Standardization of Immune Biomarkers: Lessons From the HIV Field

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CD4 and Viral Load

Standardization

Immunology Quality Assessment Program(IQA)

The IQA is a resource designed to help Immunologists evaluate and enhance the integrity and comparability of immunological laboratory determinations performed on patients enrolled in multi-site HIV/AIDS investigations (therapeutic, vaccine, prevention, etc.).

IQA Program

- **~83 Participating Laboratories**
- **6 Shipments per year (Jan, Mar, May, Jul, Sept, Nov)**
- **Included in shipment 5 Whole Blood samples**
- **Samples are shipped overnight priority via Federal Express in Ambient Temperature (Guaranteed delivery by 10:30 am next business day)**

IQA Program

- **Laboratories are divided into three or four groups**
- **Each laboratory receives 5 EDTA whole blood samples**
- **Each group will receive some type of replicate scheme (Quad, Trip, Doublet)**

IQA Program

- **All 83 laboratories will receive either 1, 2, or 3 common samples** (Depending on the replicate scheme for the particular sendout)
- **All 83 laboratories are expected to perform CD4/CD8 determination**
- **10 Advance flow laboratories are also expected to perform analysis on extended markers** (CD4, CD8, CD38, CD45RA, CD45RO, CD62L, CD28, CD95, HLA-DR)

Information Captured

- **Date and Time samples received**
- **Date and Time Samples were prepared**
- **Type of Lysing method used**
- **Type of Flow Cytometer**

Information Captured cont'd

- **Date and time samples were analyzed**
- **Flow Cytometry panel make-up**
- **Hematology analyzer used**
- **Date and time Hematology samples were analyzed**

