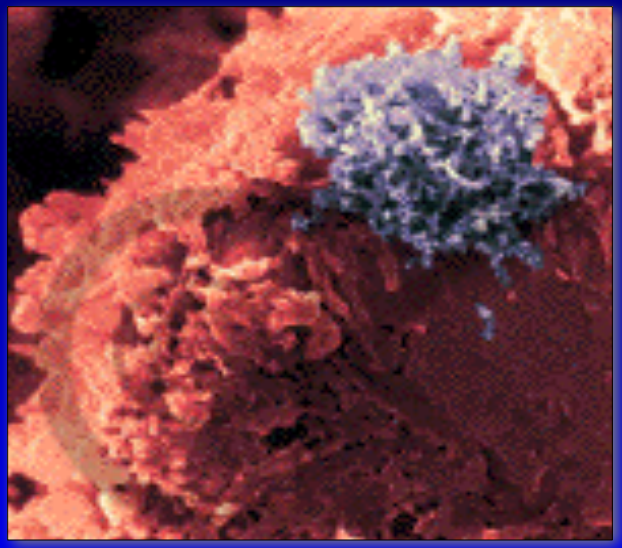
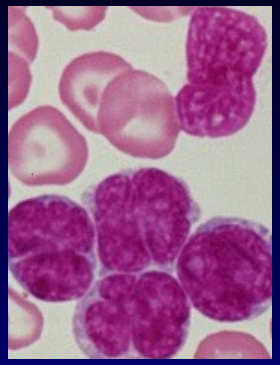
Role of Dendritic Cells in HTLV-1 Transmission and Pathogenesis

Human T-cell Lymphotropic Virus Type I (HTLV-1)

– First human retrovirus discovered (30th anniversary)
– Complex retrovirus (Tax – oncoprotein)
– Belongs to deltaretrovirus genus
  - HTLV-2
  - HTLV-3
  - HTLV-4
  - STLVs
  - BLV



Pathogenesis of HTLV-I

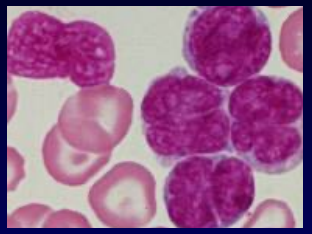


- **Adult T cell leukemia**
  - Clonal malignancy of $CD4^+$ T cells.
  - Long latency; neonatal transmission
  - Immune deficiency

- **Inflammatory syndromes:**
  - HTLV-I associated myelopathy/ Tropical spastic paraparesis
  - uveitis
  - arthropathy

- **Asymptomatic in majority of individuals:**
  - HTLV-I carriers: 5-8% lifetime risk of developing disease

Pathogenesis and Infectivity of HTLV-I



- **Adult T cell leukemia**
  - Clonal malignancy of CD4+ T cells.
  - Long latency; Immune deficiency
  - Tax and HBZ needed for transformation

- **Inflammatory syndromes:**
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  - uveitis
  - arthropathy

- **Asymptomatic in majority of individuals:**
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- Transmission of HTLV-I:
  - Mother to child, via blood products, sexually
- Tropism of HTLV-I:
  - CD4+ T cells
- Deltaretroviruses: believed to be poorly infectious
  - Cell-free virus can not infect T cells in culture
  - Efficient spread of HTLV-I require cell-cell contact
    - In vitro
    - Transmission between individuals

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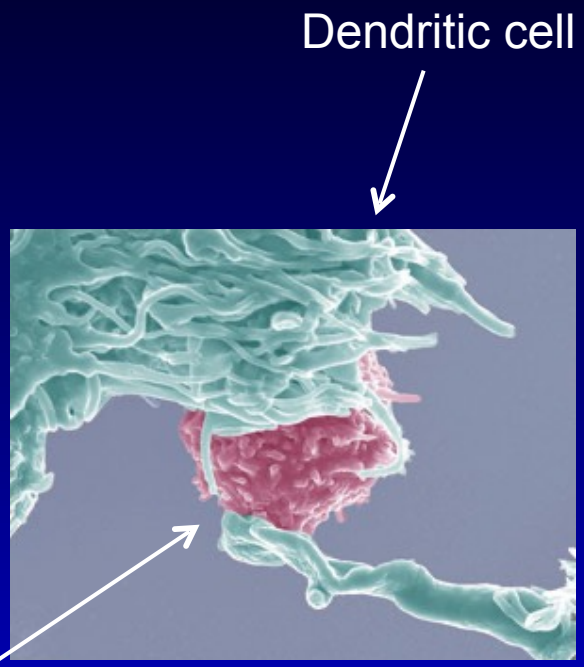
Dendritic Cells vs. Viruses

- Dendritic cells- potent antigen-presenting cells:
  - Play a central in immune responses against viruses
  - Located at sites of viral entry
    - Mucosal membranes
    - Peripheral blood
- 2 types of DC in peripheral blood:
  - Myeloid dendritic cells (conventional DC)
    - classical APCs
    - initiate the activation of T cells
  - Plasmacytoid dendritic cells
    - innate immune response (IFN-α)
    - Link innate and adaptive immunity



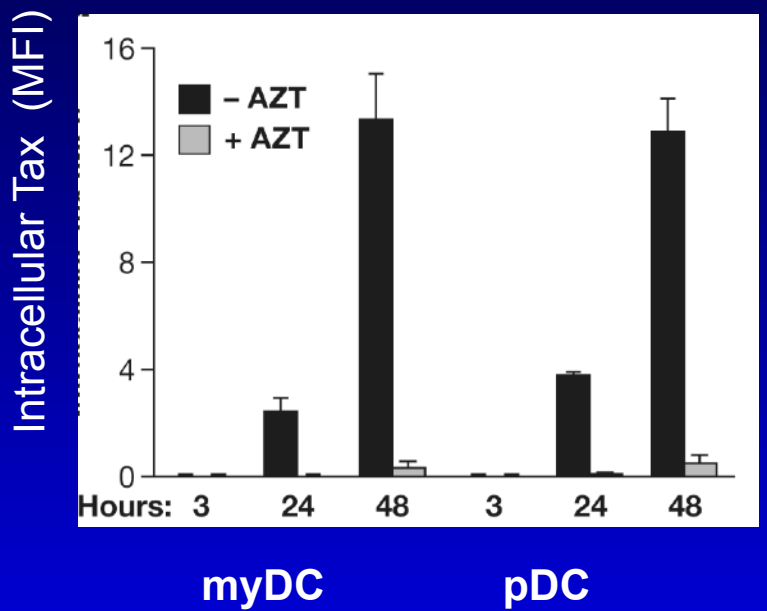
O. Schwartz; Nat Cell Bio

Viruses can interfere with immune responses

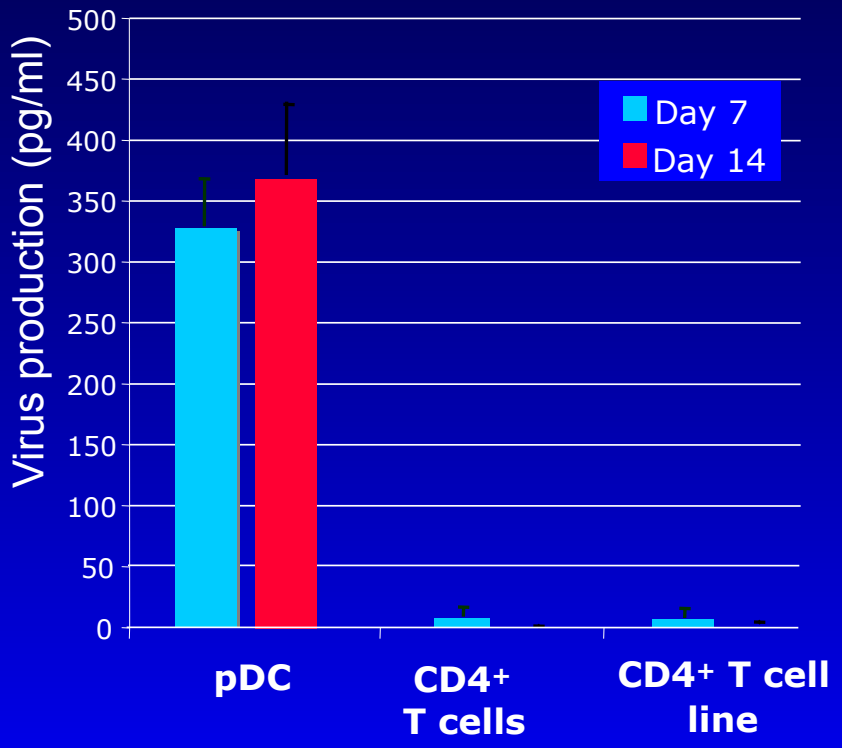
Many viruses use DC to facilitate spread

Dendritic Cells Become Infected Following Exposure to Cell-free HTLV-1

Intracellular staining (Tax)



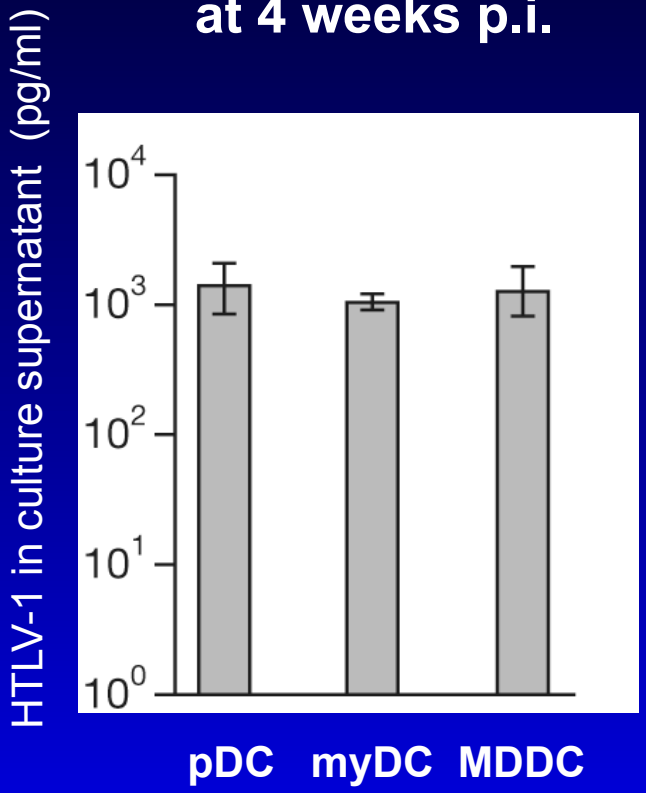
Virus production



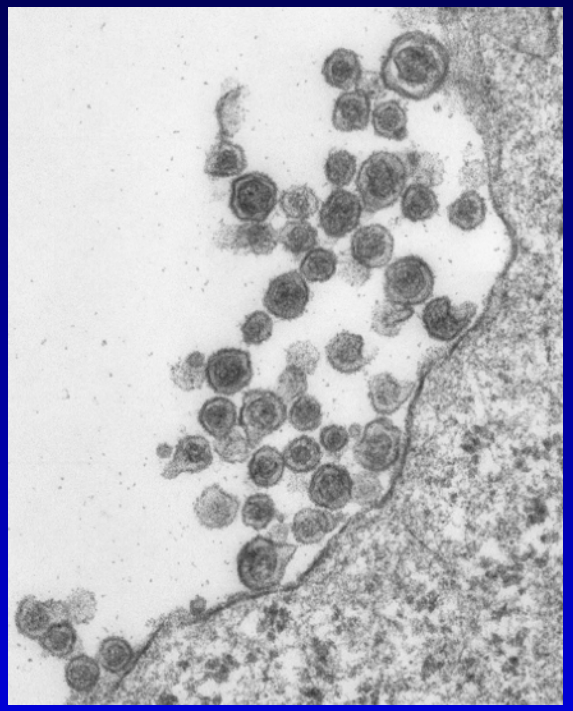
Jones et al., Nat Med 14:429, 2008

Virus Production by HTLV-1-Infected DC

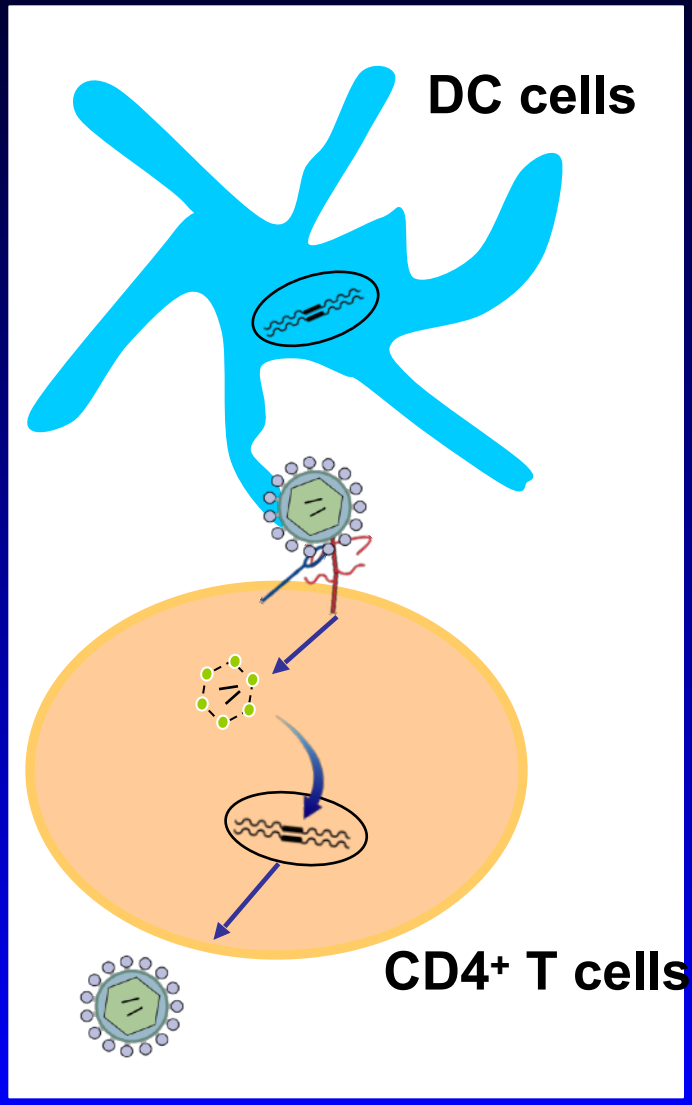
Virus production by DC at 4 weeks p.i.

