PHOSPHORYLATED HIV-1 AS POTENTIAL CYTOTOXIC T LYMPHOCYTE (CTL) TARGETS

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Abstract: *279*

Background

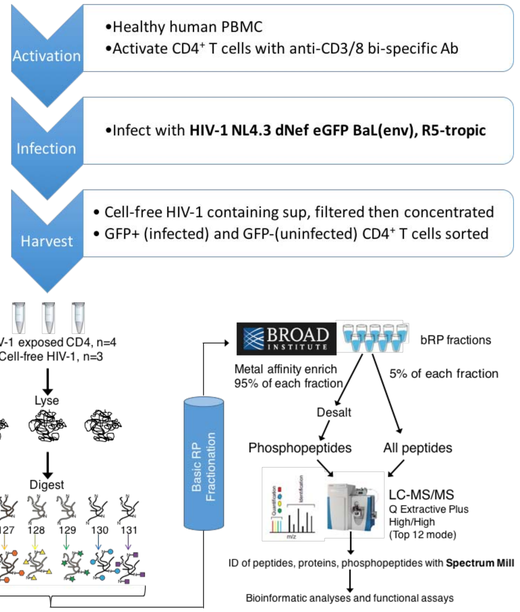
- HIV-1 is genetically conserved retrovirus
- HIV-1 depends on the host cell machinery to support its life cycle, including post-translational modifications such as phosphorylation
- Phosphorylation of HIV-1 could influence functional states of viral proteins and may influence viral replicative fitness
- How HIV-1 phosphorylation affects viral immune recognition by cytotoxic T cells is unknown at present
- Notably, recognition of phosphorylated epitopes by CD8 T cells is well documented in the context of lymphoma and melanoma

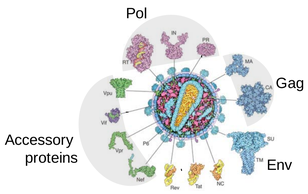
Key questions:

1. Is there site-specific phosphorylation of HIV-1 during infection of CD4 T cells and is it sustained in the cell-free virus?
2. Can phosphorylated HIV-1 peptides be presented in the context of HLA Class I?
3. Can phosphorylated HIV-1 peptides elicit immune responses in CD8 T cells?

Detection and coverage of HIV-1 proteins

Harvesting infected cells and virus for proteomic and phosphoproteomic analysis





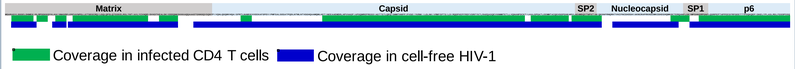
HIV-1 structures and proteins
Adapted from Johnson *et al,* 2015

HIV-1 Sequence coverage

HIV-1 proteins	% AA Sequence Coverage		
	Infected CD4⁺T cells	Cell-free Virus	
			+Asp-N, Label-free
Gag	66%	74%	85%
Pol	46%	44%	53%
Env	12%	5%	18%
Rev	47%	16%	35%
Tat	66%	-	20%
Vif	20%	-	23%
Vpr	-	28%	7%

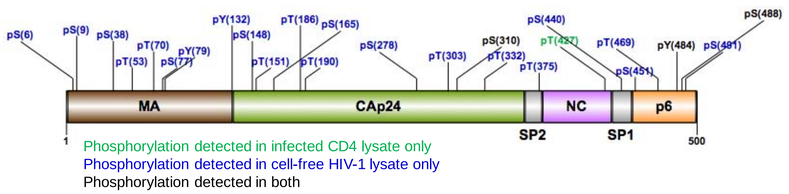
Proteins with detectable phosphorylation

Sequence coverage of Gag polyprotein: 98.5% of the available Ser/Thr/Tyr covered



Site-specific phosphorylation of HIV-1

Map of phosphorylated sites detected in HIV-1 Gag polyprotein



Phosphorylation detected in infected CD4 lysate only
Phosphorylation detected in cell-free HIV-1 lysate only
Phosphorylation detected in both