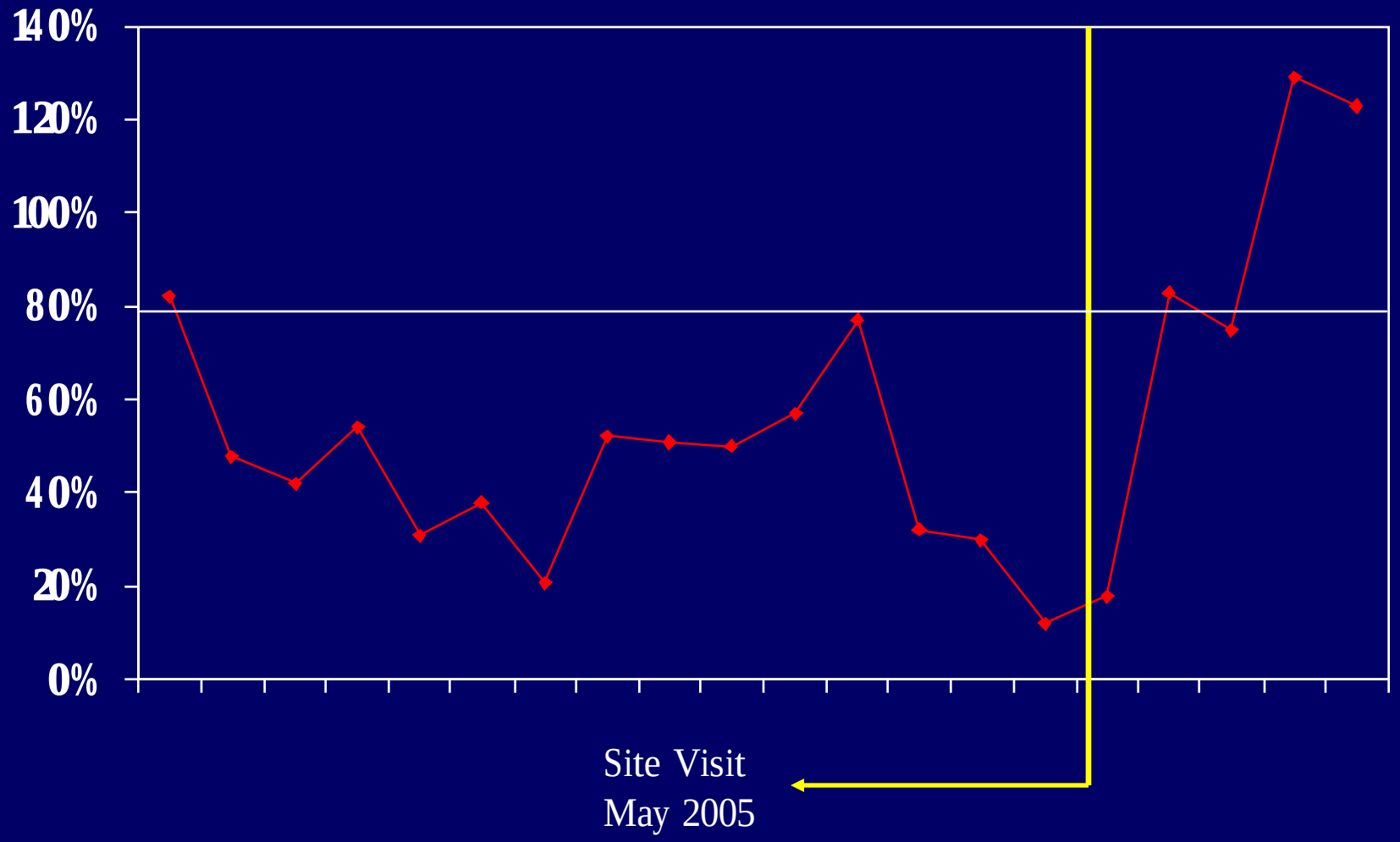
IQA Feedback

- **IQA reviews statistical report and identifies SITES that are having difficulty performing assays**
- **Poor performers are contacted via telephone or email to discuss specific problems**
- **Laboratories are requested to fax histogram results to the IQA for review**

IQA Feedback cont'd

- **If there is an analysis problem, the IQA will mail a virtual sample (List mode file saved on a CD) for analysis.**
- **If problem is not clear, the IQA may share samples with the faltering site with specific instructions on staining, acquisition and analysis.**
- **If problem is not solve, the IQA will offer to have a technologist visit the IQA for training or have an IQA staff member visit the faltering laboratory.**

Historical Performance
Site B



Cell Freezing

QC

How often do participating sites should submit samples?

- **Every three months (March, June, September and December)**, frozen PBMCs must be shipped from the participating laboratory to the NIAID Immunology Quality Assessment Program (IQA)

How is the assessment accomplished?

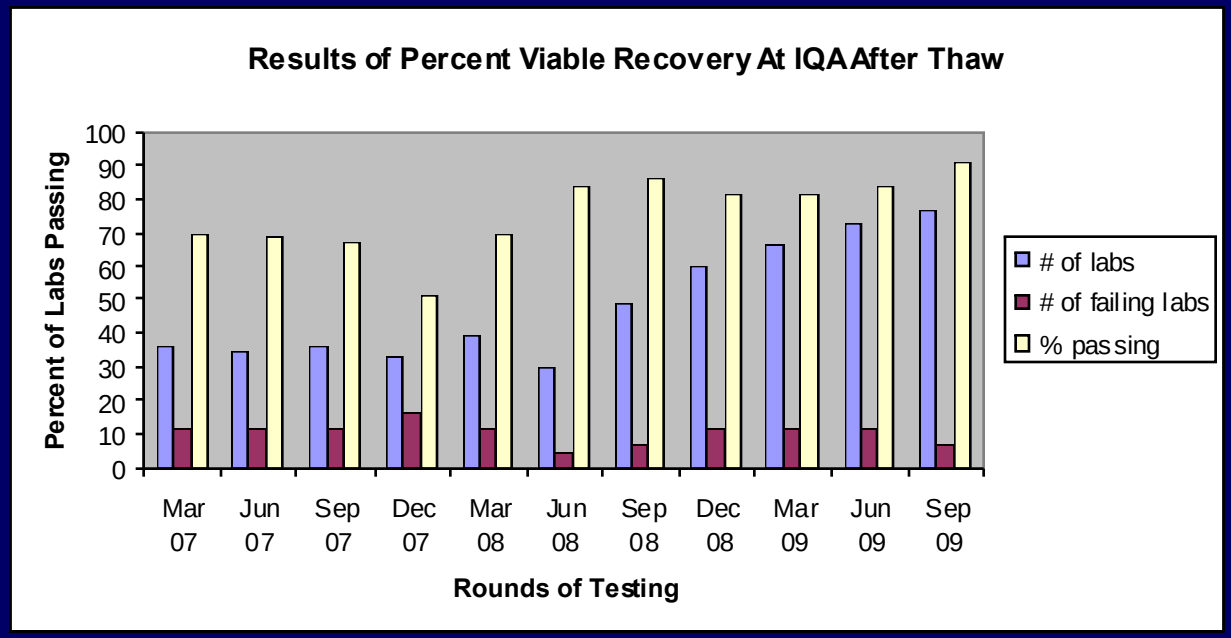
- The assessment is accomplished by comparing the viability of PBMCs and the viable yield before freezing and after thawing.