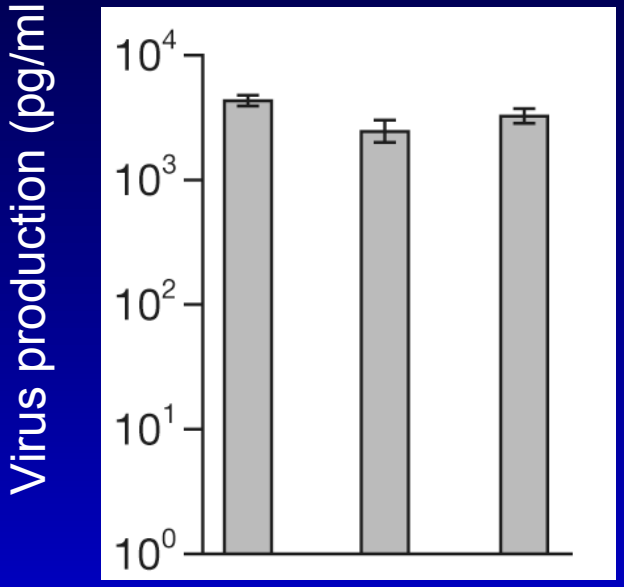


Transmission EM of HTLV-1-infected DC



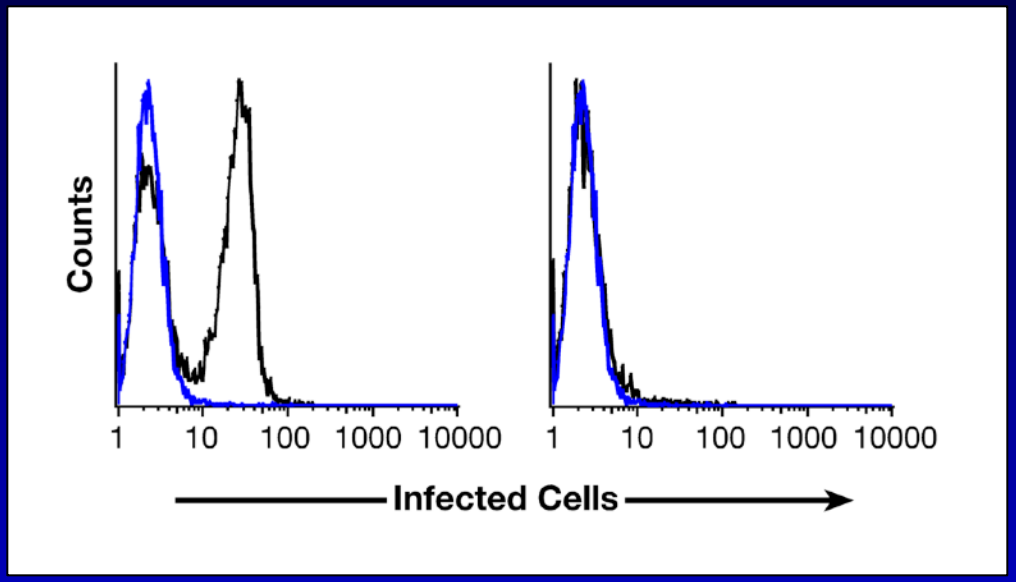
CD4+ T Cells Are Efficiently Infected with HTLV-1 Via Dendritic Cells





Infected via: pDC myDC MDDC

Uninfected pDC Do Not Enhance HTLV-I Infection of CD4+ T cells



Co-cult with pDC/HTLV-I

Co-cult with uninfected pDC + cell-free HTLV-I

Summary of DC-T cell Transmission Studies

- CD4+ T cells are efficiently and productively infected with HTLV-1 via DC:
  - Autologous and heterologous CD4+ T cells
  - CD4+ T cell lines
- Transmission from DC to T cells can occur both:
  - *In trans:* DC capture and transmit HTLV-1 prior to becoming infected
  - *In cis:* transmission of de novo produced virus from infected DC
- CD4+ T cells infected with HTLV-1 via DC can be transformed
  - Transformed CD4+ T cells share phenotypic characteristics with malignant T cells from individuals with ATL

Productive infection of DC stimulated rethinking of current paradigms of HTLV-1 transmission and disease

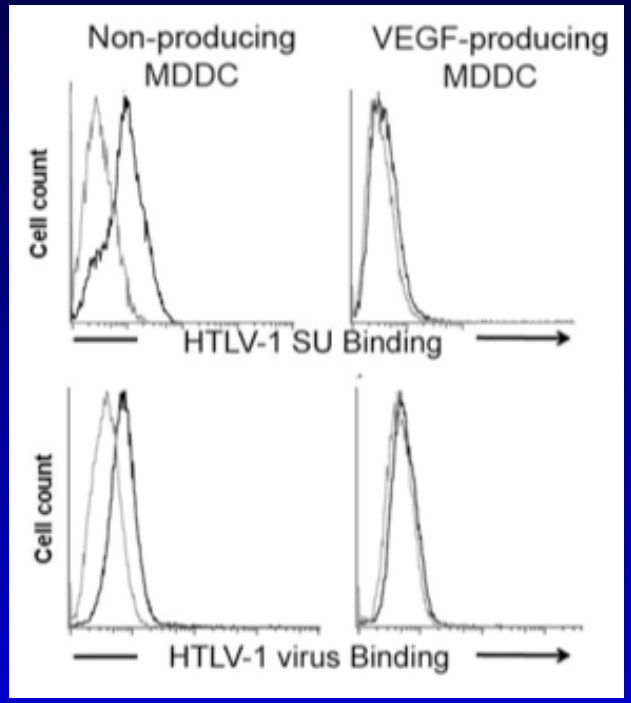
1. Use of in vitro infection assays to better characterize HTLV-1 receptor complex on DC cells and T cells (Pique)

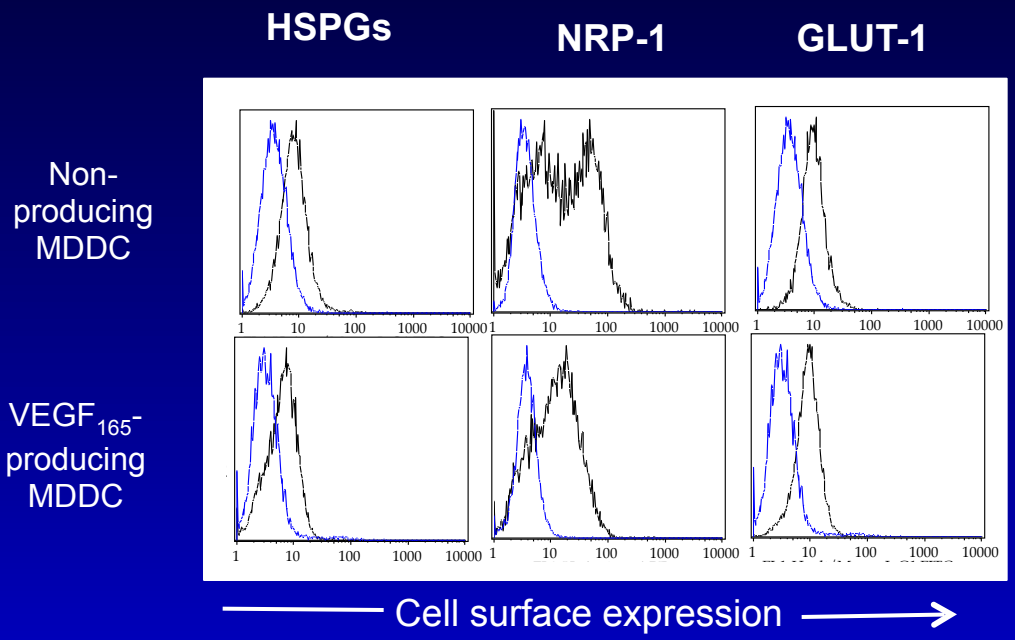
2. Examine pDC from ATL patients for presence of virus and altered function (Janik/Moore/Waldmann)

3. Role of HTLV-1 Accessory Proteins in infection (Franchini)

   p30 and p12 mutant viruses are poorly infectious for DC

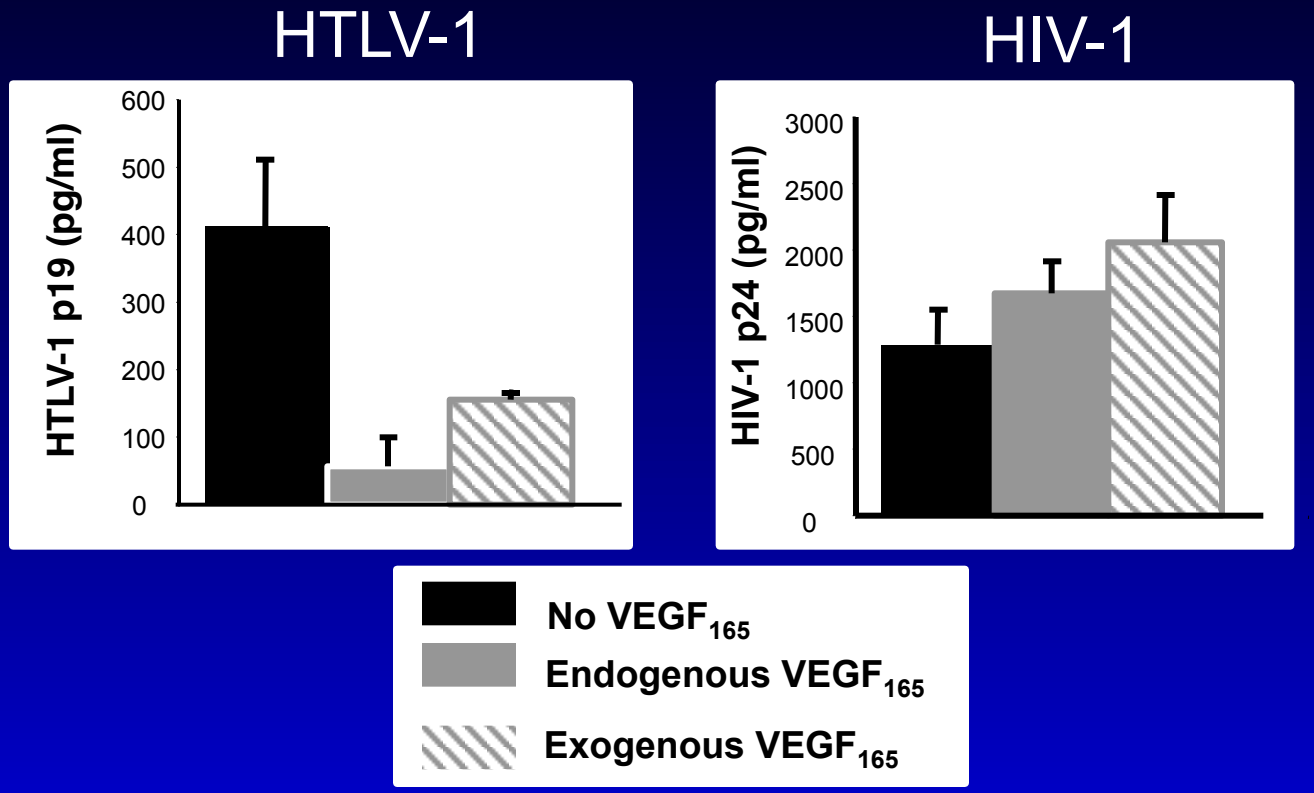
HTLV-1 Binds Poorly to Dendritic Cells Endogenously Expressing VEGF





