

HIV-1-Specific T Cell Immunity in HIV-1 Uninfected Homosexual Men with Very High Risk Sexual Activities

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ABSTRACT

Background and Methods: Individuals who are repeatedly exposed to HIV-1 but remain uninfected represent a unique cohort to study the potential mechanisms of resistance against HIV-1 infection. To investigate if specific cellular immunity contributes to protection, we enumerated T cells recognizing HIV-1 Env, Gag, Pol and Nef epitopes in 16 exposed seronegatives (ES) using dendritic cells (DC) for antigen presentation and an IFN- γ ELISPOT assay for detection.

Results: All 16 ES are healthy male homosexuals maintaining high-risk sexual activities with their known HIV-1 infected partner (100%) or multiple other partners (50%). We detected T cells specific to HIV-1 epitopes in 6 ES (38%). This finding is in contrast to our previous studies in which only 1 of the 16 ES demonstrated HIV-1-specific T cells in an IFN- γ ELISPOT assay using PBMC alone. The majority of responses were directed against Pol epitope pools (32 out of 57 total responses), followed by Env (18/57) and Nef (7/57). The magnitude of individual responses ranged from 160 spot-forming cells (SFC) to 3,900 SFC per million responder cells (mean 1,820; median 1,650). HIV-1 Gag-specific T cells were not seen. For most assays, PBMCs were purified into CD8⁺ and CD8⁻ T cell subsets and responses were present both within the CD8⁺ (34/55) and the CD8⁻ cells (21/55). We also detected responses within the CD8⁺ but not the CD8⁻ subset in 1 out of 13 low-risk HIV-1-seronegative individuals. When we stratified the risk status of the 16 ES by scoring the overall frequencies of anal receptive (AR) and anal insertive (AI) intercourse, 5 of the 6 responders (83%) fell into the highest risk category. Among the nonresponders, only 3 of 10 volunteers (30%) fell into the highest risk category, and one of these tested homozygous for the CCR5 Δ 32 mutation.

Conclusions: These findings suggest that repeated exposure to HIV-1 by unprotected anal intercourse with an HIV-1 infected partner stimulates broad and pronounced HIV-1 specific T cell immunity in some but not all individuals.

OBJECTIVE To investigate if specific cellular immunity contributes to protection in individuals who remain uninfected despite repeated exposure to HIV-1

METHODS

STUDY SUBJECTS

Healthy HIV-1 seronegative volunteers were recruited within metropolitan Seattle who reported unprotected sexual intercourse with a known HIV-1 infected person (A) greater than five times in the previous 6 months or (B) an average of two times weekly over a period of 4 months within 2 years of enrollment. Volunteers were tested for HIV-1 infection at the screening visit and in follow-up visits every 1-3 months by HIV-1/2 ELISA and Western Blot, plasma RNA RT-PCR and PBMC DNA PCR. Questionnaires concerning risk behavior are completed at each visit.

DENDRITIC CELL ENZYME-LINKED IMMUNOSPOT (ELISPOT) ASSAY FOR IFN- γ RELEASE FROM SINGLE ANTIGEN-SPECIFIC T CELLS

DCs are generated from monocyte precursors by 6 day culture with GM-CSF and IL-4 (1), and matured for an additional 2 days by addition of monocyte-conditioned medium (MCM) (2). Ten thousand mature DCs per well are transferred to anti-IFN- γ -coated 96 well ELISPOT plates and pulsed for 1 h with HIV-1 synthetic peptide pools covering the complete Gag, Pol, Env and Nef region of the consensus HIV-1 strain HXB2 or a pool of 5 irrelevant control peptides. Each HIV-1 peptide pool consists of 25 sequential 15-mer peptides overlapping by 10 amino acids. Peptides are used at a final concentration of 2 μ g/ml. Cells stimulated with staphylococcal enterotoxin B (SEB) serve as positive control. CD8⁺ and CD8⁻ responder cells are then added at a ratio of 10 responder cells to 1 DC and cultured for 16-20 h. The plates are treated and developed as described previously (3), and colored spot-forming cells (SFCs) are counted using an automated ELISPOT reader. Responses are considered positive if the number of SFCs is two-fold or greater than those in the negative control wells and if \geq 50 SFCs per 1×10^6 responder cells after subtraction of the peptide control are present.