- Proper processing and freezing is a critical component of the process for storage of viable PBMCs.

What should the participating labs achieve?

Network*	Minimum Viability (%)	Minimum Viable Recovery (%)
ACTG	80	80
IMPAACT	75	70

*Laboratories who serve both the ACTG and IMPAACT must conform to the more stringent criteria of the ACTG (80% viability and 80% viable cell recovery)


Results of Percent Viable Recovery At IQA After Thaw
Percent of Labs Passing
100
90
80
70
60
50
40
30
20
10
0
Mar 07
Jun 07
Sep 07
Dec 07
Mar 08
Jun 08
Sep 08
Dec 08
Mar 09
Jun 09
Sep 09
Rounds of Testing
# of labs
# of failing labs
% passing

VQA Mission Statement

Quality Assurance

- Standardize Assays and Laboratory Procedures

Quality Control

- Monitor Sources of Error

Quality Assessment

- Proficiency Test

Assay Development

- Formulation, Development, & Validation

VQA Participants

- Enrolled Laboratories Groups Include:
  - ACTG, IMPAACT, HPTN, HVTN, MACS, CPCRA, DATRI, CIPRA, NICHD, RO1, WHO
- 63 Domestic Laboratories
  - 17 states, 1 territory
- 85 International Laboratories
  - 28 Countries, all except one continent

VQA QC Reagents

QC Reagents

- Purpose: To assist the VQA Program and the VQA Client Laboratories in monitoring the performance of their HIV-1 diagnostic assays as they are routinely performed in their laboratory.

Present VQA Reagents

- p24 Ag EIA Standard Curve Reagents
- p24 Ag EIA Medium/Serum Matrix Controls
- HIV-1 DNA PCR Copy Controls
- HIV-1 DNA PCR Blinded Pellets
- HIV-1 RNA PCR Copy Controls

VQA Proficiency Programs

Proficiency Testing

- Purpose: To monitor the performance of laboratories performing assays for NIAID clinical trials and epidemiology cohort studies, and to analyze the resultant data sets to identify sources and magnitude of variability in HIV-1 assays