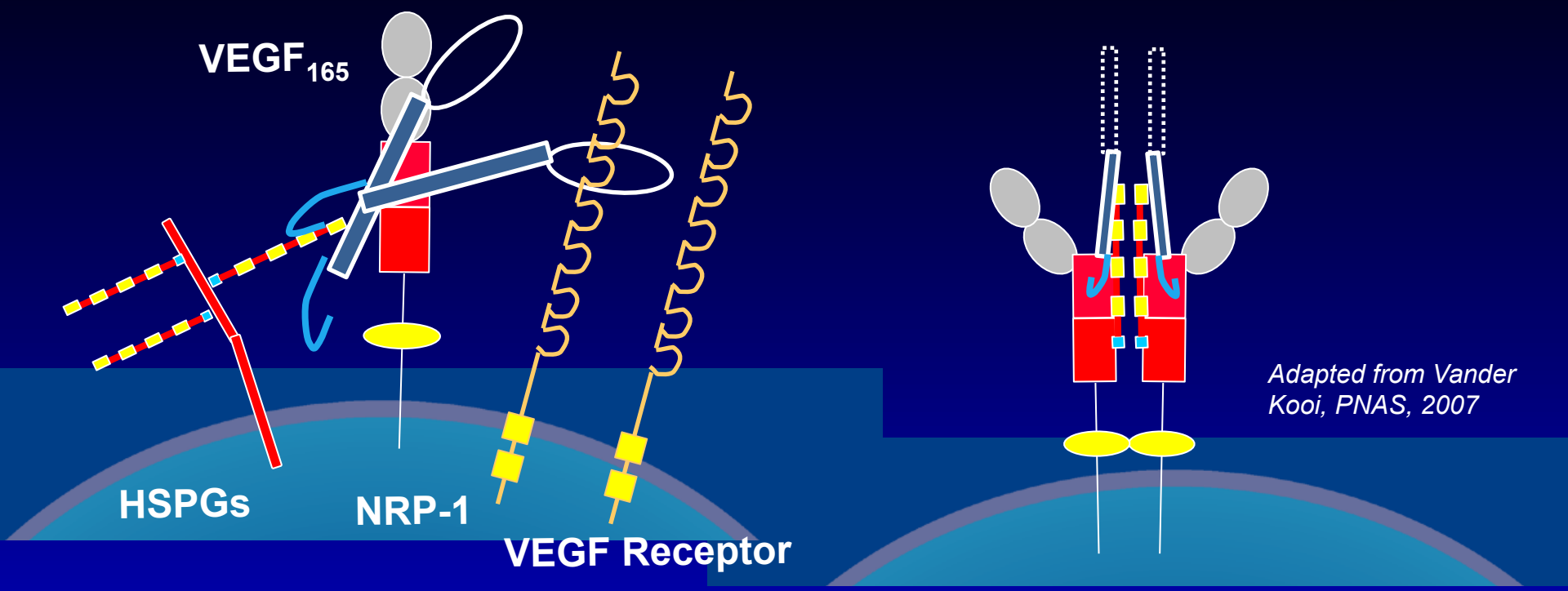
Lambert et al., Blood 13:5176 2009

$VEGF_{165}$ Blocks Infection of DC by HTLV-1



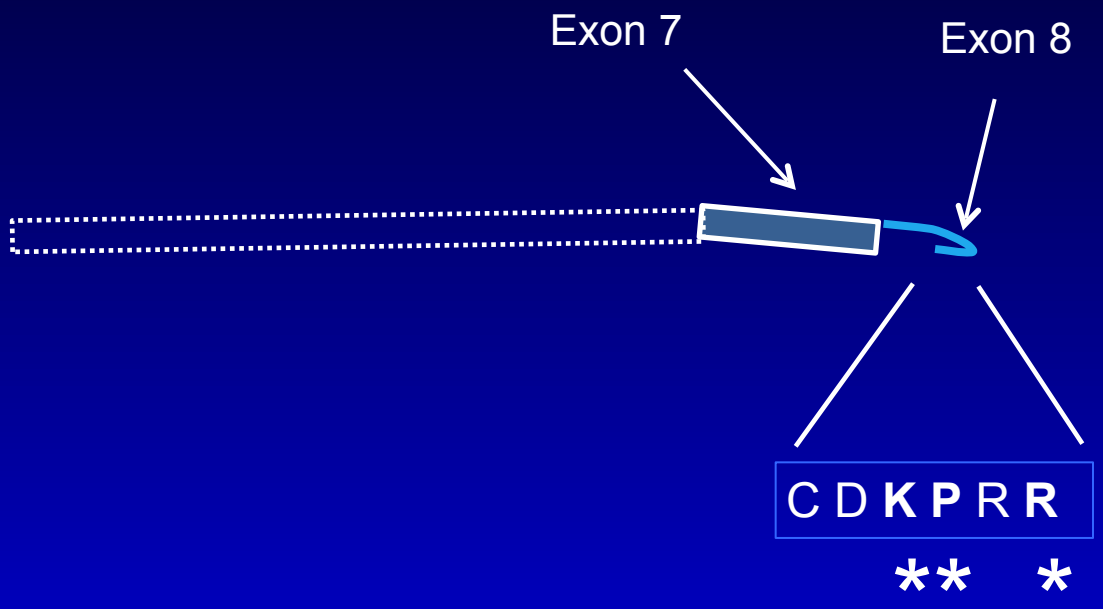
$VEGF_{165}$ blocks HTLV-1 binding, entry, and infection of DCs

NRP-1 and HSPG cooperate to bind VEGF$_{165}$



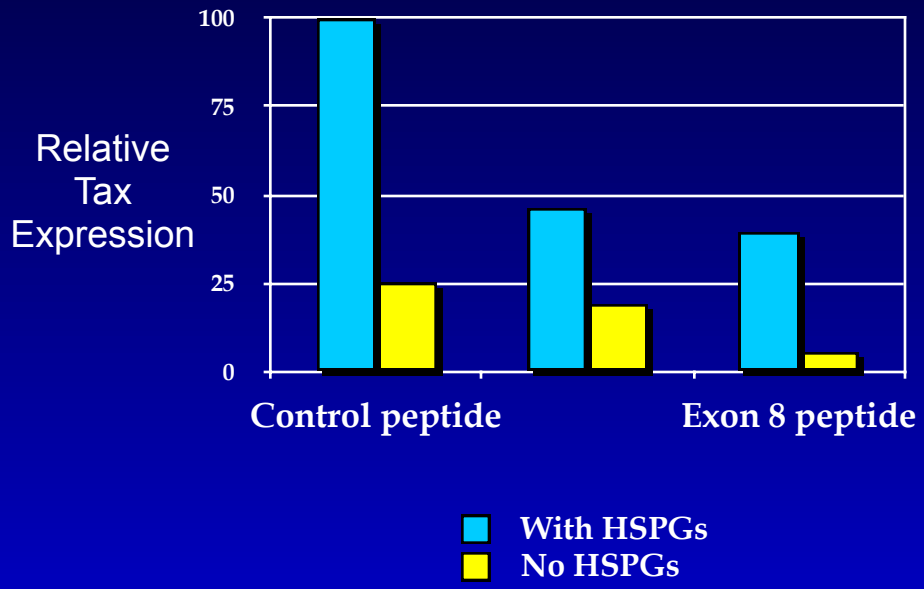
1. VEGF$_{165}$ binds to a complex of HSPGs and NRP-1
   - Binding is 20-100 fold greater in presence of HSPGs
2. HSPGs promotes NRP-1 dimerization
3. After binding to HSPG/NRP-1, VEGF$_{165}$ interacts with another receptor (VEGF-R2)

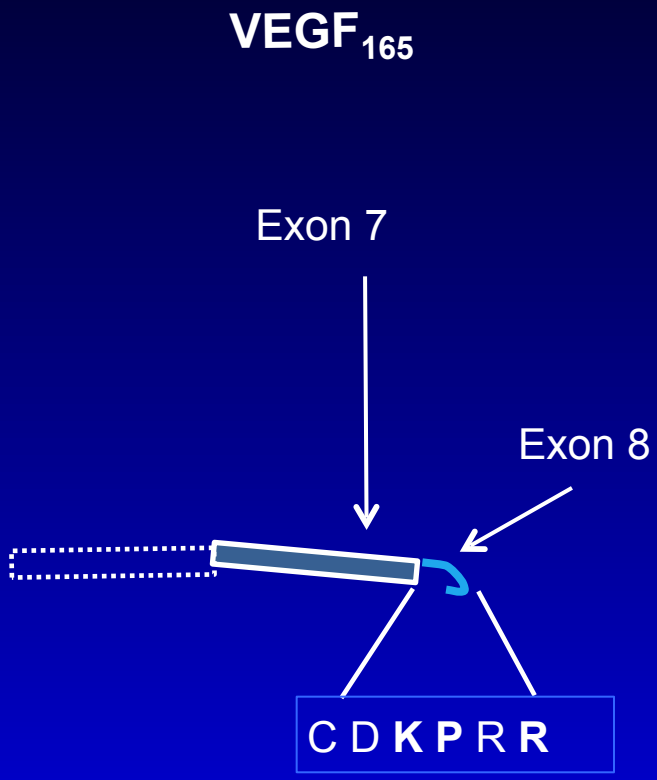
Regions of the Ligand $VEGF_{165}$ Involved in Binding NRP-1



1. Mutagenesis of the 3 conserved residues reduce $VEGF_{165}$ binding to NRP-1
2. Different peptides containing the KPxR motif bind directly to NRP-1 (Biacore)

Both Exon 7 and Exon 8 Peptides Block Infection of Primary CD4⁺ T cells via DC





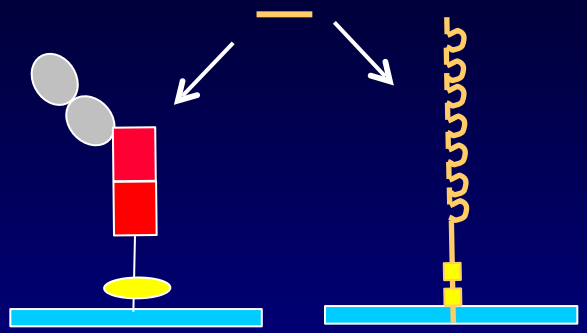
Does HTLV-1 SU contain sequences homologous to the regions of $VEGF_{165}$ required for NRP-1 binding?

HTLV/STLVs SU Contains a VEGF exon 8-like Motif

		** *
$VEGF_{165}$ exon 8	C D	**K P** R **R**
HTLV-1 SU (90-94)	K	**K P** N **R**

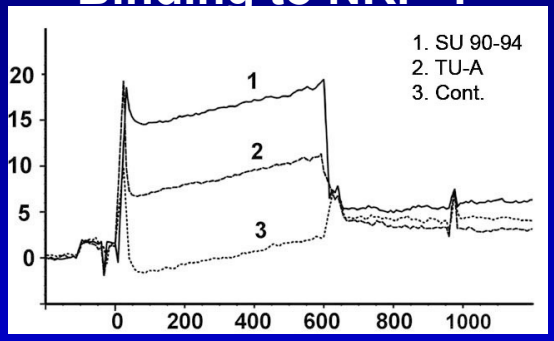
- HTLV-1 SU has a region homologous to $VEGF_{165}$ exon 8
- Consensus motif (KPxR) found in 99% (308/311) of HTLV and STLV SU
- This region contains the 3 residues in $VEGF_{165}$ that directly interact with NRP-1
- Arg 94 residue of SU- critical for infection (Delamarre, J. Virol, 1997)

Peptide Homologous to HTLV-1 SU aa 90-94 Binds Directly to NRP-1



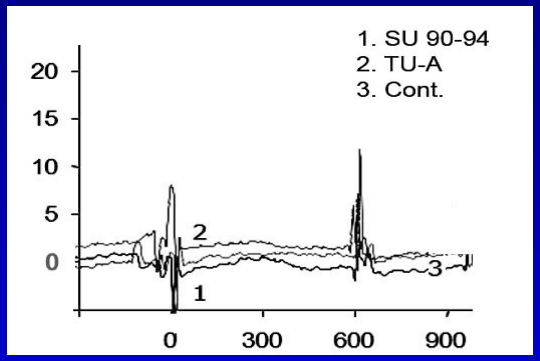
1. SU (90-94) K **K P** N **R**
2. Tuftsin analogue T **K P** P **R**
3. Control L T R K D

Binding to NRP-1



Time (sec)

