

Online Resource

Table 1. Clade A HIV gag peptide pools used in the ELISpot assay

Pool1	HIVA1	PIVQNAQQGQMHQALS	Pool 2	HIVA23	PIPPGQMREPRGSDI
	HIVA2	AQGQMHQALSPRTL		HIVA24	GQMREPRGSDIAGTT
	HIVA3	GQMHQALSPRTLNAWV		HIVA25	EPRGSDIAGTTSTLQ
	HIVA4	ALSPRTLNAWVKVIE		HIVA26	SDIAGTTSTLQEIQIG
	HIVA5	RTLNAWVKVIEEKAF		HIVA27	GTTSTLQEIQIGWMTS
	HIVA6	AWVKVIEEKAFSPEV		HIVA28	TLQEIQIGWMTSNPP
	HIVA7	VIEEKAFSPEVIPMF		HIVA29	EQIGWMTSNPPIPVGD
	HIVA8	KAFSPEVIPMFSALS		HIVA30	MTSNPPIPVGDIYKR
	HIVA9	PEVIPMFSALSEGAT		HIVA31	PPIPVGDIYKRWIIL
	HIVA10	PMFSALSEGATPQDL		HIVA32	VGDIYKRWIILGLNK
	HIVA11	ALSEGATPQDLNMML		HIVA33	YKRWIILGLNKIVRM
	HIVA12	GATPQDLNMMLNIVG		HIVA34	LNKIVRMYSPV
	HIVA13	PQDLNMMLNIVGGHQ		HIVA35	LNKIVRMYSPVSILD
	HIVA14	MMLNIVGGHQAAQM		HIVA36	VRMYSPPVSILDIRQG
	HIVA15	IVGGHQAAQMQLKT		HIVA37	SPVSILDIRQGPKEP
	HIVA16	HQAAMQMLKDTINEE		HIVA38	ILDIRQGPKEPFRDY
	HIVA17	MQMLKDTINEEAAEW		HIVA39	RQGPKEPFRDYVDRF
	HIVA18	KDTINEEAAEWDRHL		HIVA40	KEPFRDYVDRFFKTL
	HIVA19	INEEAAEWDRLHPVHA		HIVA41	RDYVDRFFKTLRAEQ
	HIVA20	AEWDRLHPVHAGPIP		HIVA42	DRFFKTLRAEQATQE
	HIVA21	RLHPVHAGPIPPGQM		HIVA43	KTLLRAEQATQEVKNW
	HIVA22	VHAGPIPPGQMREPR		HIVA44	AEQATQEVKNWMTET
Pool 3	HIVA45	TQEVKNWMTETLLVQ	Pool 4	HIVA68	SRELERFALNPSLLE
	HIVA46	KNWMTETLLVQNANP		HIVA69	ERFALNPSLLETAEG
	HIVA47	TETLLVQNANPDCKS		HIVA70	LNPSLLETAEGCQQI
	HIVA48	LVQNANPDCKSILRA		HIVA71	LLETAEGCQQIMEQL
	HIVA49	ANPDCKSILRALGPG		HIVA72	AEGCQQIMEQLQSAL

