

Defining Immune Correlates of Protection: HIV-specific CD4⁺ Cytotoxic T cells

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Abstract

HIV-specific T helper (Th) cells may play an active role in controlling HIV replication, though the extent of their contribution is unknown. Comprehensive screening of CD4⁺ T cell IFN- γ responses from acutely, chronically or long-term non-progressively (LTNP) infected individuals identified 36 individuals with frequencies of HIV-specific CD4⁺ T cells greater than 1% directed to HIV peptide pools spanning Env, Gag or Nef. The prevalence of CD4⁺ cytolytic activity in these individuals with disparate levels of viral control will be measured. These studies represent a first step in identifying a new immune correlate of protection from HIV, which would provide a much-needed advance in vaccine development and evaluation.

Introduction

- HIV-specific Th cells may play an active role controlling HIV replication.
- HIV-specific Th cell proliferative responses and IL-2 secretion correlate with viral load in an inverse fashion, reflecting HIV-mediated destruction of these cells, or alternatively, robust virus-specific Th responses may help control the virus through undefined mechanisms.
- Direct cytolytic effector functions mediated by CD4⁺ CTL have been demonstrated for viral infections including HSV, HBV & HIV.

Subjects

Rare individuals with high CD4⁺ IFN- γ responses were identified from the UCSF SCOPE (chronic HIV, elite suppressors, LTNP and PCAT-infected individuals) and OPTIONS (acutely infected individuals) cohorts. LTNP have been infected for at least 10 years with viral loads <5000 copies/ml off antiretroviral therapy. PCAT subjects have partial control of viral replication on antiretroviral therapy. Elite suppressors control viral load <500 copies/ml but have been HIV infected for <10 years. Acute patients were identified prior to or within 6 months of seroconversion.

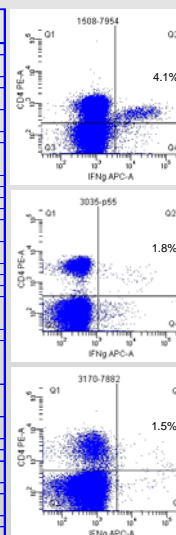
Methods

Identification of target epitopes: Overlapping peptides spanning the HIV proteome in pools of 10, with each peptide present in 2 pools were used in a high-throughput ELISPOT assay measuring IFN- γ release from PBMCs. **CD4-restriction of responses:** Was confirmed by intracellular cytokine staining for IFN- γ production following overnight stimulation with peptides using standard fix-perm techniques. At least 100,000 events per condition were collected on a BD LSR II. **Cytotoxicity:** Was measured in a standard overnight ⁵¹Cr release assay.

Results

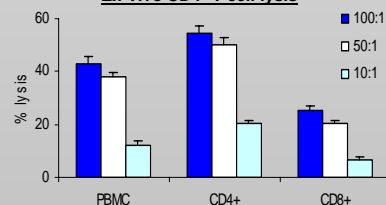
Individuals with high frequency HIV-specific Th cell responses

Subject	Status	ART	CD4 count (cells/ml)	Virus Load (RNA copies/ml)	% CD4 ⁺ IFN γ *	Antigen
S-3166	Progressor	Yes	234	46361	1.07	Gag
S-3169	Progressor	Yes	209	13309	2.2	MN
S-3170	Progressor	Yes	265	21594	1.5	Gag
S-3035	PCAT	Yes	260	3225	0.8	MN
S-3040	PCAT	Yes	280	8856	1.2	Gag
S-2004	PCAT	Yes	275	485	1.75	Gag
S-1504	LTNP	No	959	157	0.3	MN
S-1511	LTNP	No	536	<75	1.55	Gag
S-3052	PCAT	Yes	208	1891	1.14	Gag
S-3042	PCAT	Yes	232	183	1.04	Gag
S-3167	PCAT	Yes	392	7531	1.06	Gag
S-1516	LTNP	No	728	97	0.7	Gag
S-1507	LTNP	No	747	<75	1.22	Nef
S-3005	PCAT	Yes	174	2717	1.25	Nef
S-3109	PCAT	Yes	174	44727	20.6	Nef
S-1502	LTNP	No	995	<75	0.8	Nef
S-1504	LTNP	No	790	1592	0.3	MN
S-1508	LTNP	No	763	301	4.1	Gag
S-1523	LTNP	No	562	229	0.5	p55
S-1113	Elite	No	1081	<75	0.2	Gag
S-1114	Elite	No	608	92	0.2	MN
O-829	Acute	Yes	646	201	1.03	Gag
O-815	Acute	No	572	46600	1.36	Env
O-809	Acute	No	264	9372	0.4	p55
O-847	Acute	Yes	870	50	0.2	Env
O-625	Acute	Yes	788	35120	0.2	Gag
O-396	Acute	Yes	435		0.5	Gag
O-1339	Acute	No		490549	1.3	Env