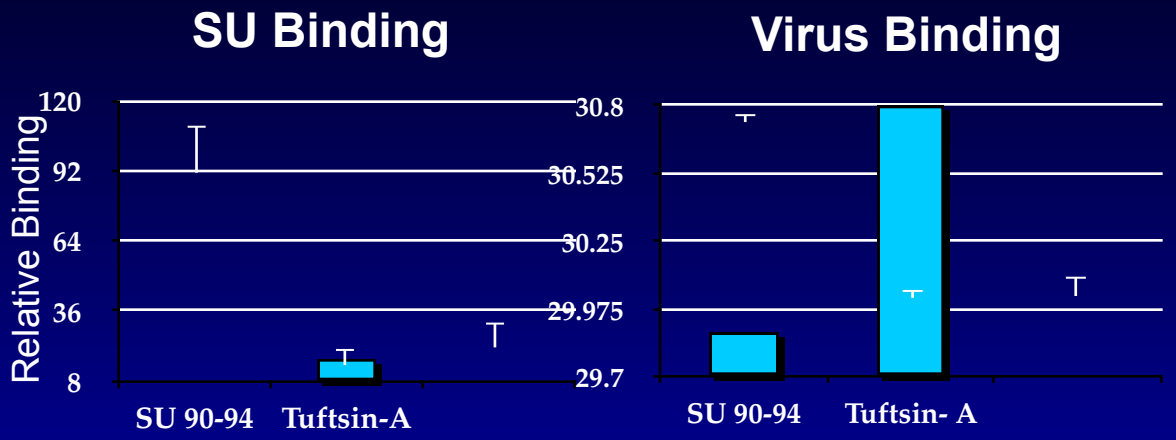
Binding to Control Protein

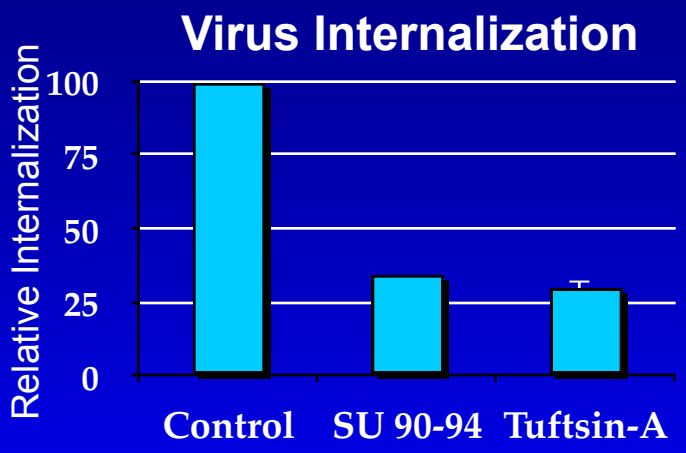


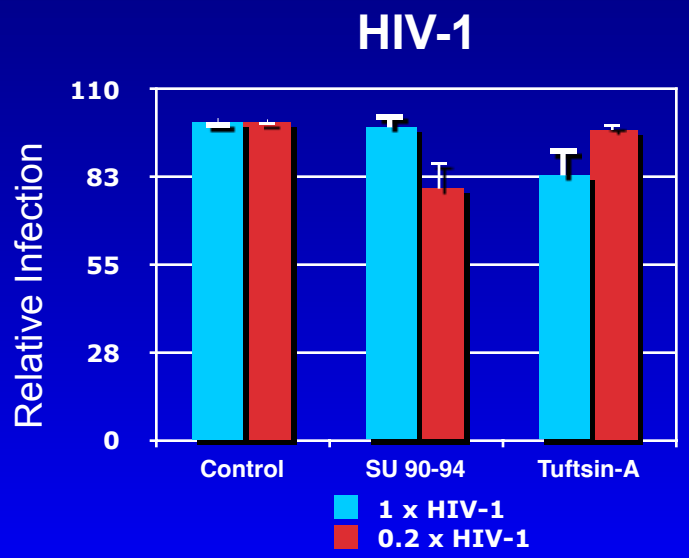
Time (sec)

Peptides containing KPxR Motif Block HTLV-1 Binding and Internalization into CD4+ T Cells

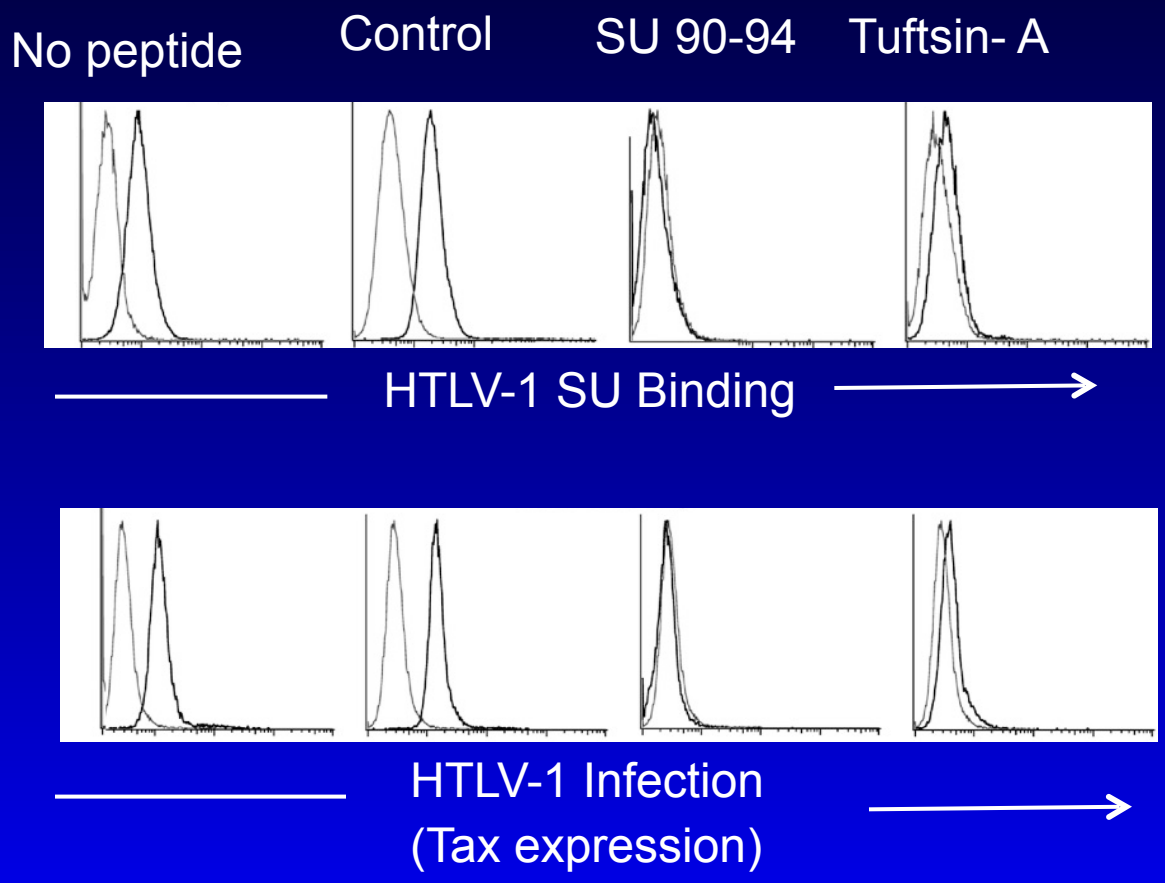
SU (90-94)	K **K P** N **R**
Tuftsin- A	T **K P** P **R**
Control	L **T R** K **D**







Peptides containing KPxR Consensus Motif Block HTLV-1 Binding to and Infection of Dendritic Cells



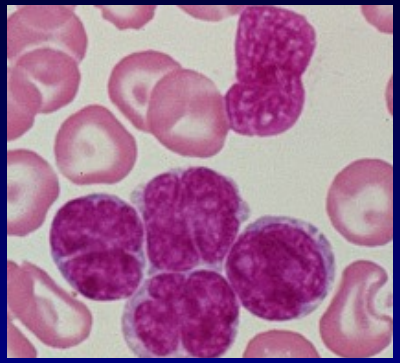
HTLV-1 SU Uses Molecular Mimicry of the Neuropilin-1 Ligand VEGF-A_{165} To Enter Target Cells Via HSPG and Neuropilin-1 Complexes

Same mechanism is employed for both cell-free and cell to cell transmission

Does HTLV-1 Infection of pDCs Play a Role in ATL?

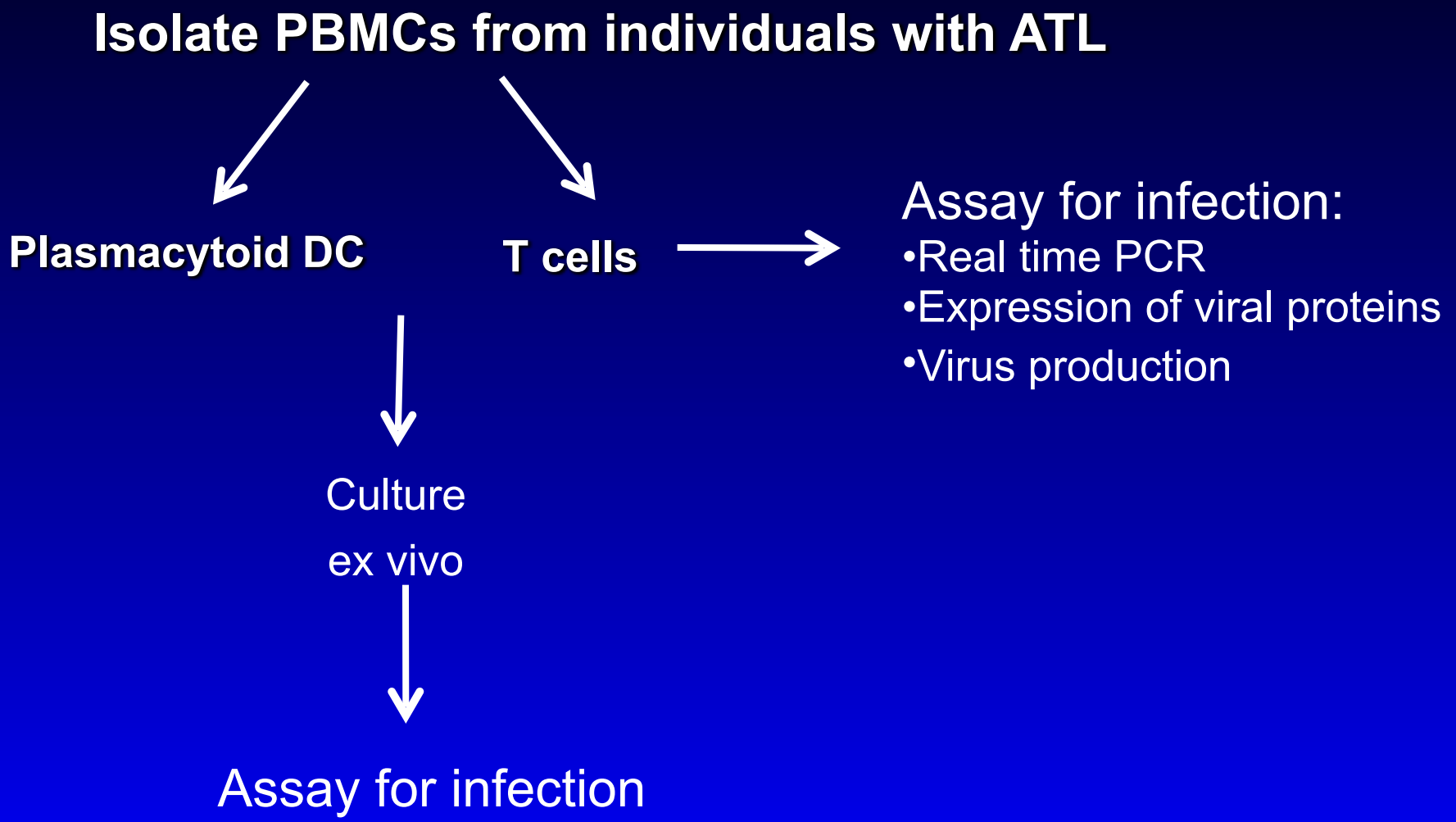
Adult T Cell Leukemia/Lymphoma (ATL)

- Clonal malignancy of T cells (usually $CD4^+$ $CD45RO^+$)
- Associated with severe immunodeficiency
- Poor prognosis (mean survival <12 months)