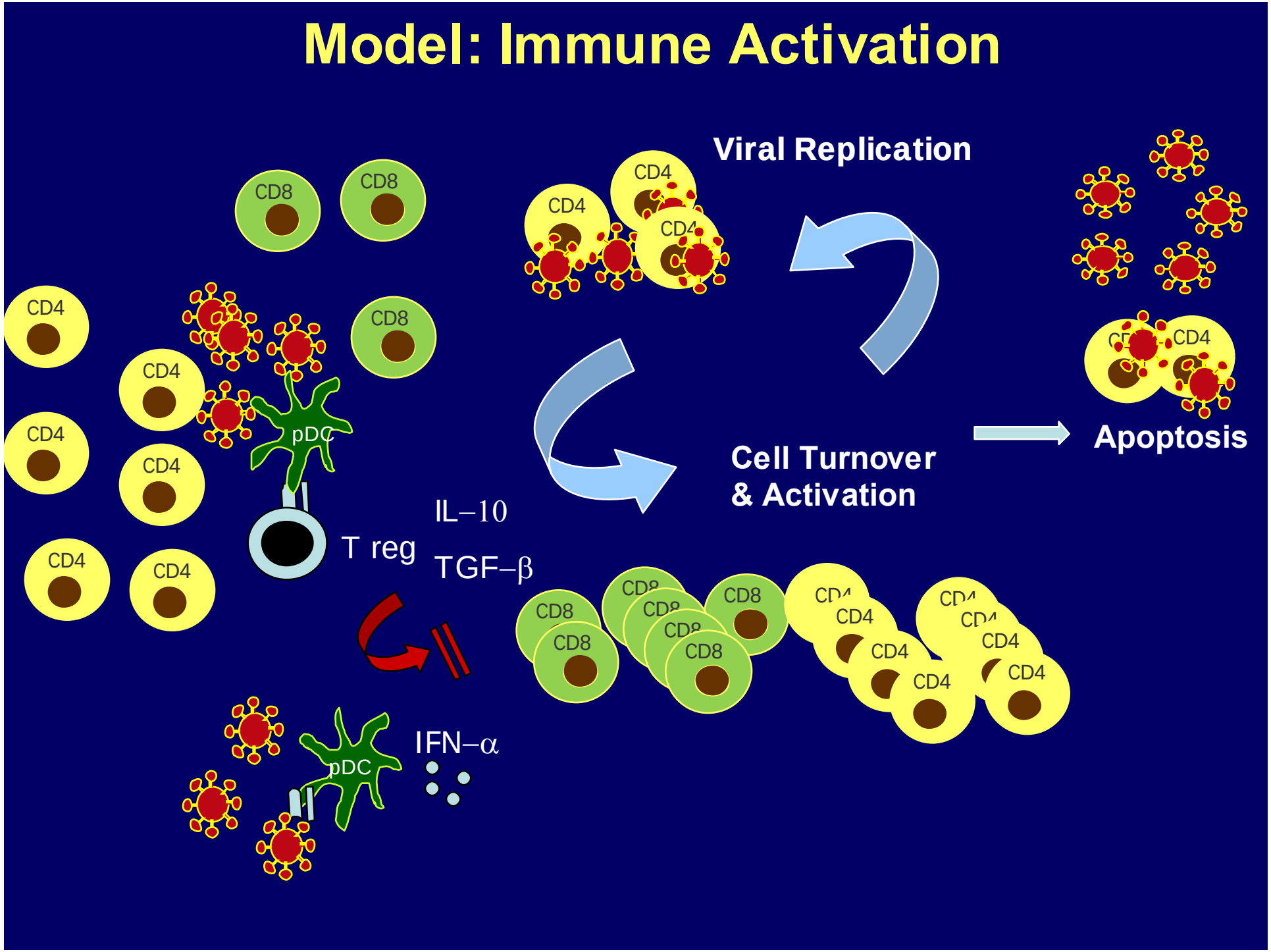
Present VQA Proficiency Programs

- Qualitative HIV-1 Macro/Micrococultures (?)
- Qualitative HIV-1 DNA PCR
- Quantitative HIV-1 RNA
- HIV-1 Genotypic Drug Resistance

Beyond CD4 + Viral Load

- Soluble + Cellular Markers of Immune Activation + Inflammation

Model: Immune Activation



Flow Cytometry Panels

HIV Ag Specific T cells:
CD3, CD4, CD8, IFNγ, IL-2, IL-10, IL-17, CD14, Aqua Live Dead

Cell Turnover/Activation Apoptosis:
CD3, CD4, CD8, KI-67, CD38, HLA DR, Caspase-3, Bcl-2, Aqua Live Dead

T regulatory Cells:
CD3, CD4, CD25, CD45RA, CD127, FoxP3, CTLA-4, CD14, Aqua Live Dead

Soluble Biomarkers

- Samples from early and late time points from subjects with extreme HIV phenotypes (LTNP vs. rapid progressors) will be tested in a pilot project. Factors associated with control will be followed-up in a larger sample.