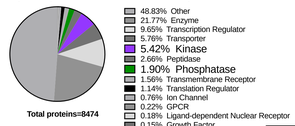
Host kinases can phosphorylate HIV-1 protein *in vitro*: e.g. pS(165)

Prediction of kinases activity

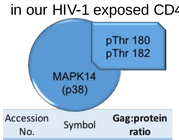
1. NetPhosK
2. KinasePhos
3. GPS3.0

Predicted Kinases for site-specific phosphorylation
p42 MAPK
p38 MAPK
GSK-3
CDK1/CyclinB
CDK2/CyclinE
CDK7/CyclinH
p25-CDK5
PKC-Theta
CK-2
CK-1
PKA
PKC-Beta
PKB
CDK2/CyclinA
p35-CDK5

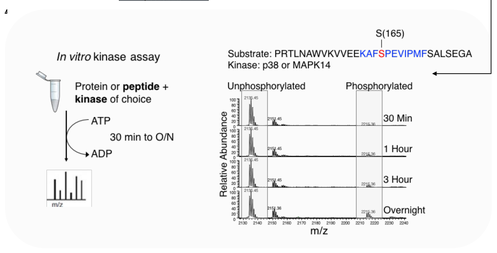
We detected numerous kinases and phosphatases in HIV-1 exposed CD4 T cells



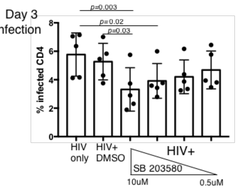
Activated p38 detected in our HIV-1 exposed CD4 T cells



Accession No.	Symbol	Gag:protein ratio
Q16539	MAPK14	50.61



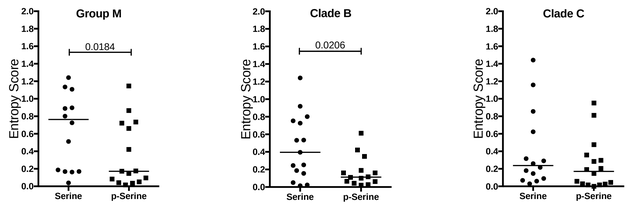
Inhibition of p38 function using the small molecule inhibitor SB 203580 reduces HIV-1 infection *in vitro*



Friedman test with Dunn's multiple comparison

Serine residues with phosphorylation detected in Gag are more conserved in Group M & Clade B of HIV-1

Entropy score
Higher → more mutations, less conserved
Lower → less mutations, more conserved



All serine residues of HIV-1 Gag were grouped as either phosphorylated or unphosphorylated based on detection in our current study. Entropy scores were determined as reported in the LANL database. Statistical differences were determined using Mann-Whitney test.

Phosphorylated sites can be detected in optimal CTL epitopes

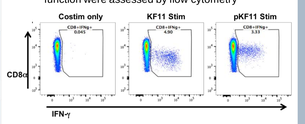
- **Phosphorylated residues were detected on 34 out of 74 optimal CTL epitopes as identified in the LANL database**
- **We selected five epitopes from Gag for further analysis based on *in vitro* binding assays and sample availability. Additionally, we tested three predicted epitope as well from Rev protein.**
- **This poster includes data for two of these epitopes**

Peptide Sequence*	Protein (optimal epitope name)	Total No. of Patients Tested	HLA tested	CTL responsive patients
KAF**S**PEVIPMF	Gag (KF11)	41	B*5701 B*5703	B*5701, n= 17 B*5703, n= 12
QA**S**QEVKNW	Gag (QW9)	39	B*5301 B*5701	B*5301, n= 12 B*5701, n= 10
ISPR**T**LNAWV	Gag (IW9+1)	17	B*0702 B*5701 B*5703	B*0702, n= 4 B*5701, n= 1 B*5703, n= 1
SLYNTVATL	Gag (SL9)	2	A*0201	A*0201, n= 2
KEL**Y**PLT**S**L	Gag (KL9)	28	B*4001 B*0801	No responses detected
IR**S**ISERILSTYL	Rev (predicted)	7	B*4001 B*4002	No responses detected