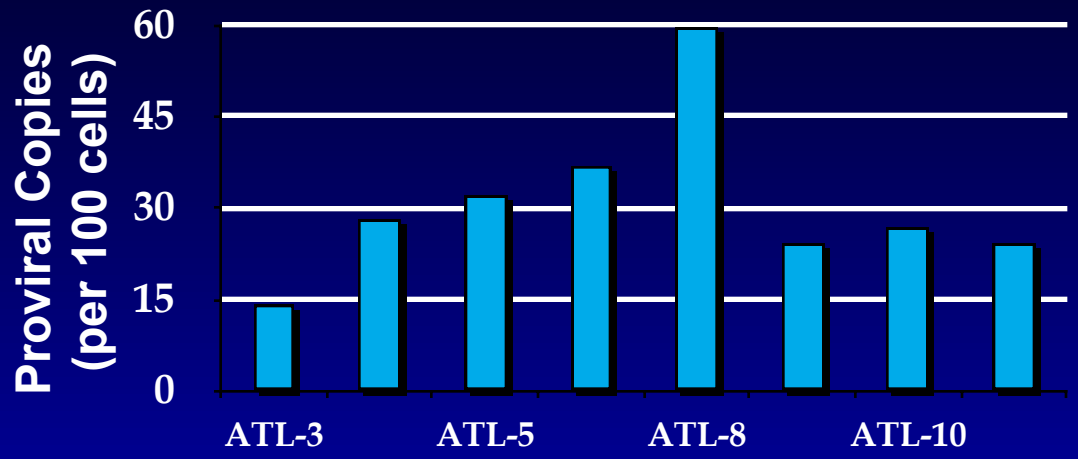


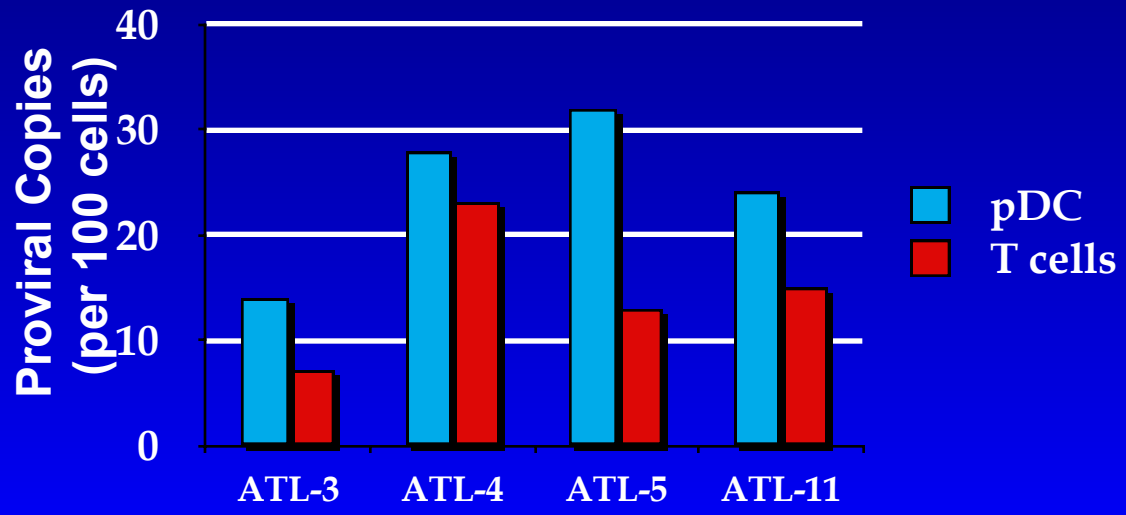
- Prolonged survival/remission can be obtained by treatment with IFN-α and AZT
- All malignant cells contain HTLV-1 proviral sequences
  - Low/undetectable expression of virus
  - ATL cells express HTLV-1 after ex vivo culture

Experimental Approach

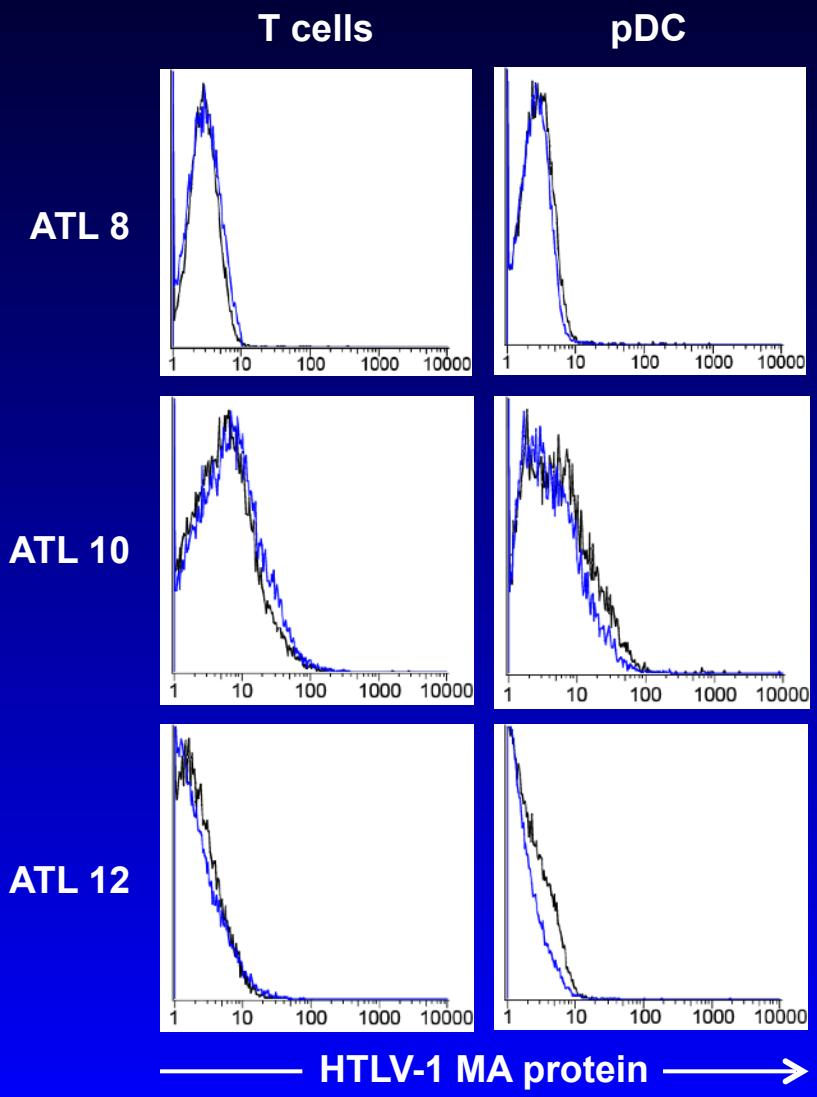


pDC Isolated from ATL Patients Are Infected with HTLV-1

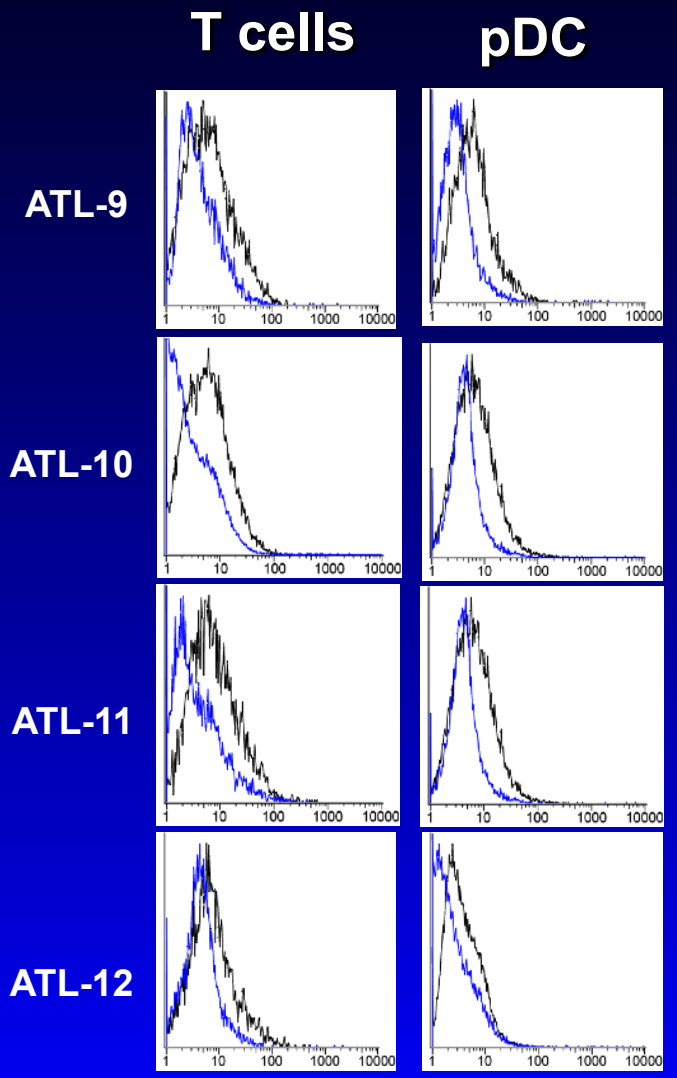




Little or No Viral Expression in pDC from ATL Patients Immediately After Isolation

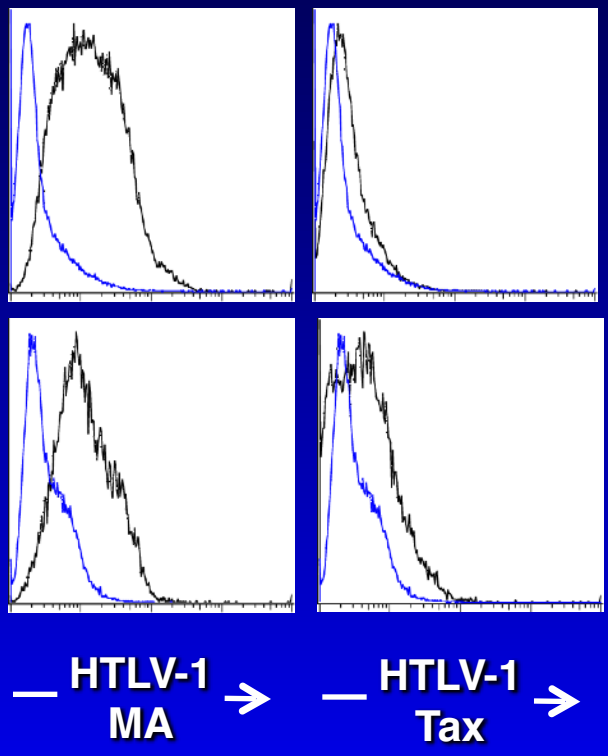


Viral Expression Increases in pDC and CD4+ T cells Following Ex Vivo Culture of ATL