- Pro-inflammatory- IL-1α, IL-1β, IL-2, IL-6, IL-7, IL-8, IL-12, IL-15, IL-17, IL-23, TNF-α, IFN-γ, GM-CSF, sCD40L

- Anti-inflammatory- IL-1ra, IL-4, IL-5, IL-10, IL-13, G-CSF

- Chemoattractants/receptors-MIP-1α, MIP-1β, RANTES, MCP-1, IP-10

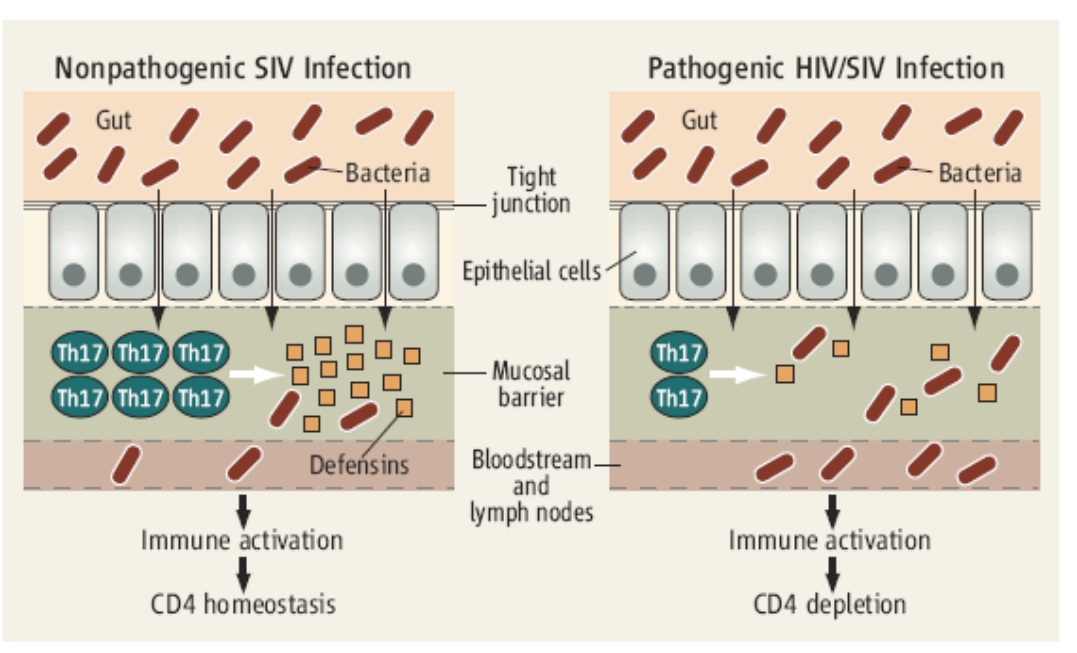
- Growth factors- PDGF

Why Multiplex Assays?

	ELISA	Multiplex
Number of Cytokines	10	10
Number of samples	40	40
# cytokine data	400	400
Number of plates	10	1
Results per plate	40	400
Time required	>40 hr	4-6 hr
Sample volume	2 - 3 ml	0.2 ml
Lower Limit of Detection (LLD)	0.2 – 8 pg/ml (varies by analyte)	0.2 – 4 pg/ml (varies by analyte)