RESULTS

Figure 1. Sensitivity of detection of IFN- γ release from single HIV-1 specific T cells by ELISPOT assay using DCs as antigen-presenting cells versus not using DCs. A. In preliminary experiments in four HIV-1 infected individuals, we determined the effect of antigen presentation by DCs differentiated and matured over varying periods of time on the sensitivity of the ELISPOT assay. A representative experiment in one HIV-1⁺ patient responsive to the HIV-1 gag 27 peptide is shown and demonstrates that the addition of DCs greatly increases the number of gag27-specific IFN- γ SFCs in comparison to PBMCs alone. In addition, day 8 DCs, matured for two days with MCM, most commonly yield the highest absolute response and the strongest increase over cultures without DCs. In other experiments, a ratio of DC to responder cells of 1:10, a final peptide concentration of 2 μ g/ml, and a positive selection strategy for CD8⁺ responder cells was determined optimal. These conditions were therefore used throughout the ES cohort. B. The predicted optimal suitability of day 8 DCs for uncovering HIV-1 peptide specific T cells in an ELISPOT assay was first confirmed in ES individual #40, who did not show any positive response at this time point when PBMCs alone were tested, but demonstrated T cells specific to the HIV-1 Env1 and Env3 peptide pools when DCs were added for peptide presentation.