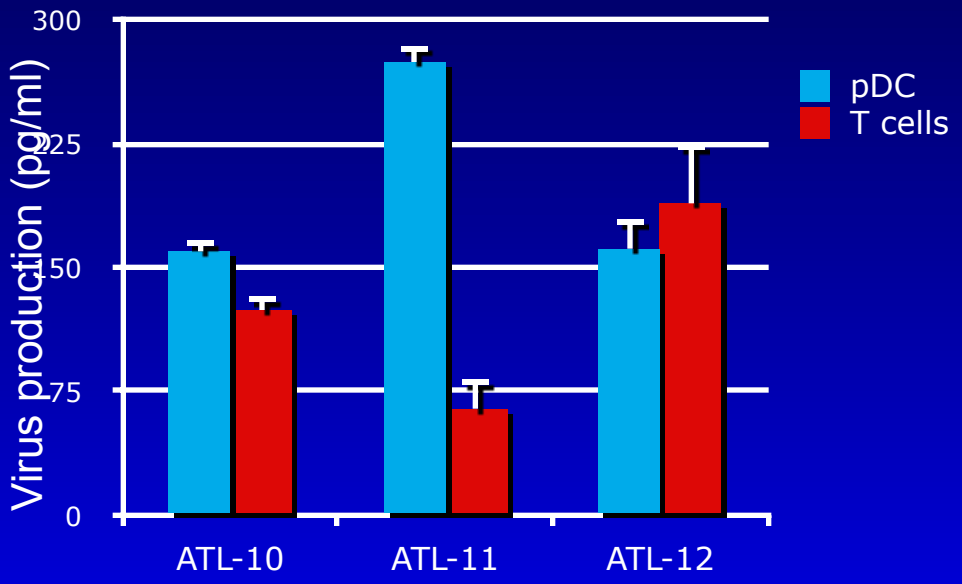


Viral Production In pDC and CD4+ T cells from ATL Patients After Ex Vivo Culture

Viral Protein Expression



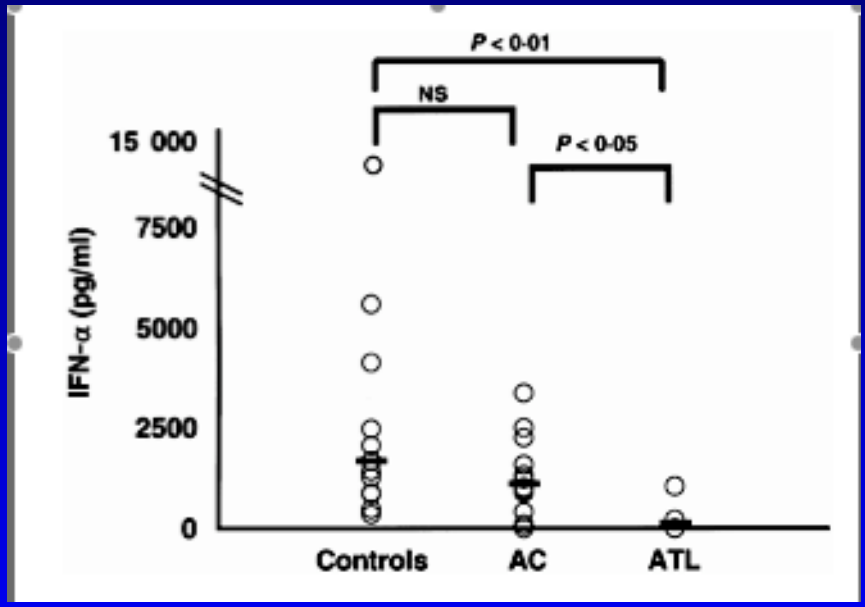
Virus Production



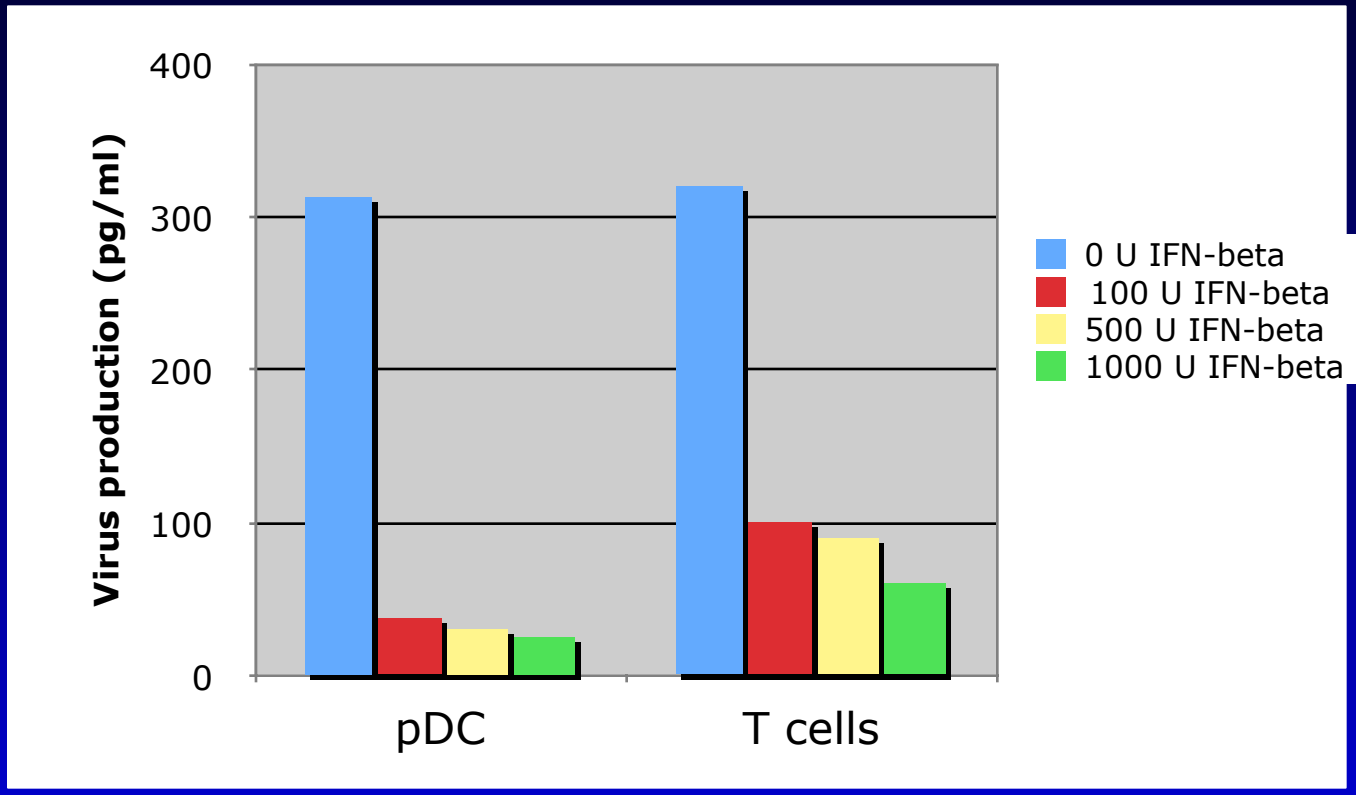
HTLV-1 Infection of pDC and Pathogenesis: Role for Type I IFN?

- Many viruses counteract innate immune response by blocking:
  - Production of Type I IFN
  - Binding of Type I IFN to receptors
  - Signalling by Type I IFN

IFN-α production capacity of PBMCs from HTLV-1-infected individuals

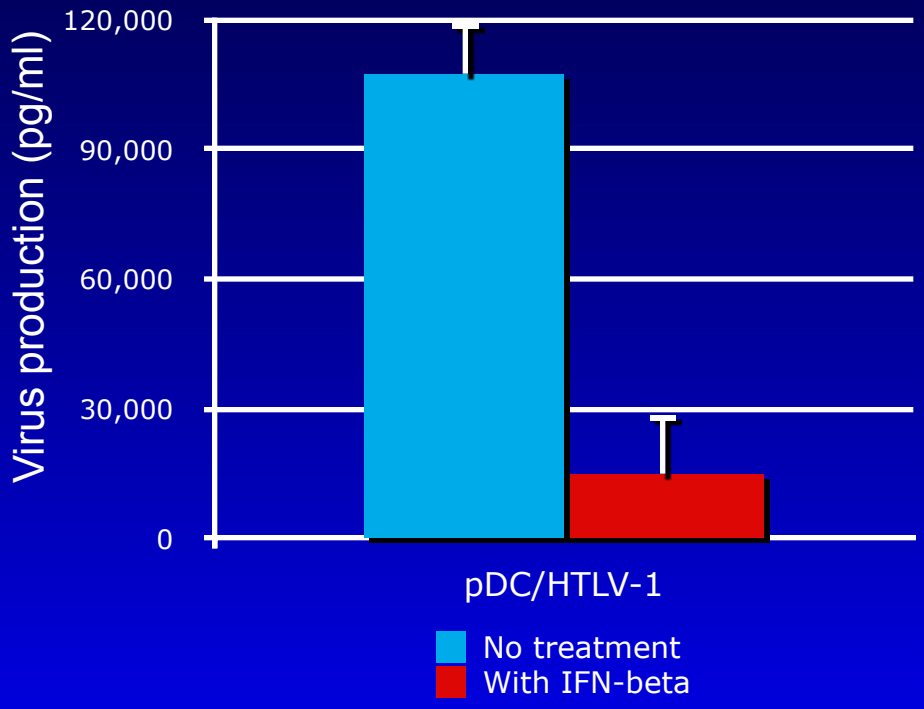


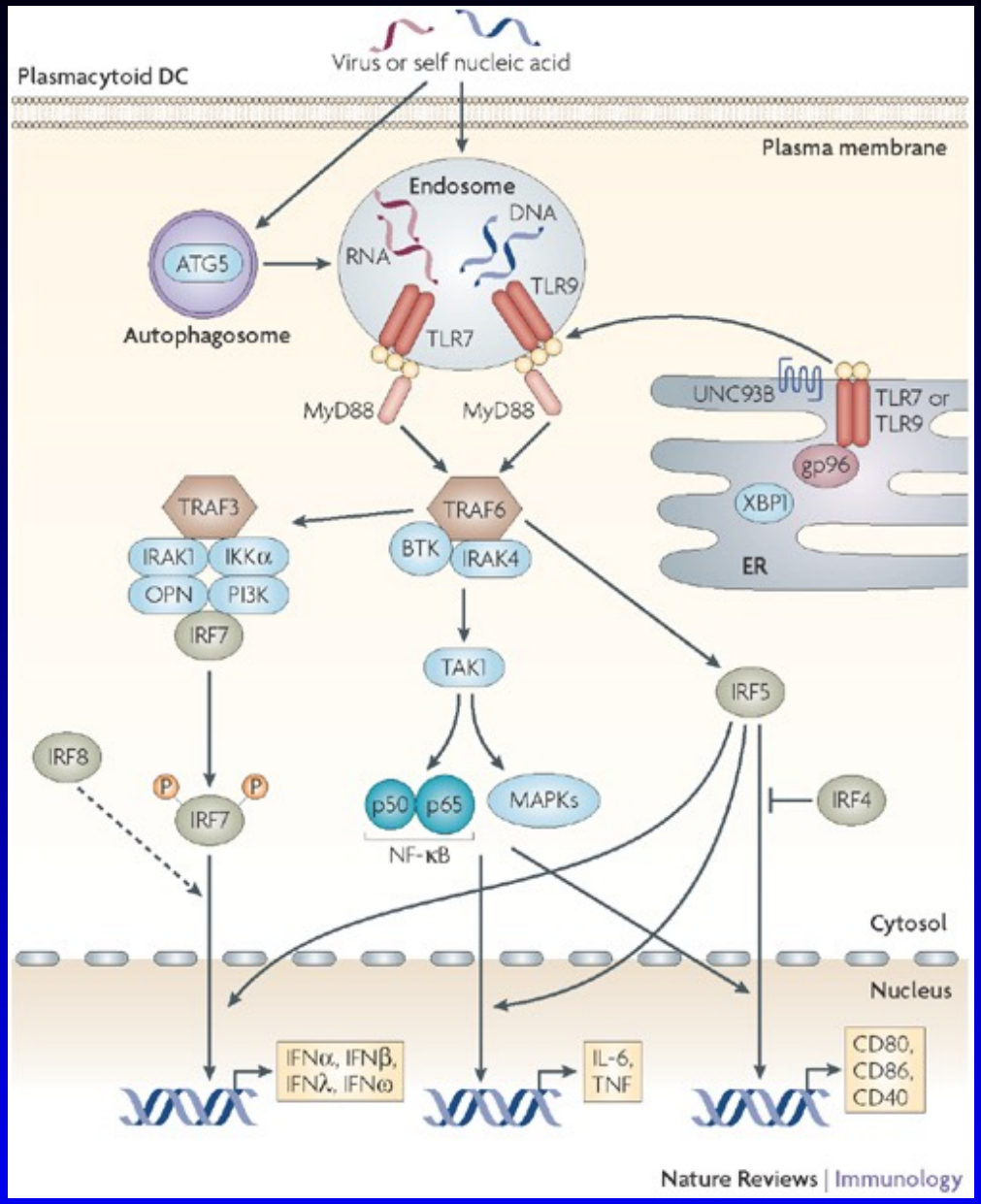
Effect of Type I IFN on HTLV-1 Production From pDC and T cells From ATL Patient



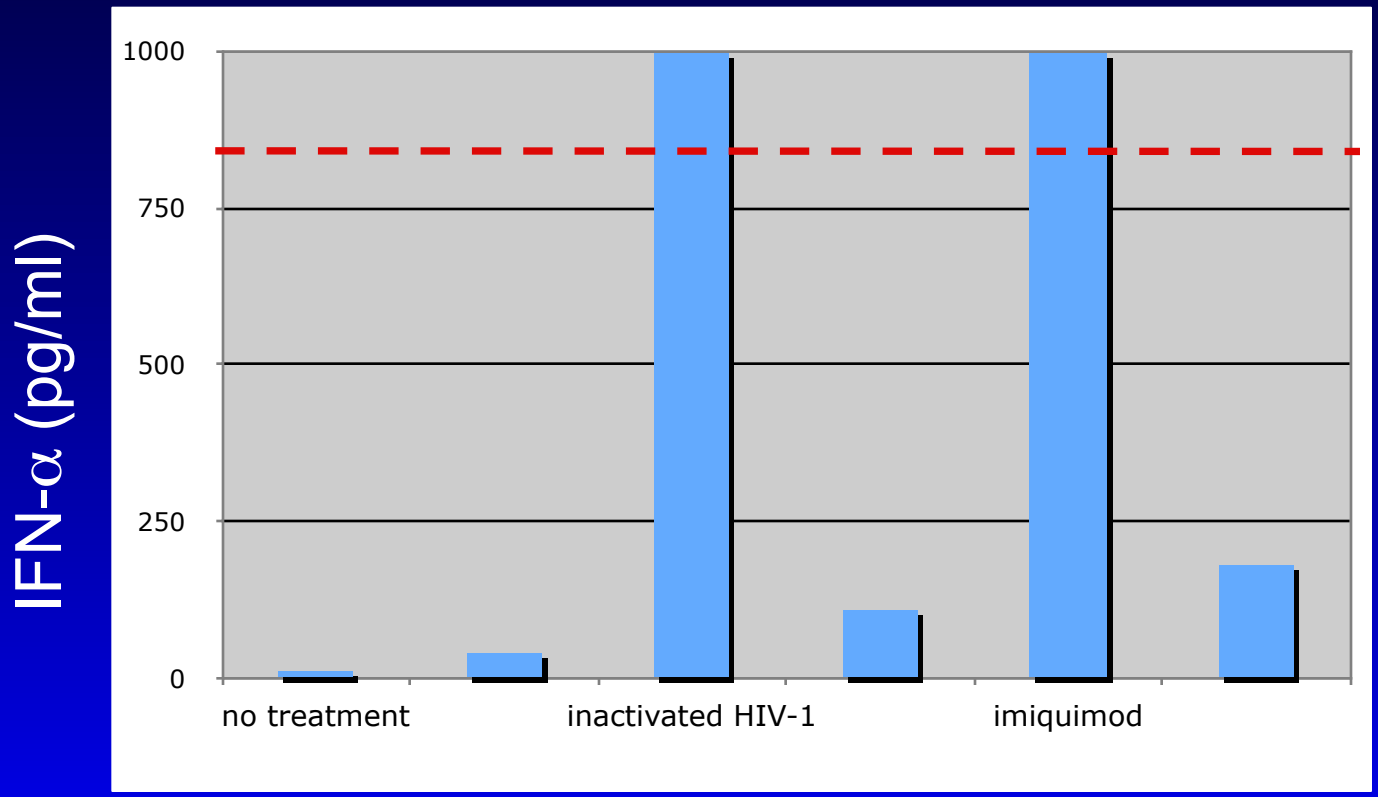
- Recently reported that Type I IFN controls HTLV-1 expression by T cells from ATL patients/ ATL-derived cell lines (Kinpara et al., J Virol., 83:5101, 2009)