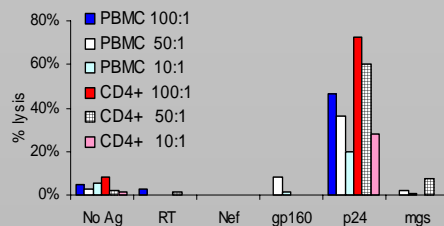


• Of over 180 individuals screened for HIV-specific CD4⁺ IFN γ production, 36 have been identified for further screening, of which 14 individuals have responses greater than 1% to HIV-specific peptides. Representative flow plots of CD4 versus IFN- γ staining, gated on CD3⁺ cells, are shown for 3 individuals.

Ex Vivo CD4⁺ T cell lysis

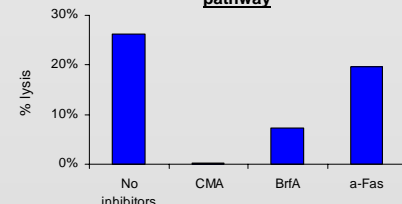


• Enrichment for CD4⁺ or CD8⁺ T cells and lysis of targets pulsed with whole p24 protein. CD4⁺ = PBMC enriched for CD4⁺ T cells, CD8⁺ = PBMC enriched for CD8⁺ T cells. Enrichment for CD4⁺ T cells led to an increase in lysis and enrichment for CD8⁺ T cells led to diminution of lysis.



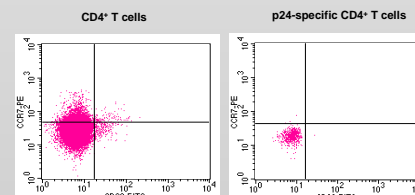
• Overnight killing assay with freshly isolated PBMC or CD8⁺ T cell depleted PBMC and B cell targets pulsed with HIV-1 proteins (RT, Nef, and gp160) or a peptide pool (p24).

CD4⁺ T cell cytolytic activity is mediated by the perforin pathway



• Assays were performed in the presence of inhibitors of perforin (concanamycin A) or Fas-Fas ligand lysis (brefeldin A or anti-Fas blocking antibody). Blocking the perforin mediated pathway completely abrogated lysis, while blocking the Fas pathway partially inhibited lysis.

Phenotype of CD4⁺ cytolytic T cells



• HIV-specific CD4⁺ T cells express an effector phenotype.

Left plot = CD4⁺ T cells
Right plot = HIV-specific (IFN- γ) CD4⁺ T cells

Conclusions

- Ex vivo CD4⁺ cytolytic activity has been detected in one LTNP-infected individual.
- CD4⁺ cytolytic activity was mediated by the perforin pathway.
- CD4⁺ cytolytic T cells had an effector phenotype.

Future Plans

- The prevalence of CD4⁺ cytolytic activity in the individuals identified with high numbers of HIV-specific IFN- γ CD4⁺ T cells.
- Correlate the presence of cytolytic CD4⁺ T cells with levels of viral control.
- Characterization of phenotypic and cytolytic marker expression on cytolytic CD4⁺ T cells to allow identification of this unique population.

Acknowledgements

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