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HIV-1 gp120 T-Cell Responses Correspond to Infection Outcomes in the Global iPrEx Chemoprophylaxis Trial

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Abstract: Association of HIV-1–specific T-cell responses to infection risk in seronegative individuals is controversial. We quantified and phenotypically characterized gp120-specific T-cell responses in HIV-1 exposed, but uninfected subjects enrolled in the global Pre-exposure Prophylaxis Initiative (iPrEx) chemoprophylaxis trial. IFNγ ELISpot responses were detected in 24% of subjects irrespective of infection outcome. HIV-1 gp120 envelope-specific T-cell responses were more uniformly IFN-γ+TNF-α+Mip-1β+ in persistently seronegative subjects relative to subjects who later seroconverted (median frequency of 76.5% and 66.5%, respectively). IFNγ responses targeted the V2 loop for subjects who remained seronegative. HIV-1 gp120 envelope V2 loop-specific $CD8^+$ T-cell responses may help to protect against HIV-1 acquisition.

Key Words: HESN, polyfunctionality, T cell, $CD8^+$, vaccine

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INTRODUCTION

The RV144 immune correlates analysis was an important achievement, establishing the framework on which future prophylactic HIV-1 vaccines are being developed. The analysis showed that a humoral immune response of the right immunoglobulin class to the V1V2 region of gp120 was associated with vaccine efficacy.[1] The vaccine regimen induced T-cell responses, in particular $CD4^+$ polyfunctional T cells,[2] but this was not associated with infection outcomes in the analysis of primary variables despite suggestions of an association among secondary variables.[1]

The association of T-cell responses and reduced infection risk is controversial. Inducing T cells using an adenovirus-vectored Gag/Pol/Nef vaccine failed to protect human vaccines from HIV-1 infection and may have increased infection risk.[3,4] In contrast, results from cytomegalovirus-vectored simian immunodeficiency virus vaccination of nonhuman primates showed vaccine-elicited $CD8^+$ T cells, which associated with viral suppression.[5] We have also recently shown a reduced infection risk associated with naturally acquired T-cell responses in the iPrEx trial.[6]

More recently, a reanalysis of the RV144 T-cell response data suggested that HIV-1 gp120 envelope-specific T-cell responses are part of the protective immune response in vaccines. This reanalysis used the novel, open source analytical tool COMPASS, enabling a more thorough dissection of the complexities of T-cell polyfunctionality and overall response in RV144. The results indicated that the $CD4^+$ T-cell responses had protective associations that were comparable in magnitude with the previously reported humoral responses.[7] The totality of the RV144 data indicated that vaccine-induced HIV-1 prophylactic efficacy is linked to both humoral and cellular immunity and that the qualitative characteristics are critical in determining outcomes. The results also suggested that a response against a single protein antigen, with the optimal qualitative characteristics, may be sufficient for protection.

The RV144 analyses were constrained to immune responses mechanistically related to the vaccination and study-related variables and thus not designed to address other mechanisms of reduced HIV-1 infection risk. One mechanism of potential-reduced infection risk, vaccine-induced HIV-1–specific $CD8^+$ T-cell responses, could not be rigorously tested in the correlates analysis because of the low frequency, consistent with the vaccine's design. We have shown that HIV-1–specific T-cell responses are present in some HIV-1–exposed seronegative (HESN) subjects and that certain responses were associated with reduced HIV-1 infection risk.[6] In that initial study, responses to the gp120 protein antigen were not assessed.

In light of the RV144 results and evidence of protective naturally acquired T-cell immunity, it may also be possible that $CD8^+$ gp120-specific T-cell responses could contribute to protection. In support of this are reports of naturally induced T-cell responses specific for gp120 described in cohorts of

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HIV-1 exposed but persistently seronegative individuals, both from sexual and occupational HIV-1 exposure.[8–11] We hypothesized that gp120-specific $CD8^+$ T-cell responses, quantitatively or qualitatively, would be associated with infection risk among comparably exposed placebo-treated individuals in the global Pre-exposure Prophylaxis Initiative (iPrEx) chemoprophylaxis trial.[12]