Type I IFN Reduces HTLV-1 Production By HTLV-1-Infected pDC Infected In Vitro

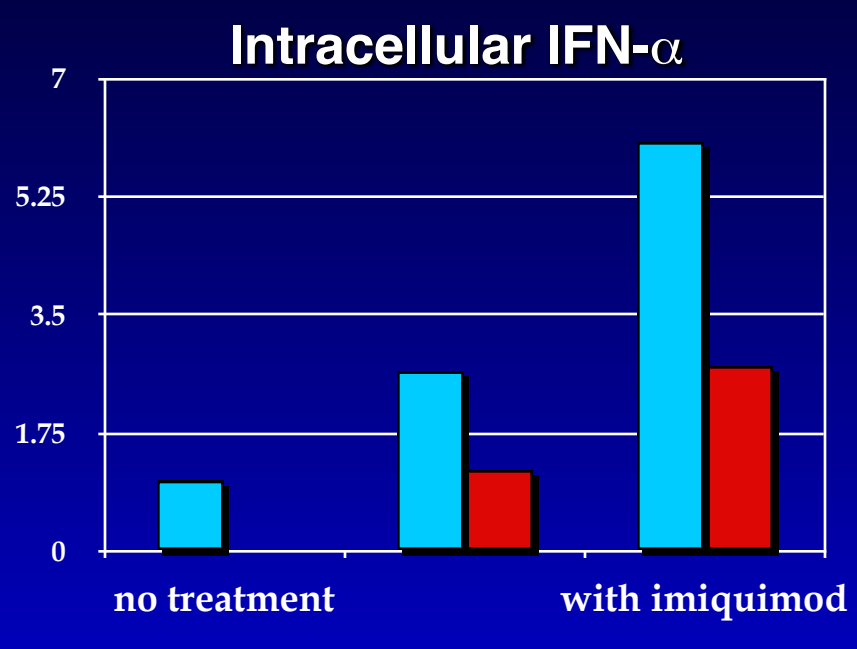


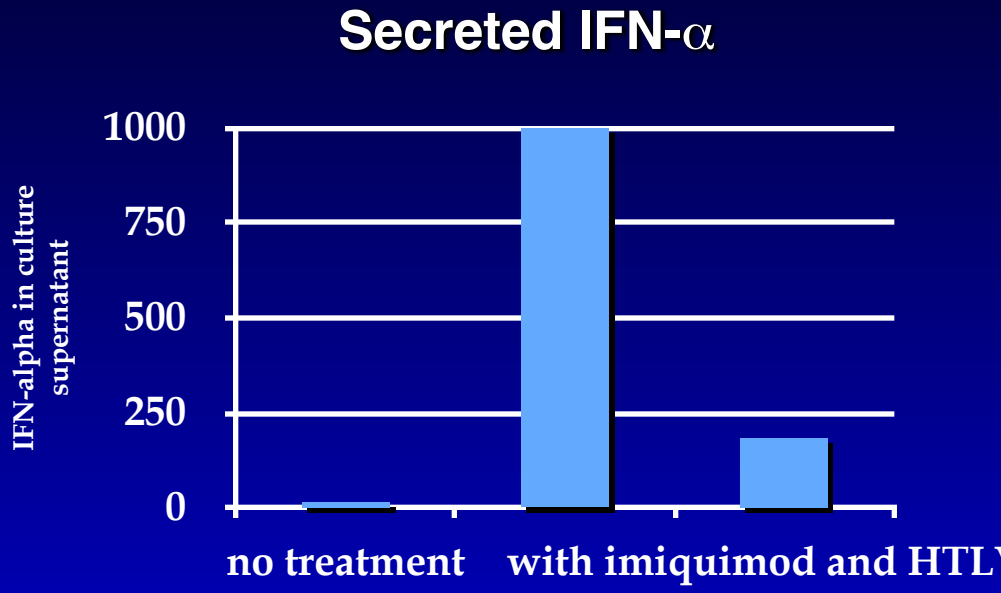

Plasmacytoid DC
Virus or self nucleic acid
Plasma membrane
Endosome
DNA
RNA
TLR9
TLR7
ATG5
Autophagosome
MyD88
MyD88
UNC93B
TLR7 or
TLR9
gp96
XBP1
ER
TRAF3
TRAF6
IRAK1
IKKα
OPN
PI3K
IRF7
BTK
IRAK4
TAK1
IRF5
IRF8
P
P
IRF7
p50
p65
MAPKs
NF-κB
IRF4
Cytosol
Nucleus
IFNα, IFNβ,
IFNλ, IFNω
IL-6,
TNF
CD80,
CD86,
CD40
Nature Reviews | Immunology

Level of IFN-α Secreted by pDC Exposed to Viruses and TLR7 Agonist



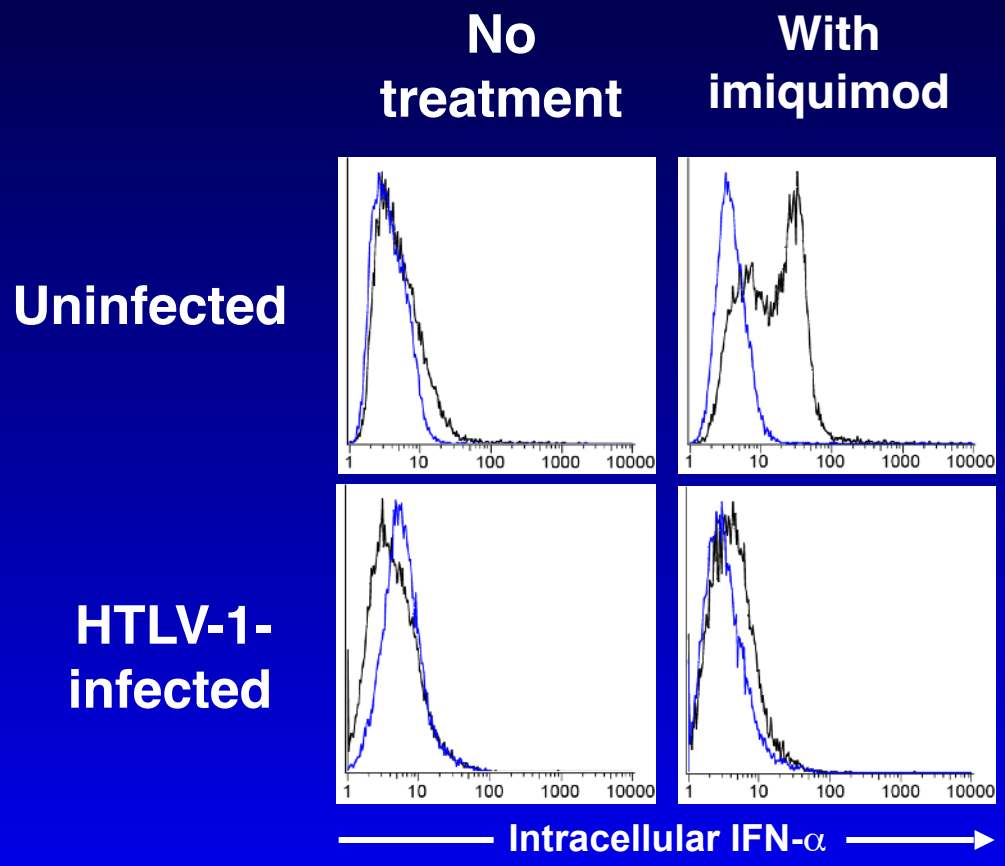
HTLV-1 Reduces Production of IFN-α in pDC Exposed to TLR7 Agonist



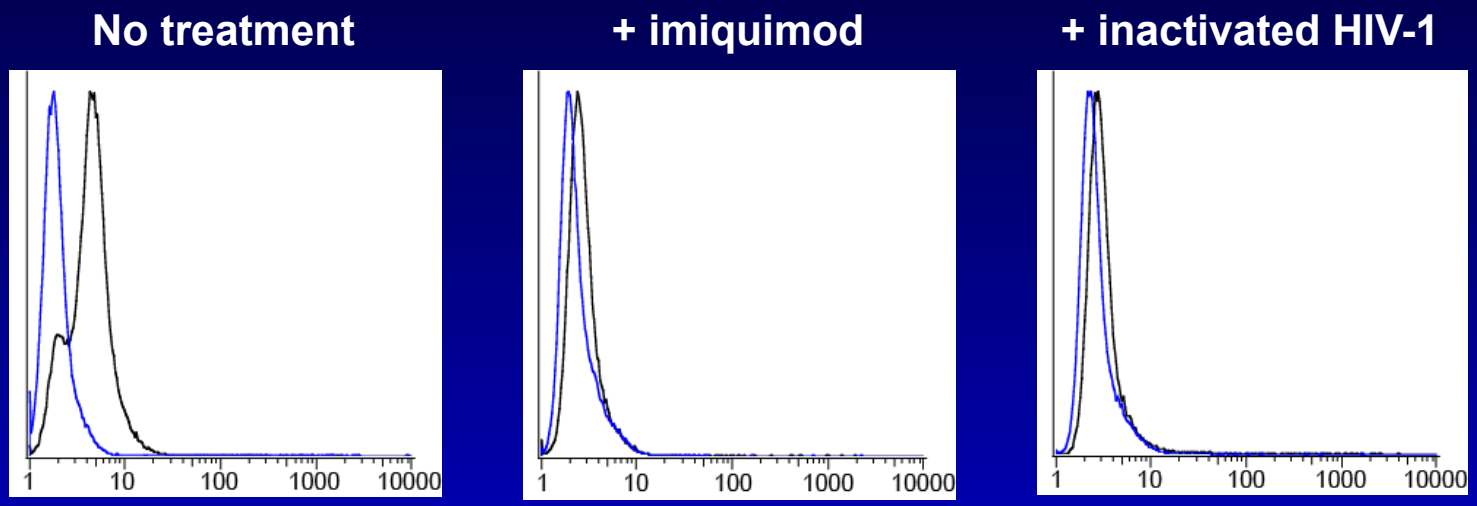


no HTLV-1
with HTLV-1

IFN-α Production Is Blocked in pDCs Chronically Infected with HTLV-1



TLR activation Blocks Infection by HTLV-1



Summary –IFN control and HTLV-1 Production

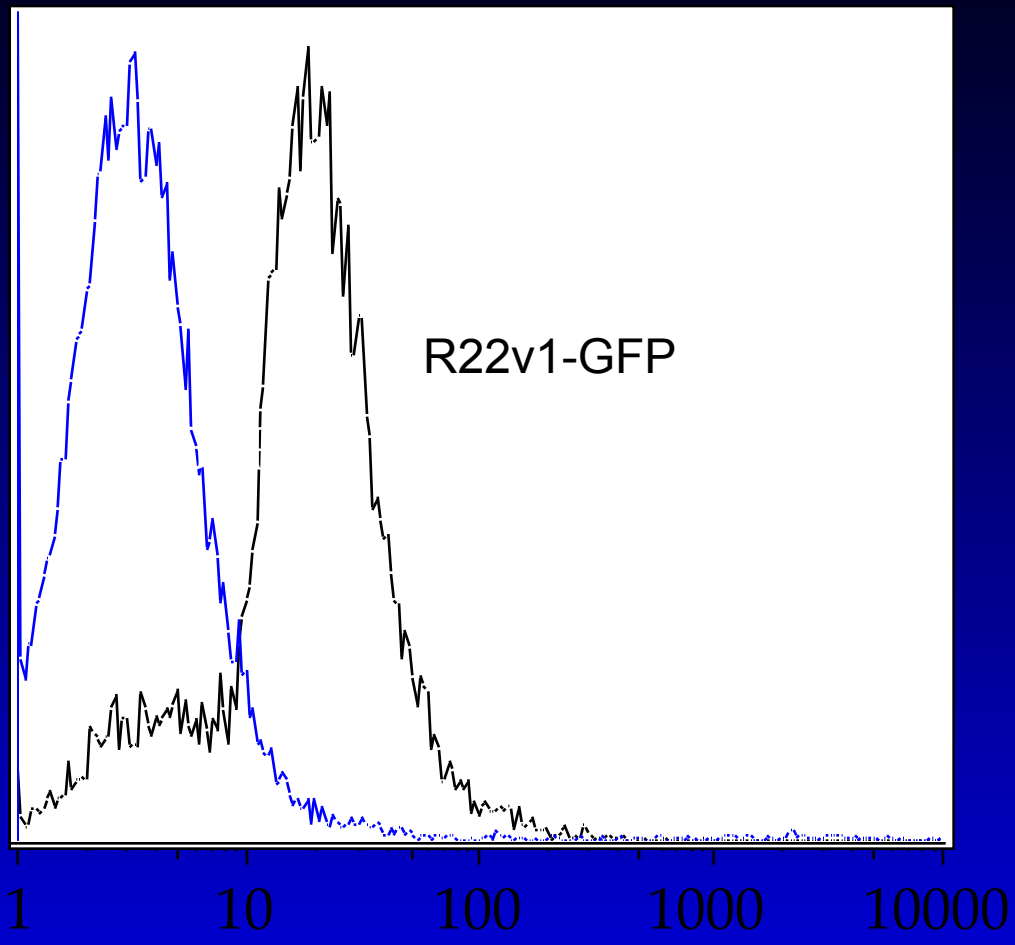
1. pDC in the peripheral blood of individuals with ATL are infected with HTLV-1

2. HTLV-1-infected pDC from ATL patients, like T cells:
   - Express low or undetectable levels of viral proteins
   - Express virus after ex vivo culture
   - Virus expression- controlled by Type I IFN (Tetherin?)

