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TLR7 Agonist GS-986 Markedly Activates T & B Cells From ART-suppressed Donors

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Introduction

TLR7 is expressed within plasmacytoid dendritic cells and B cells, and senses viral RNA to link innate and adaptive immune responses. The TLR7 agonist GS-986 (an analog of GS-9620) induces viral blips after 3-4 biweekly doses in SIV$^+$ rhesus macaques on ART by unknown mechanisms.

We investigated the immunologic and virologic effects of GS-986 *ex vivo* using cells from HIV-infected donors on long-term ART.

Methods

PBMCs and CD8$^+$ T cell or B cell-depleted PBMC derived from HIV$^+$ donors on ART were isolated from fresh blood or leukopaks and cultured for 5 days with or without 100nM GS-986.

Cell-associated HIV-1 DNA (CA-DNA), unspliced RNA (CA-RNA) and virion release were measured by qPCR (Hong *et al*, J Clin Micro 2016)

Immune cell phenotype, cytokine production, proliferation, and antibody levels were evaluated by flow cytometry, Luminex, CFSE, and ELISA assays, respectively.

After 5 days of culture, resting and total CD4$^+$ T cells were isolated and assayed for the number of cells releasing virions following maximal stimulation with PMA/ionomycin (iono).

Results

PBMC were obtained from 9 donors (5 blood; 4 leukopak) on suppressive ART (median duration: 7 years). Exposure to 100nM GS-986 for 5 days did not consistently alter CA-DNA or CA-RNA per 1M CD4$^+$ cells, virion release, or the number of cells releasing virions after subsequent PMA/iono stimulation. CD8- or B cell-depletion did not affect virologic responses (**Fig 1**).

GS-986 was immunologically active in all donors, doubling the proportion of CD4/CD8 double-positive (DP) T cells and having the greatest effects on CD25, CD69 and HLA-DR in this population (mean absolute increases of 11.4%, 11.9%, 18.4%, respectively) (**Fig 2**).

11% of naïve B cells became activated and recall of memory plasma B cells was induced to 29%, with large increases in secreted IgM (mean 60-fold) and IgG (mean 34-fold increase), and induction of 6 rounds of proliferation by CFSE (**Fig 3**). GS-986 induced multiple cytokines, most notably IL-1β, IL-6 and IL-10 with mean increases of 6.4-, 250- and 8.2-fold, respectively (**Fig 4**).

Figure 1 **Median Virologic Measures**

	Control	GS-986	Control CD8 Depletion	GS-986 CD8 Depletion	Control B cell Depletion	GS-986 B cell Depletion
Single Copy Assay (SCA) Avg HIV-1 RNA cp/mL	<1	<1	1	<1	<1	<1
Cell-Associated HIV-1 DNA Normalized per 1M CD4	4171 ± 2469	5658 ± 3443	4534 ±	2887 ±	6768 ±	3399 ±
Cell-Associated HIV-1 RNA Normalized per 1M CD4	927 ± 1185	125 ± 3131	677 ±	988 ±	344 ±	353 ±

Figure 2 **Lymphocyte Proportions and Activation Status After 5 Day Stimulation**