Identifying New Biomarkers in Pathogenesis Studies

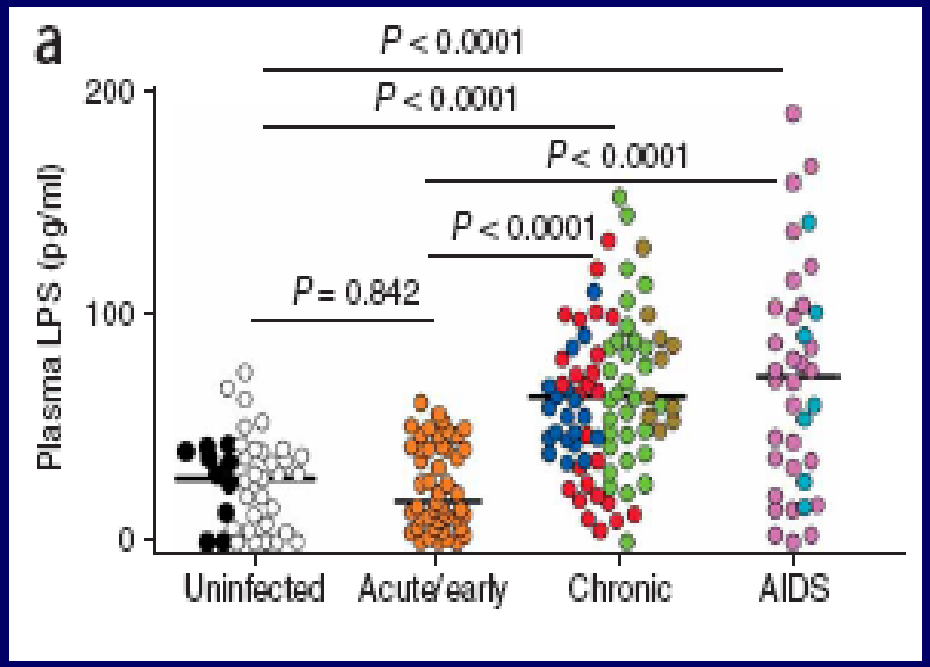
Microbial translocation due to a leaky gut