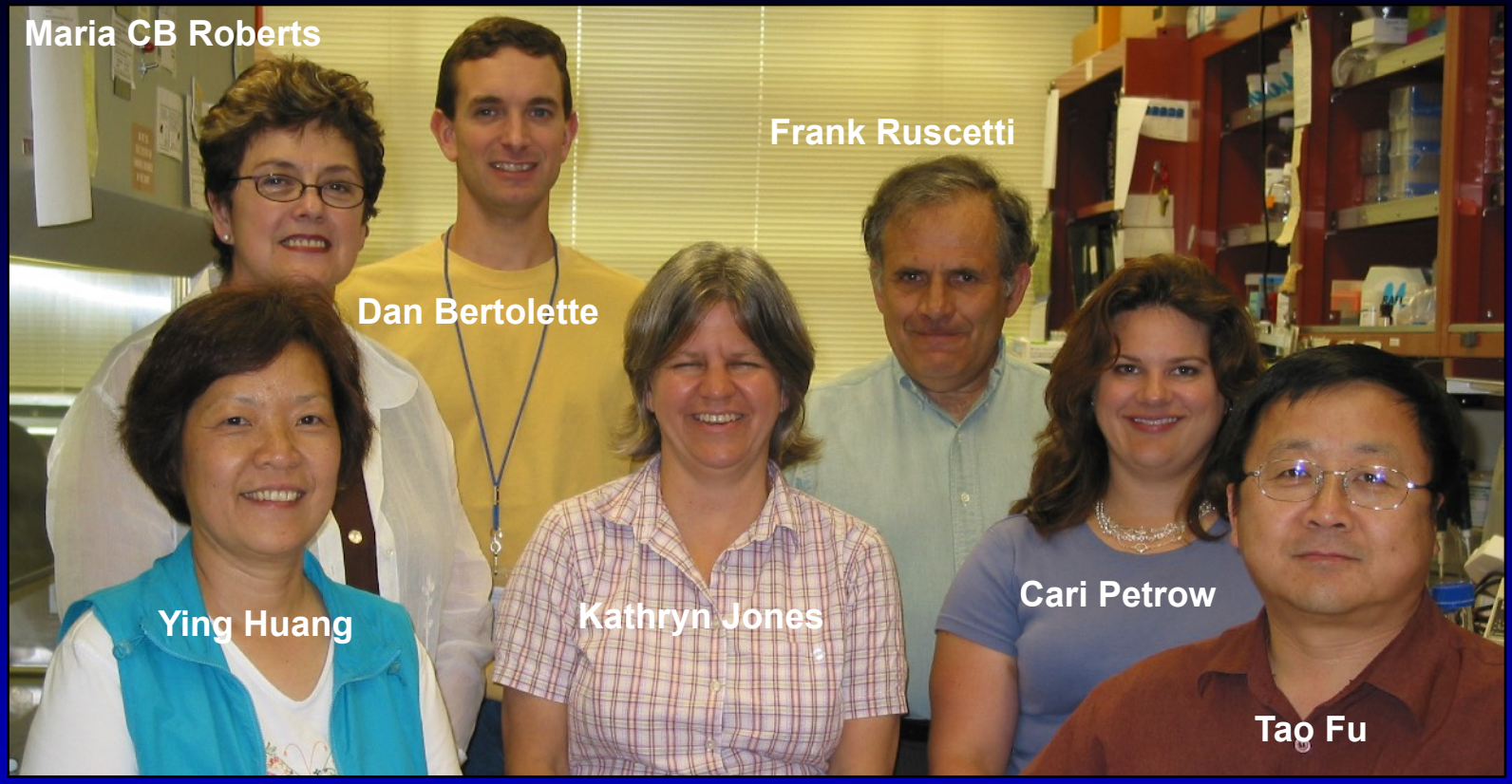
3. HTLV-1-infected pDC have reduced capacity to produce Type I IFN in response to TLR7 agonists

4. These data suggest possible roles for pDC in:
   - viral transmission and/or persistence
   - ATL disease progression
   - immune dysfunction

XMRV infects plasmacytoid DC



Latest results on XMRV detection are presented in poster #62 (Mikovits et al)



Leukocyte Biology Section, LEI

Acknowledgments

Sophie Lambert
Claudine Pique
Institut Cochin

Sébastien A. Chevalier
Renaud Mahieux
Institut Pasteur

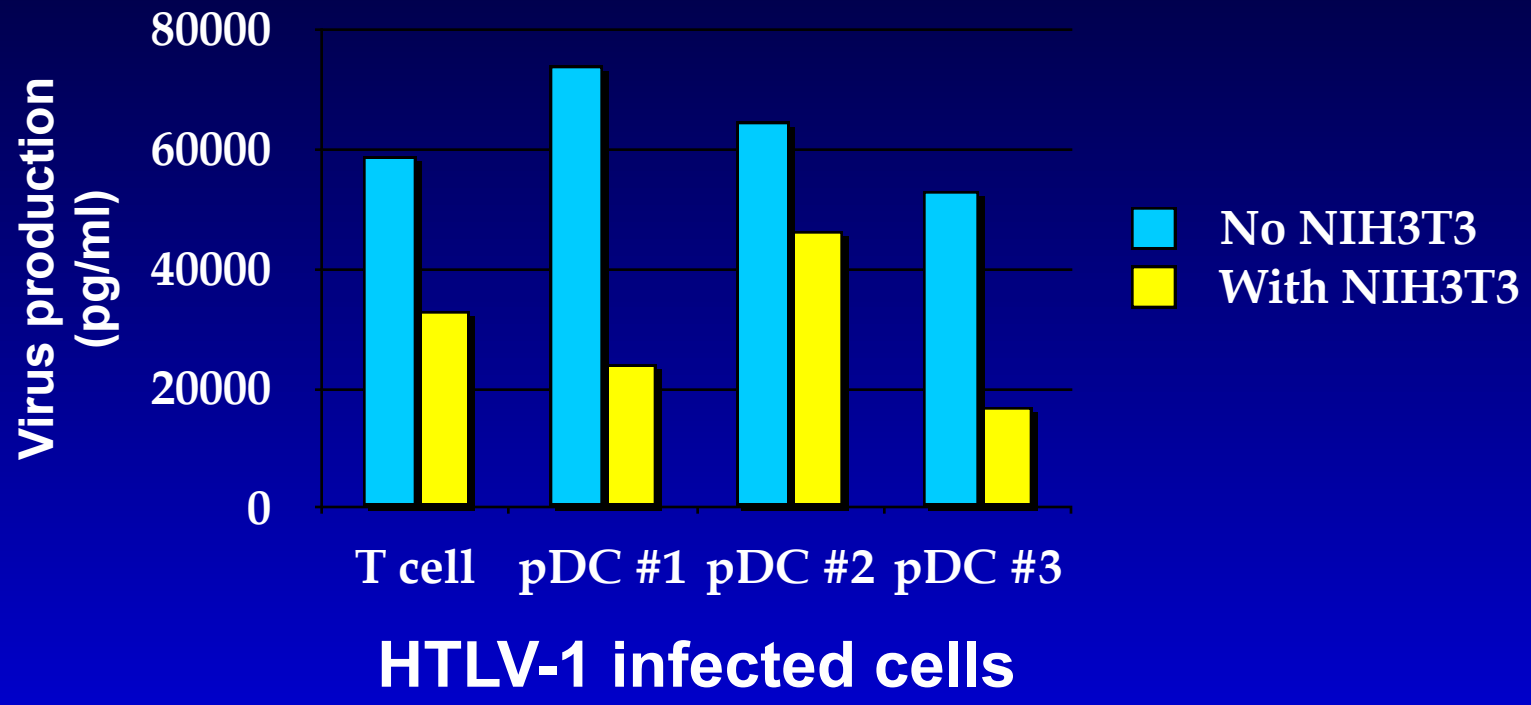
Vibeke Andresen
Nancy van Prooyen
Veffa Franchini
National Institutes of Health

John Janik
John Moore
Tom Waldmann
National Cancer Institute

Oh Unsong
Steve Jacobson
National Institutes of Health

Chou-Zen Giam
Uniform Services University of Health Science

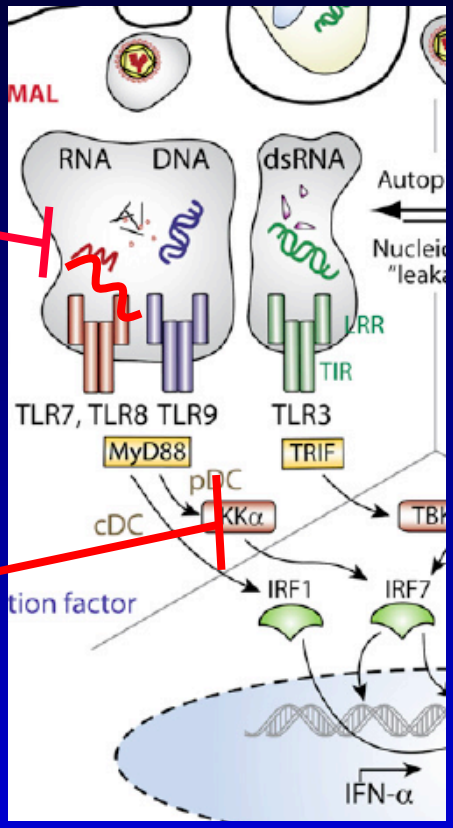
Coculture with IFN-β-producing Cells Reduces Virus Production from HTLV-1-infected pDC

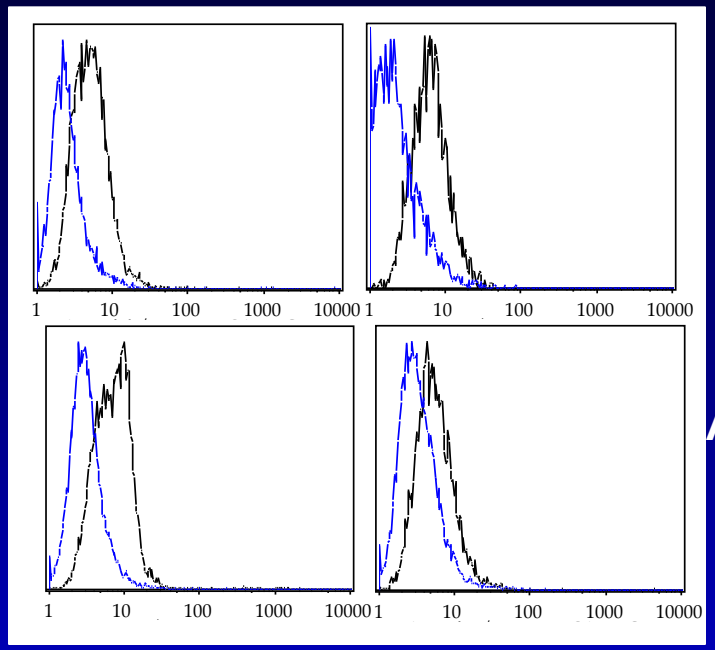


Is IFN-α Production in pDC Exposed to HTLV-1 Blocked by Competition for TLR Signaling?

HTLV-1 RNA?

HTLV-1 protein?





Exposed to AT-2 HIV

Exposed to AT-2 HIV and HTLV-1

Intracellular IFN-α

Appears block to IFN-α production is subsequent to TLR signaling