METHODS

Our study was designed as a case–control cross-sectional analysis of gp120-specific IFNγ cellular immune responses with phenotypic characterization of positive responses. We examined gp120-specific cellular immune responses from preinfection-cryopreserved peripheral blood mononuclear cells (PBMCs) from 25 subjects who seroconverted during the follow-up period of the iPrEx trial (median time-on-study at the time of documented infection was 561 days for this group). The most proximal HIV-1–negative time point relative to diagnosis of HIV-1 infection was used for the analyses with HIV-1–negative status at this time point determined by a combination of HIV-1 antibody and HIV-1 RNA testing. These subjects were designated seroconverters–before infection (SC-BI). Each SC-BI was matched to 3 persistently HIV-1–negative trial participants (HESN; n = 75) from the same enrollment site with comparable time-on-study as the SC-BI participant (n = 75). All PBMC specimens used in the study were obtained from consented participants of the iPrEx trial completed in 2010 (registered with ClinicalTrials.gov, NCT00458393). All subjects were randomized to placebo in the trial.

We used a prespecified 2-step process first screening with an IFN-γ ELISpot assay followed by phenotypic characterization and response confirmation with multiparametric flow cytometry (MFC) where PBMCs were available from the same aliquot.

IFN-γ ELISpot assays were conducted with peptide pools consisting of 15-mer peptides overlapping by 11 amino acids (AA) from NIH AIDS Research and Reagent Program (Rockville, MD) that spanned gp120 protein antigen with sequences corresponding to HIV-1 consensus B sequence. A gp120 pool and series of matrix peptide pools along with individual peptide preparations were used in the experiment. All peptides were reconstituted, pooled, and used at a final concentration of 5 μg/mL. PBMCs were plated at a concentration of 1×10^5 cells/well and incubated overnight (16–18 hours). Two positive controls (phytohaemagglutinin, and a peptide pool consisting of cytomegalovirus, Epstein Barr virus, and influenza virus peptides) were also included. No reassays were permitted and all data included in the analyses to ensure objectivity. A positive gp120 response in ELISpot was defined as ≥55 spots per million PBMCs after subtracting twice the spot count in the negative control well.

MFC was performed on the same PBMC aliquot from which ELISpot responses were detected and within 1 day of thawing. All runs included an unstimulated negative control condition. Costimulatory anti-CD28 and CD49d mAbs were added for every 1×10^6 cells in all conditions. Peptides were added at 5 μg/mL incubated for 1 hour at 37°C after which 50 μL of Brefeldin A was added then incubated over night. Cells were stained in the presence of human IgG (Sigma) with Pacific Blue anti-CD3 (BioLegend), Brilliant Violet 605 anti-CD8 (BioLegend), Brilliant Violet 650 anti-CD4 (BioLegend), and allophycocyanin/cyanine 7 anti-CD14 and anti-CD19 (Biolegend). Amine Aqua (Life technologies) was included in each stain to exclude dead cells. The cells were fixed, washed, pelleted, and permeated. Cells were then stained intracytoplasmically with allophycocyanin anti-IFNγ (BD Pharmingen), TNF-α with Alexa Fluor (BD Pharmingen), and Mip-1β with PE-A (BD Pharmingen). Cells were washed, pelleted, and fixed a final time. Samples were analyzed on a 4-laser LSR II flow cytometer (BD Biosciences, San Jose). Data analysis was performed using FlowJo Version 9.6.1 software (TreeStar, Ashland, OR). A minimum number of 50 events were required before the population could be considered.

RESULTS

All subjects mounted IFN-γ responses to either the positive peptide pool or phytohaemagglutinin control; no statistically significant differences were noted between SC-BI and HESN. The frequency of positive gp120-specific responses was equal between groups at 24%. Median (interquartile range) spots per million counts for SC-BI and HESN were 775 (188–995) and 160 (106–569), respectively (Fig. 1A). The difference in magnitude of response for SC-BI and HESN did not meet statistical significance.