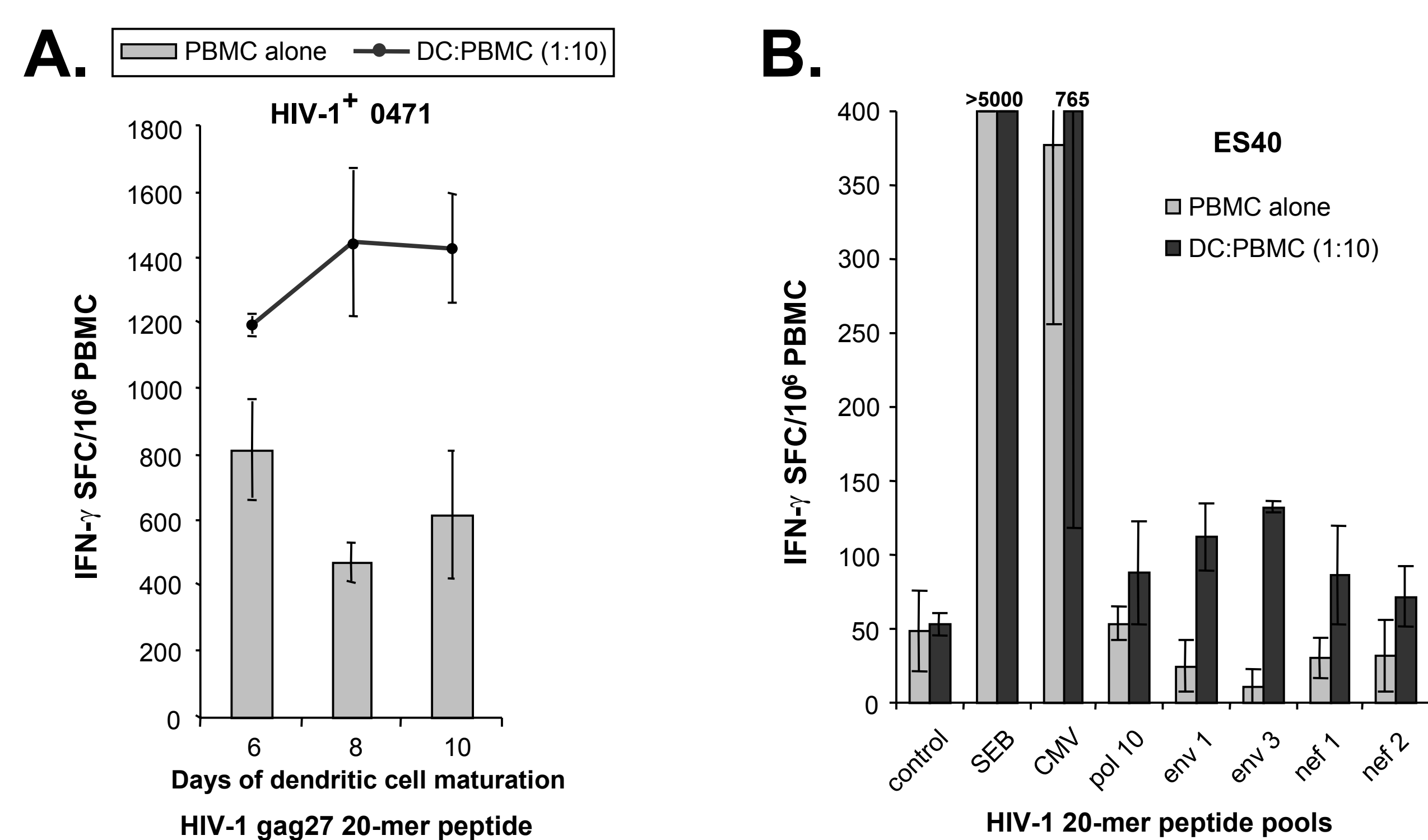


Figure 2. High risk exposures of ES

Figure 2. High risk exposures of ES individuals over time. This figure summarizes the risk activity of all individuals recruited into the ES cohort who continued to practice high risk behavior after enrollment into the study. High risk exposures include unprotected anal sex, unprotected vaginal sex and needle sharing with a known HIV-1 infected person. Each colored dot indicates that the individual reported high risk activity since his or her last visit in the clinic. ES individuals are sorted by the number of periods between two visits for which they reported high risk behavior. The color of a dot categorizes the frequency of high risk activities since the last visit (● <1 high risk event per month, ● 1-5 high risk events per month, ● >5 high risk events per month). Today's date is marked with a vertical line, the seroconversion date of the five seroconverters so far is marked by an 's'. ES individuals with colored ID numbers were tested for HIV-1-specific T cell responses using the dendritic cell ELISPOT assay, and the date of the assay is indicated by a black circle. Individuals with red ID numbers exhibited T cell responses against HIV-1 epitopes, individuals with blue ID numbers did not.