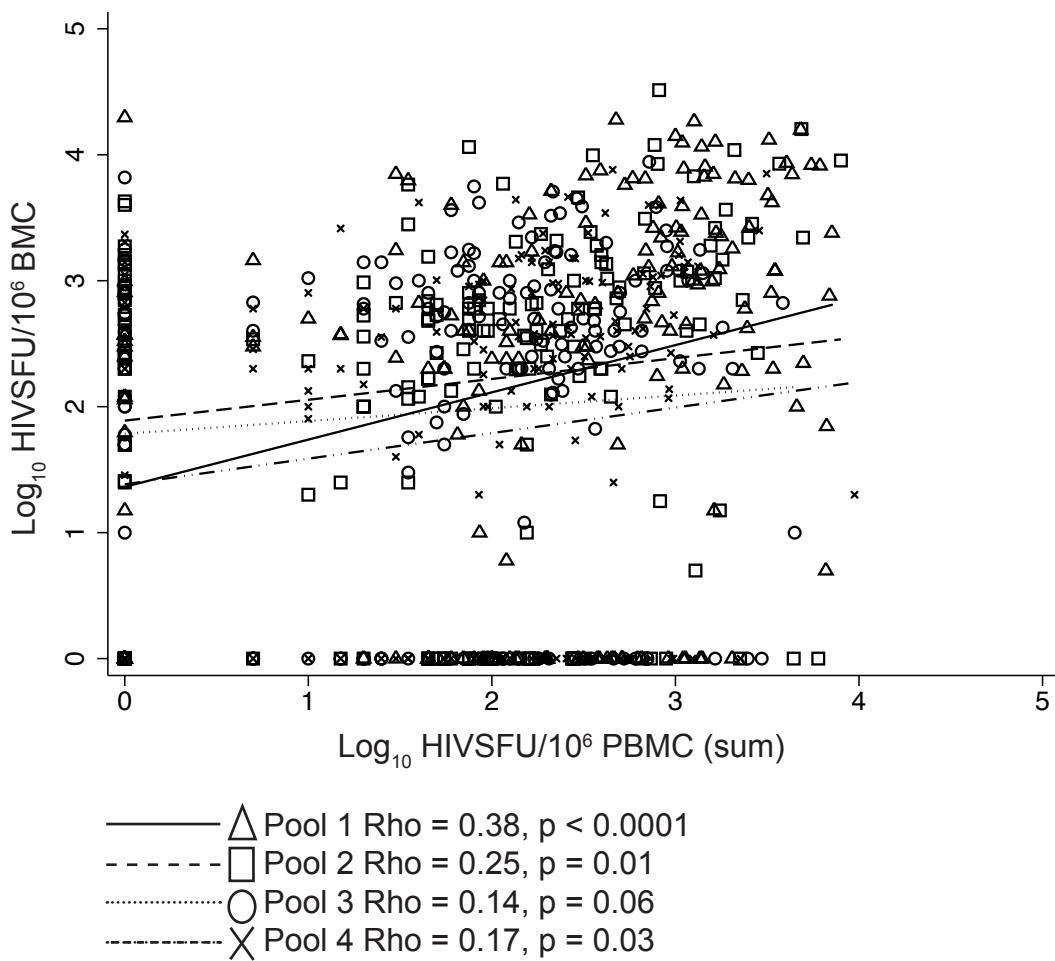
HIVA50	CKSILRALGPGATLE	HIVA73	CQQIMEQLQSALKTSE
HIVA51	LRALGPGATLEEMMT	HIVA74	EQLQSALKTSEELKS
HIVA52	GPGATLEEMMTACQG	HIVA75	SALKTSEELKSLFNT
HIVA53	TLEEMMTACQGVGGP	HIVA76	TSEELKSLFNTVATL
HIVA54	MMTACQGVGGPGHKA	HIVA77	LKSLFNTVATLYCVH
HIVA55	CQGVGGPGHKARVLG	HIVA78	FNTVATLYCVHQRID
HIVA56	GGPGHKARVLGTGAR	HIVA79	ATLYCVHQRIDVKDT
HIVA57	HKARVLGTGARASVL	HIVA80	CVHQRIDVKDTKEAL
HIVA58	VLGTGARASVLSGGK	HIVA82	KDTKEALDKIEEIQN
HIVA59	GARASVLSGGKLDAW	HIVA83	EALDKIEEIQNKSQK
HIVA61	GGKLDWEKIRLRPG	HIVA84	KIEEIQNKSQKTQQ
HIVA64	RPGGKKKYRLKHLVW	HIVA85	IQNKSQKTQQAAADTQSS
HIVA65	KKKYRLKHLVWASRE	HIVA86	SKQKTQQAAADTQSS
HIVA66	RLKHLVWASRELRF	HIVA87	TQQAAADTQSSKVS
HIVA67	LVWASRELRFALNP	HIVA89	TQSSSKVSQNYALKHR
		HIVA90	KVSQNYALKHAYEL

Supplemental Digital Content 2

Analysis of concordance of HIV specific IFN- γ responses in peripheral blood and breast milk.

Spearman's correlation was used to test for independence between immune responses in breast milk and blood. Overall, the magnitude of responses to individual peptide pools was weakly correlated in the blood and breast milk. This relationship was strongest in pool 1, which contained the greatest number of previously defined CD8+ T cell epitopes (Supplemental Digital content 3, rho=0.38, p<0.0001). The paired sign-rank test were used to compare the number of peptide pools recognized in blood and breastmilk; a larger range of peptide pools were recognized in blood (median 2.0 pools recognized, IQR=1-4) compared to breastmilk (median 2.0 pools recognized, IQR=0-3, respectively, p=0.0006).

When magnitude was summarized as the sum of peptide pool responses, the response magnitude was similar in blood and breast milk (mean $3.0 \pm SD 0.70$ versus $3.1 \pm SD 1.0 \log_{10}$ HIVSFU/ 10^6 cells, respectively, paired t-test p=0.4). The magnitude of responses was also similar between blood and breastmilk when summarized by the maximum or mean of responses to the 4 peptide pools (data not shown). However, summarizing the data obscured heterogeneous trends in the individual pools; pool 1 responses were similar in magnitude between breastmilk and blood (mean $2.2 \pm SD 1.4$ versus $2.3 \pm SD 1.1 \log_{10}$ HIVSFU/ 10^6 cells, respectively, p=0.5), pool 2 responses were greater in breastmilk versus blood ($2.2 \pm SD 1.3$ versus $1.9 \pm SD 1.1 \log_{10}$ HIVSFU/ 10^6 cells, respectively, p=0.005), pool 3 responses were similar in breastmilk and blood ($2.0 \pm SD 1.3$ versus $1.8 \pm SD 0.97 \log_{10}$ HIVSFU/ 10^6 cells, respectively, p=0.2), and pool 4 responses were lower in breastmilk compared to blood ($1.8 \pm SD 1.4$ versus $2.1 \pm SD 0.89 \log_{10}$ HIVSFU/ 10^6 cells, respectively, p=0.03).



Supplemental data Figure 1. Correlation between breast milk and blood HIV-specific IFN- γ responses.
 Scatter plot shows log10 transformed responses to individual peptide pools in PBMC and breast milk mononuclear cells (BMC). Correlation coefficients and P-values were generated using Spearman's correlation.