

Antibody- Dependent Cellular Phagocytosis of HIV-1-infected cells is efficiently triggered by IgA targeting HIV-1 envelope subunit gp41

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Supplementary data

Supplementary figure 1: HIV-1-specific-IgA and IgG are unable to mediate ADCP of HIV-1-infected CEM-NK_r CCR5 CD4⁺T cells by monocytes.

CD4⁺T cells CEM-NK_r CCR5 were infected with JR-CSF clade B HIV-1 for 72hrs and incubated with 0.5µg/ml of 2F5-IgA/2F5-IgG/2G12-IgG or hIgA/IgG for 30min at 37°C. Primary monocytes were stained with 0.1µM intracellular CellTracker™ Deep Red Dye, added to opsonized infected target cells and allowed to phagocyte for 3hrs. Cells were washed, fixed, permeabilized before intracellular staining with anti-p24 FITC Ab and analyzed by flow cytometry. ADCP percentage is determined as described in the Method section. Values represent means of HIV-1-specific infected cells ADCP % ± SEM, from 3 independent experiments performed in triplicate, NS: $p > 0.05$, unpaired Student's *t*-test.

Supplementary figure 2: Live but not apoptotic HIV-1-infected primary CD4⁺T

lymphocytes are phagocytosed by monocytes triggered by 2F5-IgA.

Primary CD4⁺T cells were infected with JR-CSF clade B HIV-1 for 72hrs. Dead cells were then removed from target cells or not before incubation with 0.05µg/ml or 0.2µg/ml of 2F5-IgA for 30min at 37°C. Primary monocytes were stained with 0.1µM intracellular CellTracker™ Deep Red Dye, added to opsonized infected target cells and allowed to phagocyte for 3hrs. Cells were washed, fixed, permeabilized before intracellular staining with anti-p24 FITC Ab and analyzed by flow cytometry. ADCP percentage is determined as described in the Method section. Values represent means of HIV-1-specific infected cells ADCP % ± SEM, from at least 2 independent experiments performed in triplicate, NS: $p > 0.05$, unpaired Student's *t*-test.

Supplementary figure 3: 2F5-IgA and 2F5-IgG do not cooperate to increase ADCP of HIV-1-infected primary CD4⁺T lymphocytes by monocytes.

Primary CD4⁺T cells were infected with JR-CSF clade B HIV-1 for 72hrs and incubated with indicated concentrations of 2F5-IgA and/or 2F5-IgG for 30min at 37°C. Primary monocytes were stained with 0.1µM intracellular CellTracker™ Deep Red Dye, added to opsonized infected target cells and allowed to phagocyte for 3hrs. Cells were washed, fixed, permeabilized before intracellular staining with anti-p24 FITC Ab and analyzed by flow cytometry. ADCP percentage is determined as indicated in the Method section. Values represent means of HIV-1-specific infected cells ADCP % ± SEM, from at least 2 independent experiments performed in triplicate, NS: $p > 0.05$, unpaired Student's *t*-test.

Supplementary figure 4: Anti HIV-1 envelope-mediated IgG ADCP of HIV-1 infected CD4⁺T lymphocytes by neutrophils is inefficient.

Primary CD4⁺T cells were infected with HIV-1 and opsonized with 1 μg of 2F5-IgA, 2F5-IgG or 2G12-IgG for 30min at 37°C. Primary neutrophils were pre-stained with 0.1 μM intracellular CellTracker™ Deep Red Dye, added to opsonized infected target cells and allowed to phagocytose for indicated times. Cells were washed, fixed, permeabilized, stained intracellularly with anti-p24 FITC Ab and analyzed by flow cytometry as in Figure 4. ADCP percentage in each condition is determined as indicated in the Method section. Values represent means of HIV-1- infected cell specific ADCP % ± SEM, from at least 2 independent experiments performed in triplicate.

Supplementary figure 5: Gating strategy to quantify ADCP of HIV-1-infected cells by flow cytometry. (A) Live cells are selected based on SSC/FSC profile. (B) Doublets are excluded to discard CD4⁺T cells/effector cells conjugates. (C) Effector cells (EC) labeled with CellTracker™ Deep Red Dye (DR) are selected based on the DR fluorescence. (D) Finally, the percentage of EC that have phagocytosed HIV-1-infected CD4⁺T cells is determined by establishing a gate excluding the 5th percentile of negative control (in the absence of antibody) allowing the calculation of HIV-1-specific ADCP by subtracting background events, as indicated in the Method section.