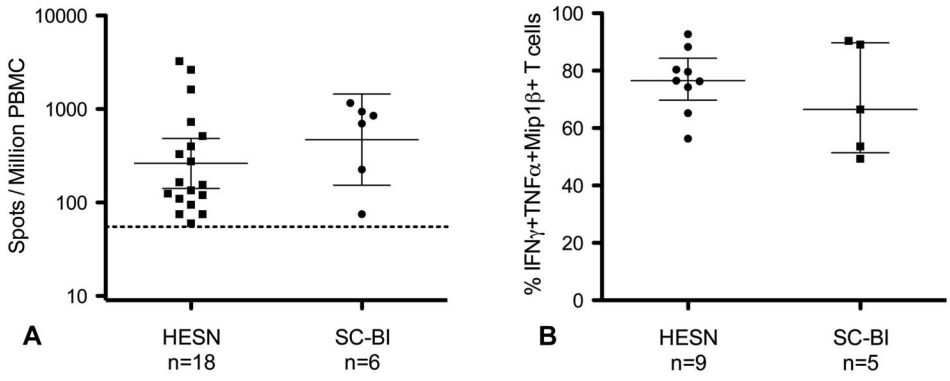

FIGURE 1. ELISpot and polyfunctionality. A, ELISpot results. Of the 25 SC-BI tested, 6 (24%) had counts above the 55 spot per million threshold (indicated by the dotted line) with a median [interquartile range (IQR)] of 775 (188–995). An equal frequency (24%) of HESN, or 18/74, had counts above the threshold with a median (IQR) of 160 (106–569). B, Multiparametric flow cytometry. Median (IQR) percent of IFNγ+TNFα+Mip-1β+ T cells of 76.5 (69.8–84.4) in 9 HESN and 66.5 (51.5–89.8) in 5 SC-BI.

IFN-γ responses to the gp120 peptide pool were confirmed by MFC in each of the 5 SC-BIs and 9 HESNs who had a positive T-cell response in ELISpot and sufficient PBMC to conduct the testing (Fig. 1B). Figure 2A shows a representative SC-BI and HESN gp120 response. Non-specific IFN-γ responses were low after incubation with cell culture medium alone in the presence of costimulation. Responses were $CD8^+$ T cell polarized in all cases tested with a trend of stronger responses in HESN when tested in MFC. Responses were detected on subsequent visits indicating persistence of responses in those tested (Fig. 2B). T-cell responses were more uniformly polyfunctional as measured by TNF-α and Mip-1-β coproduction in HESN in comparison with SC-BI with a median (interquartile range) frequency of 76.5 (69.8–84.4) and 66.5 (51.5–89.8), respectively (Fig. 1B). Insufficient numbers of subjects were studied to enable rigorous statistical comparisons of polyfunctionality.

We mapped epitope-specific responses in 1 SC-BI and 1 HESN from the same enrollment site, both at Day 144 of follow-up. We determined that the HESN subject reacted to at least 2 epitopes. One epitope comprised AA102–116 (based on the HXB2 sequence) lying proximal to the CD4 binding site and V1 loop region (Fig. 2C). The other epitope was located within the V2 loop region comprising HXB2 AA178-192, containing a glycosylation site at AA186-188. In contrast, the SC-BI subject demonstrated a weak reactivity to 2 overlapping 15-mer peptides on the gp120 pool. Both peptides spanned AA213-231, outside the V1V2 loop. The weaker response was against the peptide with a partial overlapping of an NKT glycosylation site (Fig. 2C). A second HESN from another enrollment site responded to a V2 loop epitope, HXB2 AA171-185 (Fig. 2C), proximal to the AA186-188 glycosylation site.

DISCUSSION

The importance of gp120 responses (both humoral and cellular) in the protection from a productive systemic infection with HIV-1 has been established. In the RV144 trial, T-cell responses in vaccines were $CD4^+$, targeted the V2 loop, and were associated with a predominance of IL2 or IFN-γ production, or a polyfunctional combination of the 2 in vaccinated subjects.[2] The COMPASS analysis shed further light on the qualitative characteristics strongly suggesting a $CD4^+$ T-cell response to be contributing to reduced infection risk. A corroborating study in rhesus monkeys also showed the importance of gp120 responses to protection.[13] These studies were not designed to assess other potentially protective immune responses.

Leveraging the placebo-controlled iPrEx trial with its 3-year follow-up period together with limiting our analysis to subjects receiving placebo only provided a naturalistic setting in which to investigate risk-associated gp120 T-cell responses. This unique approach enabled an assessment of HIV-1–specific immunity that was not biased by vaccination or pharmacologic interventions. In our study, we confirmed that HIV-1 gp120-specific IFN-γ responses were present in both SC-BI and HESN. The prevalence did not differ between the groups but the medians suggested a 4.8-fold greater response magnitude in SC-BI. Such results corroborate the potential of T-cell responses to increase infection risk.[3] However, the qualitative characteristics of the responses differentiated with infection outcomes similar to the COMPASS analysis and affirming the importance of qualitative factors of immune response in the overall impact of an immune response.