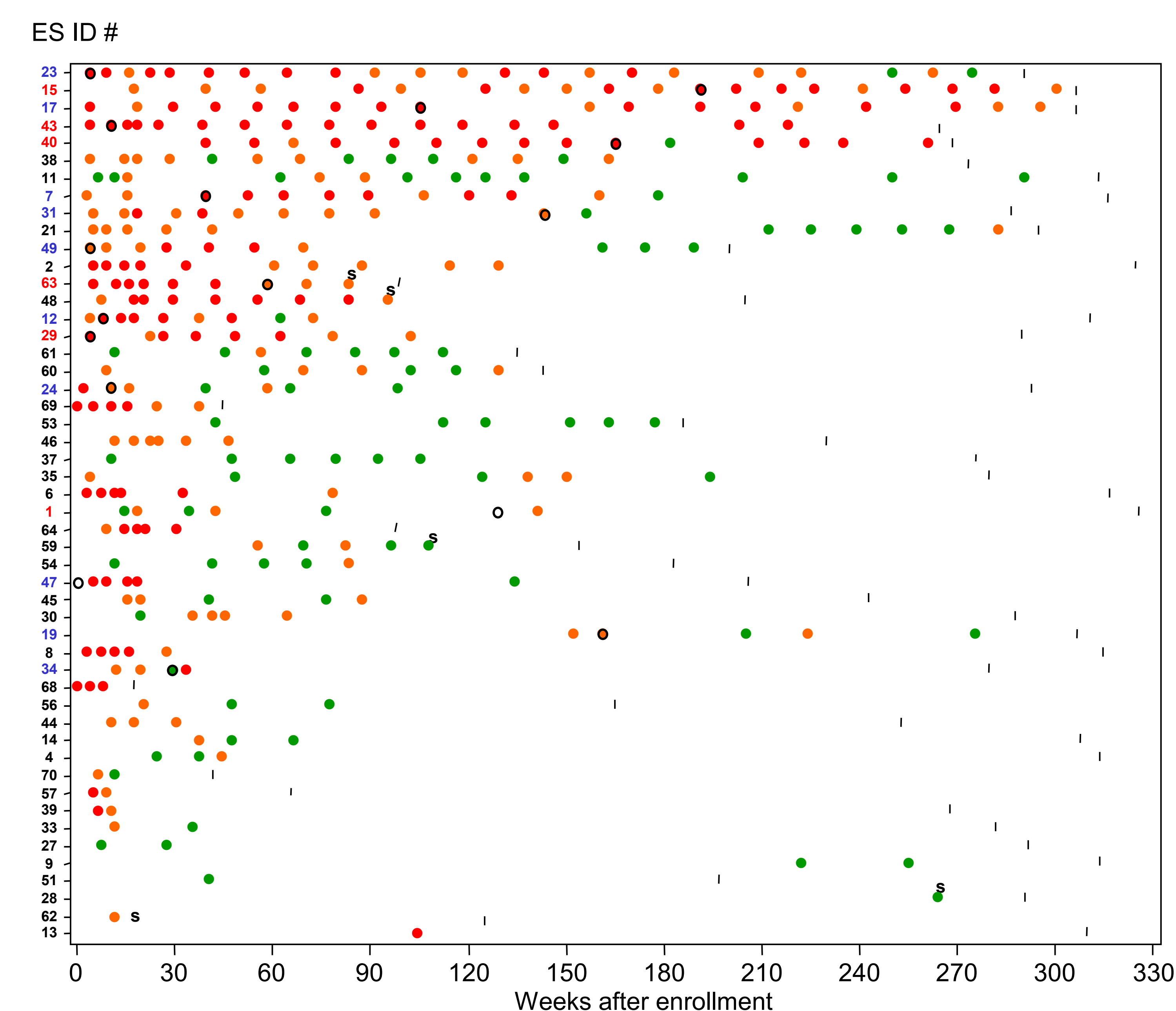
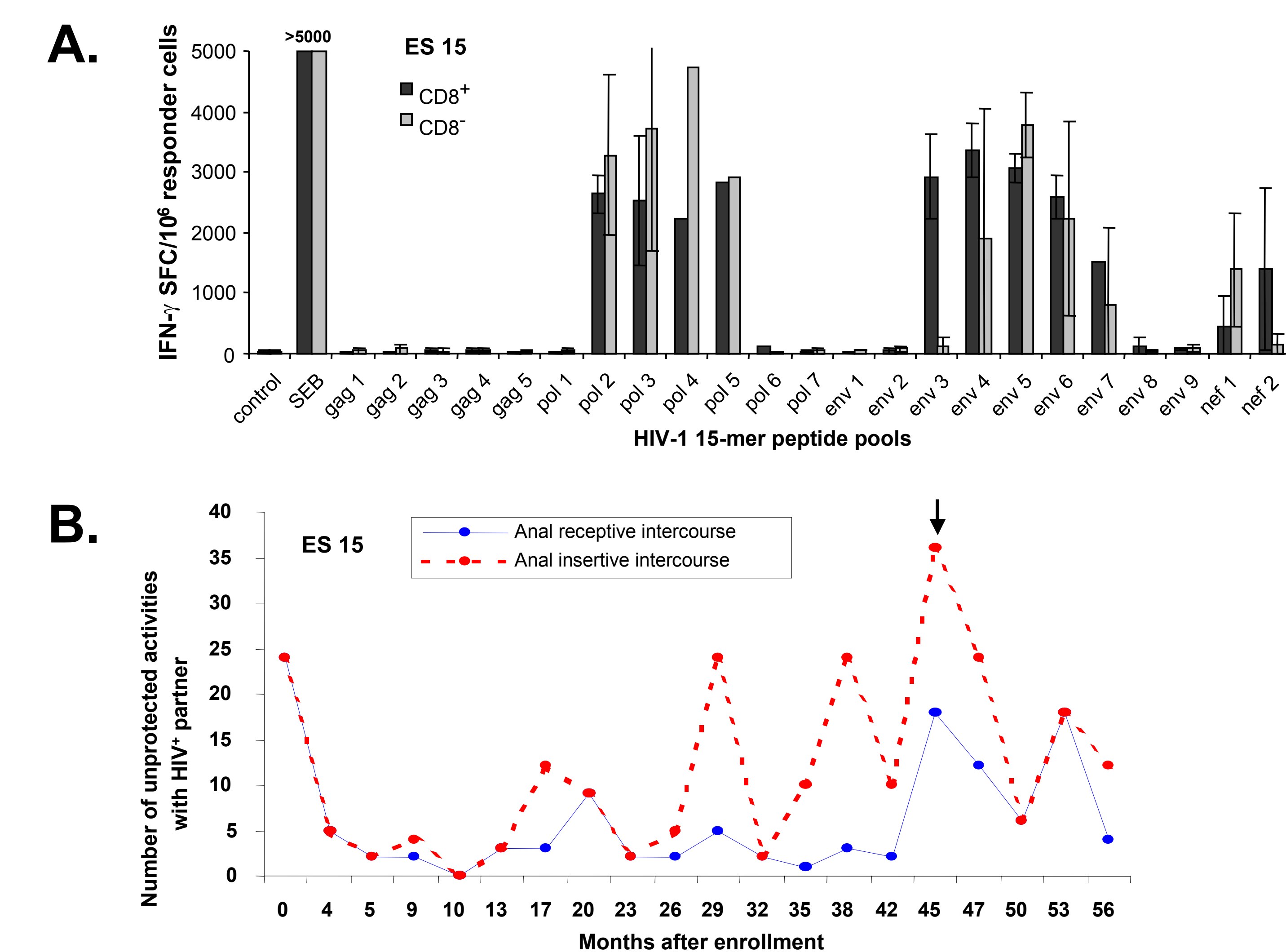
Figure 3. IFN- γ DC ELISPOT response and frequency of anal intercourse over time in ES