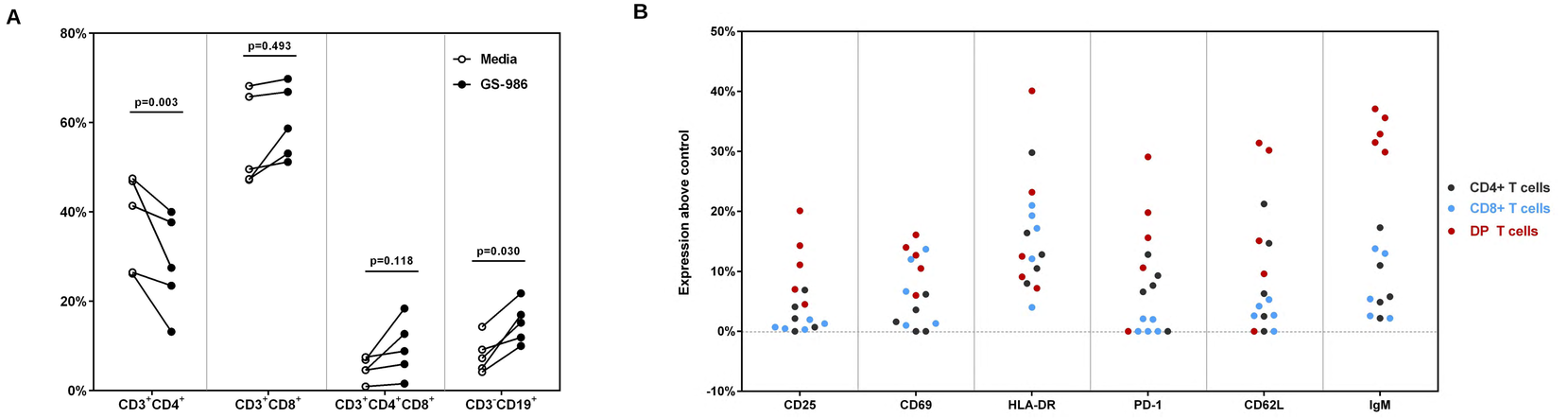


Figure 3 **GS-986 Induced Lymphocyte Proliferation and Antibody Production**

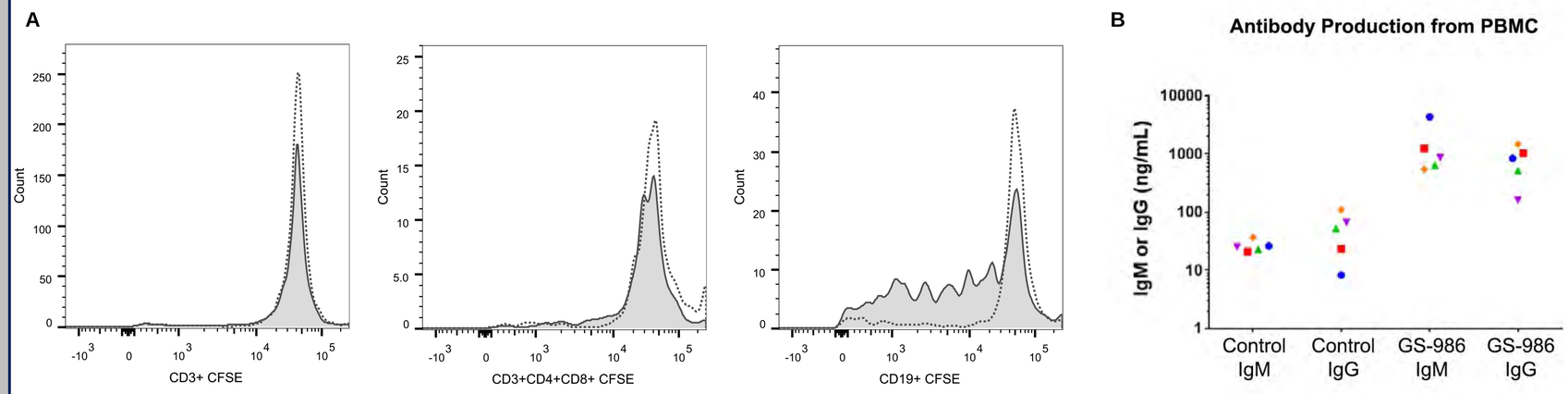
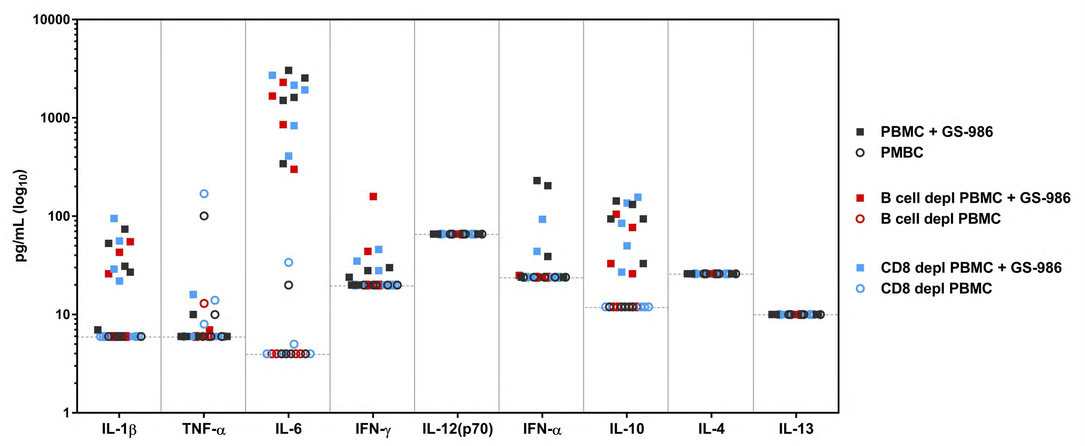


Figure 4 **Cytokine Production After 5 Day Stimulation**



Conclusions

- *Ex vivo* exposure of PBMC cultures to GS-986 for 5 days did not consistently alter virologic measures.
- *Ex vivo* culture systems involving a single exposure to GS-986 induce immunologic responses, particularly in B cells.
- However, they do not recapitulate the consistent effect of multiple oral doses of the TLR7 agonist on viremia observed in SIV-infected macaques. Cumulative responses from repeated cycles of stimulation may be required to achieve such effects.

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