cross-reactivity—did not allow to distinguish between positive and negative cells (Supporting Information Fig. 4A). Additionally, the detection of a $CD19^{+}$ $CD20^{-}$

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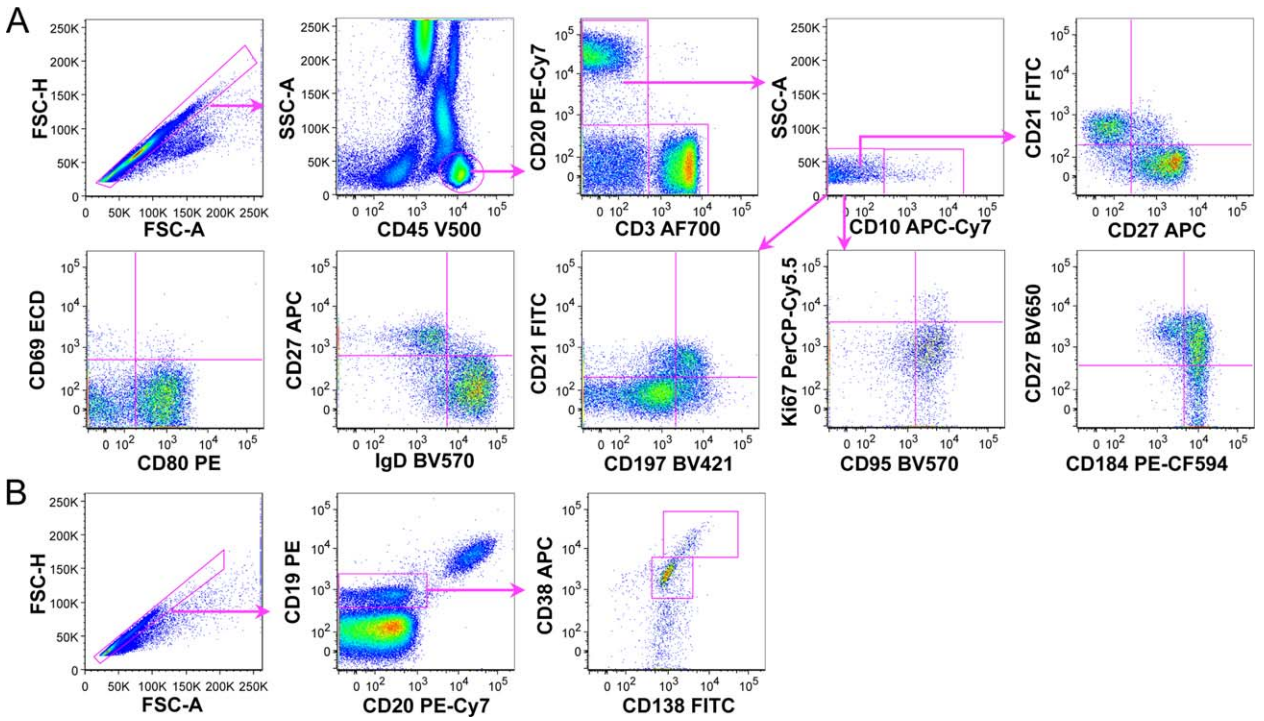

Figure 1. B and plasma cell gating strategy in rhesus macaques. **A**: B cell gating strategy. Following exclusion of duplets lymphocytes were gated based on CD45 expression. $CD3^-$ $CD20^+$ B cells were selected and immature and mature B cells were distinguished based on CD10 expression. Expression of indicated markers was analyzed on $CD10^-$ mature B cells. Data from whole blood are shown. **B**: Plasmablast and plasma cell gating strategy. Plasmablasts were defined as $CD19^+$ $CD20^-$ $CD38^+$ $CD138^+$ and plasma cells as $CD19^+$ $CD20^-$ $CD38^{++}$ $CD138^{++}$. Data from bone marrow extracted from the iliac crest are shown. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 1. Summary table for OMIP-026

PURPOSE	PHENOTYPE OF B AND PLASMA CELLS
Species	Rhesus macaque (*Macaca mulatta*); African green monkey (*Chlorocebus aethiops*)
Cell types	Fresh whole blood, PBMCs, mononuclear cells of bone marrow, lymph node, spleen, tonsil
Cross-references	None

Table 2. Reagents used for OMIP-026

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
Backbone			
CD45	D058-128	V500	Leukocyte definition
CD10	HI10a	APC-Cy7	Maturation marker
CD3	SP34-2	Ax700	Lineage
CD20	L27	PE-Cy7	Lineage
B cell staining			
CD21	B-Ly4	FITC	Differentiation
CD27	M-T271	APC	Differentiation
CD69	TP1.55.3	ECD	Activation
CD80	L307.4	PE	Activation
CD197	G043H7	BV421	Homing
IgD	Polyclonal	Biotin	Ig class switching
Plasma cell staining			
CD19	J3.119	PE	Lineage
CD27	O323	BV650	Differentiation
CD38	OKT10	APC	Differentiation; plasma cells
CD138	DL-101	FITC	Plasma cells
CD95	DX2	Biotin	Activation
CD184	12G5	PE-CF594	Homing
Ki67	B56	PerCP-Cy5.5	Proliferation
Biotin	SAV	BV570	Counterstain of Biotin-conjugated CD95 and IgD

Ax, Alexa; APC, allophycocyanin; BV, Brilliant Violet; Cy, cyanin; FITC, fluorescein isothiocyanate; PE, R-phycoerythrin; SAV, streptavidin.

cell population was only possible when using a PE- instead of a PerCP-Cy5.5-conjugated CD19 antibody (Supporting Information Fig. 4B). To discriminate between naïve and memory B cells, initially the CD27 clone O323 conjugated with PerCP was tested. Since it performed insufficiently, it had to be exchanged with an antibody conjugated with a brighter fluorochrome (Supporting Information Fig. 4C).

Recently, we have applied both staining panels for a comprehensive analysis of B and plasma cells in blood, bone marrow, lymph nodes, spleen, and tonsils of healthy rhesus macaques (5). Since these panels can also be used for African green monkeys, this will broaden their application for research on B and plasma cells in different disease models.

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