Figure 3. IFN- γ DC ELISPOT response and frequency of anal intercourse over time in ES individual #15. Sixteen homosexual individuals were selected by their relatively high frequencies of unprotected anal intercourse and tested by IFN- γ DC ELISPOT assay. Six of these 16 ES individuals demonstrated HIV-1 peptide specific T cells and ES 15 is shown in A. Error bars indicate the standard deviation of triplicate measurements. In earlier experiments set up without the addition of DCs, only one of these individuals demonstrated an HIV-1 specific T cell response at one but not two time points (ES 40, reported in (3)). B. Frequencies of unprotected anal receptive and insertive intercourse by ES 15 with known HIV-1 infected partners over time since enrollment into the study. The black arrow marks the date of the DC ELISPOT assay.



Summary Table. IFN- γ DC ELISPOT results in the 16 ES and 14 control individuals tested so far. One control individual (c009), who had a broad response to several HIV-1 peptide pools, revealed repeated unprotected sexual activity with an HIV-1 infected partner and is therefore listed in the ES group. One ES individual (ES 7) is a homozygote for the delta32 CCR5 mutation and is listed at the end of the ES group because no HIV-1 specific cellular immune response is expected in this person. The rest of the ES group is sorted by a score derived from the overall frequency of anal intercourse. Because anal receptive intercourse (ARI) is associated with a higher exposure risk than anal insertive intercourse (AII), the score gives more weight to individuals with higher frequencies of ARI than AII by calculating the AI score as (ARI*2 + AII) / 3. Five of the 6 responders had an AI score of >100. Among the 10 nonresponders, only 3 had an AI score of >100, and one of these was ES 7 who is homozygous for the delta32 CCR5 mutation. One ES individual (ES 16) seroconverted seven months after the assay date and we are currently evaluating if he was already infected at the time of the assay.

Subject	AI Score	CCR5 Genotype	HLA Type	Number of Positive Peptide Pools (Mean SFC*10/10 ⁶ ; lowest-highest)				
				Gag	Pol	CD8 ⁺	Env	CD8 ⁻
ES								
43*	347		A2,30/B18,62					1 (160)
17	274		A3,31/B7,57		1 (100)			2 (17; 11-23)
40**	243		A2,2/B44,62					ND
15	159	Δ 32	A2,30/B13,44	4 (169;95-264)	5 (249;50-336)			ND
63*	126	Δ 32	A26,32/B27,27	9 (199;49-380)	2 (159;60-258)			5 (53;37-91)
29	125	Δ 32	A1,1/B8,8	4 (318;160-375)	2 (251;145-360)			1 (277)
23	119	Δ 32	A1,2/B8,44					3 (157;115-218)
49	76	Δ 32	A2,2/B15,51					1 (207)
47	69		A3,03/B13,60	1 (10)				1 (45)
31	41		A2,23/B49,55					ND
12	39		ND					ND
19	38	Δ 32	A2,29/B7,44					ND
24	33		A2,24/B7,52					ND
34	29		A2,3/B7,44					ND
1	7		A2,68/B41,51	2 (49;30-68)			1 (390)	ND
c009	ND	ND	ND	4 (91;60-125)	2 (154;123-185)			ND
7	131	Δ 32/ Δ 32	A1,2/B44,45					ND
Control								
c003								ND
c004								ND
c005								ND
c007								ND
c008								ND
c011								ND
c017								ND
c018								ND
gqc18								ND
28								1 (14)
1001				2 (21;16-26)	3 (44;12-89)		1 (34)	1 (11)
1002								1 (11)
14								1 (11)
4								1 (11)
70								1 (11)
57								1 (11)
39								1 (11)
33								1 (11)
27								1 (11)
29								1 (11)
62								1 (11)
13								1 (11)

* Assays done with 20-mer peptide pools

Seroconversion 210 days after assay date

** Assays done with 20-mer peptide pools on PBMC only

Cursive font signifies borderline positive results

References

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CONCLUSION

Repeated exposure to HIV-1 by unprotected anal intercourse in homosexual men stimulates HIV-1 specific T cell immunity in some but not all individuals.

OUTLOOK

Detailed analysis of these cellular immune responses may reveal HIV-1 epitopes suitable for protective vaccination.