*Phosphorylated site is bolded and underlined

***Ex vivo* assessment of effector function of CTL from HIV-1 PBMC**

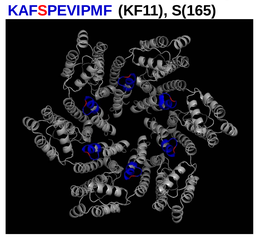
Briefly, PBMC from patients were stimulated with synthetic peptide for 12h and markers of effector function were assessed by flow cytometry



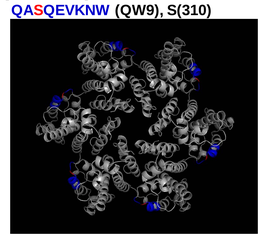
CTL response to phosphorylated HIV-1

Location of phosphorylated epitopes on p24 hexamer

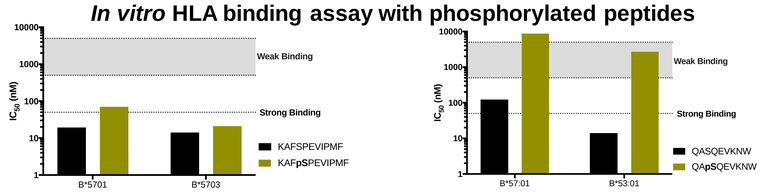
KAFSPEVIPMF (KF11), S(165)



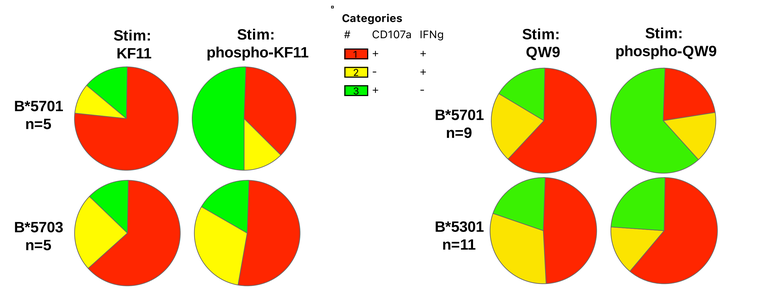
QASQEVKNW (QW9), S(310)



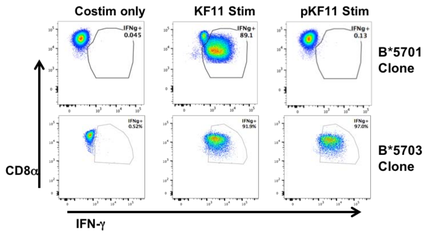
In vitro HLA binding assay with phosphorylated peptides



Ex vivo assessment of effector function of CTL from HIV-1 PBMC



CTL clonal specificity and cross reactivity of anti-phospho response



- CTL clones were isolated using IFNγ capture assay from HIV-1 infected patients
- Clones were stimulated in vitro for 6h with or without peptides before assessment of function

Conclusions and future directions

Conclusions:

- ✓ We have mapped site-specific phosphorylation of HIV-1 in infected CD4 T cell as well as cell-free virus.
- ✓ Host kinases can use viral protein as substrate for phosphorylation and blocking kinase activity broadly reduces infection rate.
- ✓ Phosphorylated HIV-1 peptide can elicit CTL immune responses in HIV-1 infected patient PBMC *ex vivo.*
- ✓ Phosphorylated epitopes potentially elicit more killing (CD107a staining) than IFNg expression in HLA B*5701 patients

Future studies will focus on functional characterization of HIV-1 phosphorylation as well as dissecting immune responses in patient cohorts

- Compare effector responses to phosphorylated epitopes between patient cohorts to determine any correlations with patient outcomes
- Identify and verify additional host kinases responsible for viral phosphorylation
- Determine effects of phosphorylation on viral fitness and replicative efficacy
  - Identify sites of viral vulnerability
  - Determine the stoichiometry of phosphorylation using Multiple Reaction Monitoring (MRM) studies
  - Identify pharmacologic targets

Acknowledgements

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