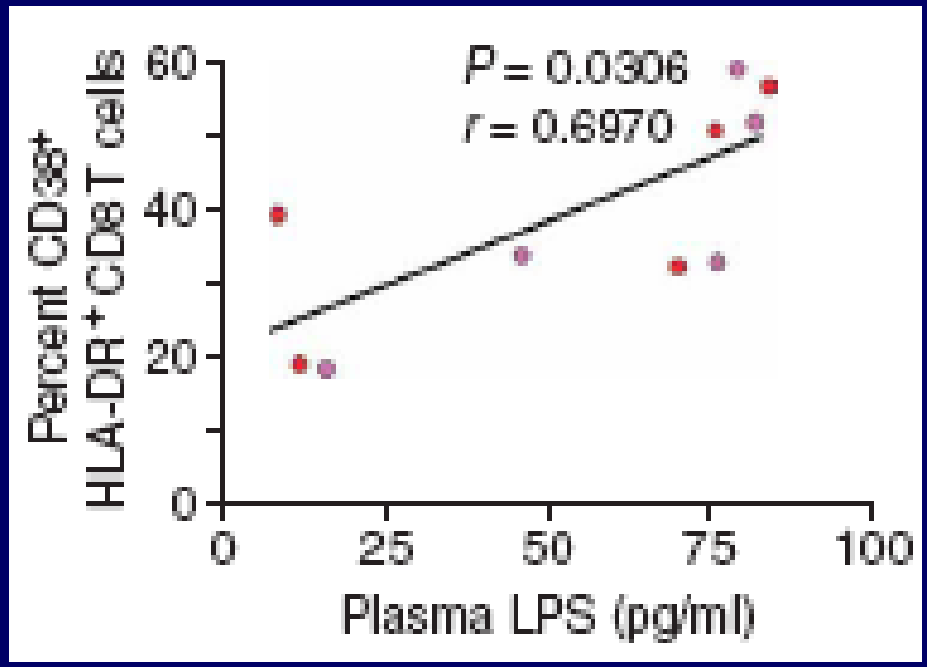


Cohen, science 2008

Mucosal Translocation of Bacterial Products

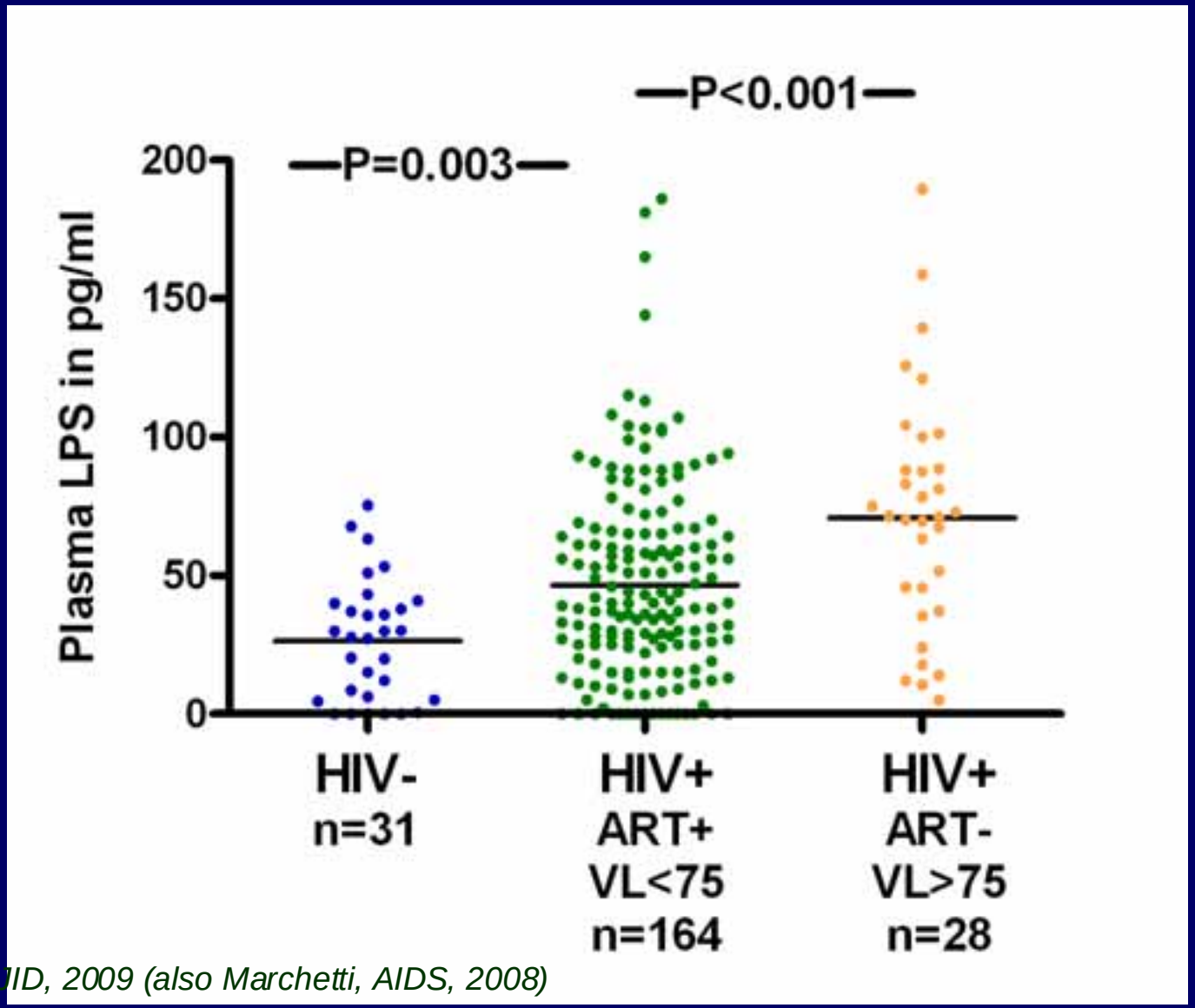
A potential cause of T cell activation in HIV





Brenchley et al, Nat Med, 2006

Microbial Translocation Decreases with HAART but Persists for Years



Jiang et al, JID, 2009 (also Marchetti, AIDS, 2008)

Markers of Microbial Translocation

- LPS – Lipopolysaccharide
- LBP - LPS binding protein
- Soluble CD14
- IFN-α
- 16s RNA

Biomarkers of activation inflammation coagulation microbial translocation

Cellular markers of activation:

↑**Cell Turnover, Activation, Apoptosis**

Panel: Caspase 3, Ki67, CD38, HLADR, CD_4 and CD8

↓ **T Regulatory Cell**

Panel: CD_4, CD25, CD_{127},$FOXP_3$, $CTLA_4$ IL_{10} TGF-β

Soluble markers of Inflammation:

S-VCAM-1, MCP-1(CCL2),hs CRP, VEGF, IL-6, TNF-α, IL1β, IP10

Coagulation markers: 2 D dimer, Prothrombin fragment (F1.2)

Markers of microbial translocation:

LPS , Soluble CD14, 16s DNA, IFN-α

Acknowledgments

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