We observed a more homogenously polyfunctional response in HESN with a greater frequency of Mip-1β+ cells in HESN. This observation is analogous to the dominance of $CD4^+$ T-cell polyfunctionality observed in RV144 vaccines,[2] although the range of detected cytokines differed between the 2 studies. Our result also mirrors analyses conducted in HIV-1 vaccines immunized with a DNA prime/rAd5 boost that showed that $CD8^+$ polyfunctionality, inclusive of Mip-1β and CD107a, was correlated to antiviral activity.[14] Although our subject numbers were limited, which impacted our ability to conduct rigorous statistical testing and may have obscured the contribution of $CD4^+$ T-cell responses in the larger population, the overall trend suggests that a more uniformly polyfunctional T-cell response may be associated with the relative resistance to HIV-1 infection.

The most impressive corroborative link between our study and the RV144 analyses is the overlap of targeted epitopes. In RV144, responses were predominantly directed to the V2 loop region and specifically HXB2 AA172-204 containing the $\alpha_4\beta_7$-binding motif and glycosylation sites at AA186-188 and AA197-199.[2] In the mapped iPrEx participants, we observed similar regional targeting for 2 HESN but not the SC-BI. Importantly, one of the HESN came from the same enrollment site and the PBMC samples from the same protocol-defined visit as the SC-BI. However, the overlap with the findings in the RV144 analyses suggests that gp120 V2 loop-specific $CD8^+$ specific T-cell responses together with humoral and $CD4^+$ T-cell immune responses may be important for protection from HIV-1 infection.

We show that qualitative characteristics of gp120-specific $CD8^+$ T-cell responses differentiate with infection outcomes. The study was principally designed to assess ELISpot IFN-γ responses and to phenotypically characterize those that were positive. Thus, the number of studied responses overall and peptide-mapped responses in particular were small, and the range of cytokines was less expansive relative to those of previous studies.[1,2,6,7] In addition, 15-mer peptides are used for detection of both class-I and class-II T-cell responses so the absence of $CD4^+$ T-cell responses is likely stochastic. The study was also not designed to assess protection; therefore, the gp120-specific responses could be a marker of exposure and not related to mechanisms of resistance. However, the striking overlap between the observations in this study, particularly in a case–control pairing, to that of others linked with vaccine efficacy may suggest that polyfunctional $CD4^+$ and $CD8^+$ gp120-specifc T-cell responses could enhance the protective effect of a prophylactic HIV-1 vaccine. Therefore, a larger study of naturalistic exposure to HIV-1 with additional polyfunctionality variables is needed to make robust conclusions.

In conclusion, we observed strong, polyfunctional $CD8^+$ T-cell responses specific for the V2 loop of HIV-1

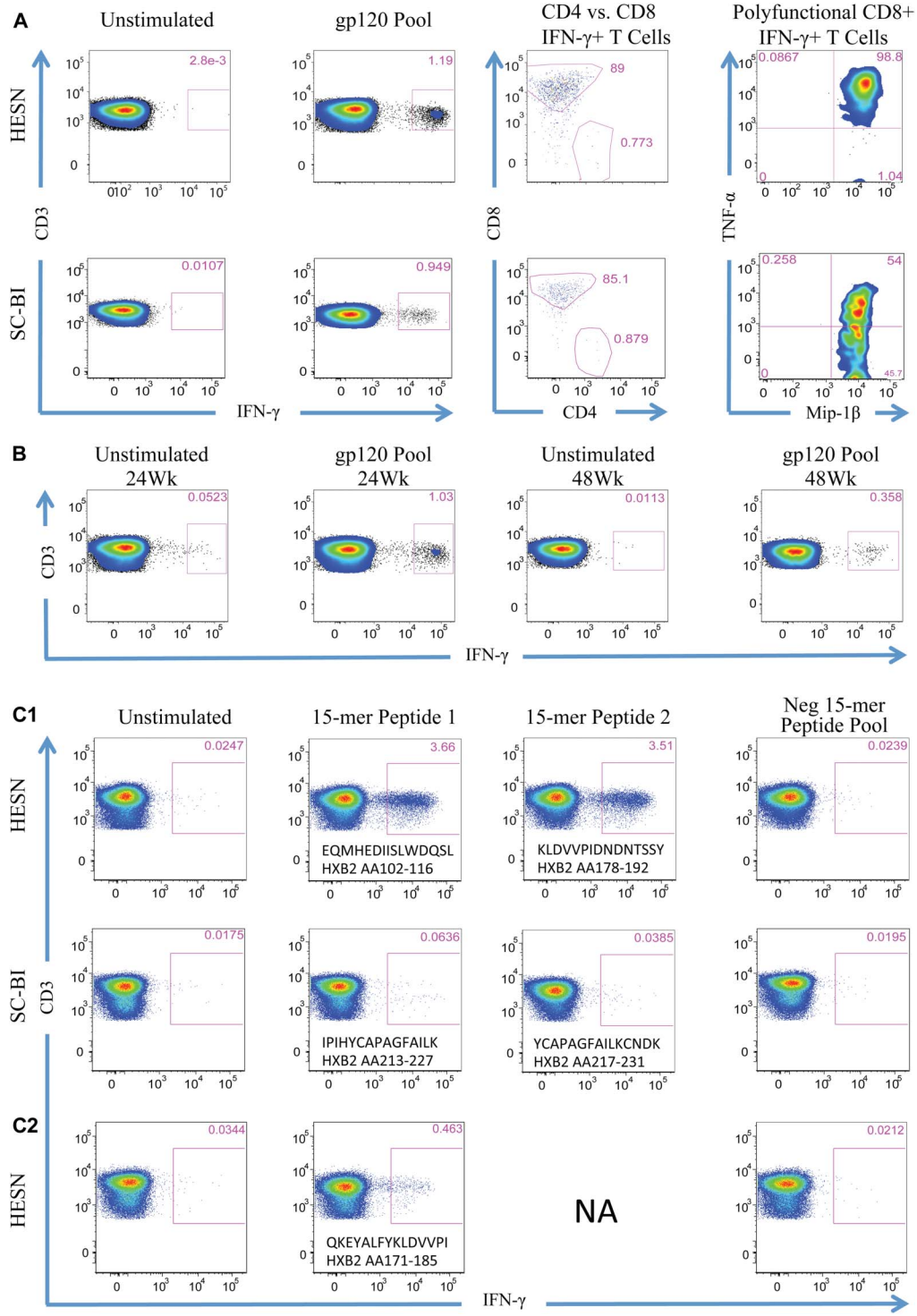

FIGURE 2. Confirmation of ELISpot responses ≥55 spots per million (SPM) with multiparametric flow cytometry. Cryopreserved PBMCs were tested first in and IFN-γ ELISpot assay with gp120 responses confirmed in MFC. HESNs or SC-BIs shown are individual subjects; flow data in this figure for each HESN or SC-BI were used once. A, Example of SC-BI and HESN responses in the absence of stimulating peptide pools and in the presence of gp120 peptide pool at 5 μg/mL, proportion of $CD4^+$ and $CD8^+$ IFN-γ–positive cells, and proportion of $CD8^+$ IFN-γ–positive cells also positive for TNF-α and Mip-1β ($CD4^+$ IFN-γ–positive T-cell frequency was too low to examine for polyfunctionality). B, Time dependency of response to gp120 peptide pool in a HESN, tested at 5 μg/mL. C1, 15-mer peptide mapping of positive gp120 response from a matrix ELISpot. The HESN individual demonstrated 2 distinct positive responses, and the SC-BI individual from the same enrollment site at the same protocol-defined visit had 2 positive responses to overlapping 15-mer peptides. C2, peptide mapping in an additional HESN. The amino acid sequence and positions are from HXB2.

gp120 in HIV-1 exposed but persistently seronegative individuals. In the subjects tested, the qualities and regional epitope specificity differed in persistently negative HESN individuals relative to those individuals who eventually became infected. The observations made in this study corroborate the ground-breaking analyses and observations of the envelope-based RV144 vaccine study regarding $CD4^+$ T-cell polyfunctionality. The concordance of the findings suggests that $CD8^+$ T-cell responses may also contribute to protection from infection and should be further studied in sufficiently powered studies, inclusive of prospectively designed retrospective studies of vaccine, and pre-exposure prophylaxis trials.

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