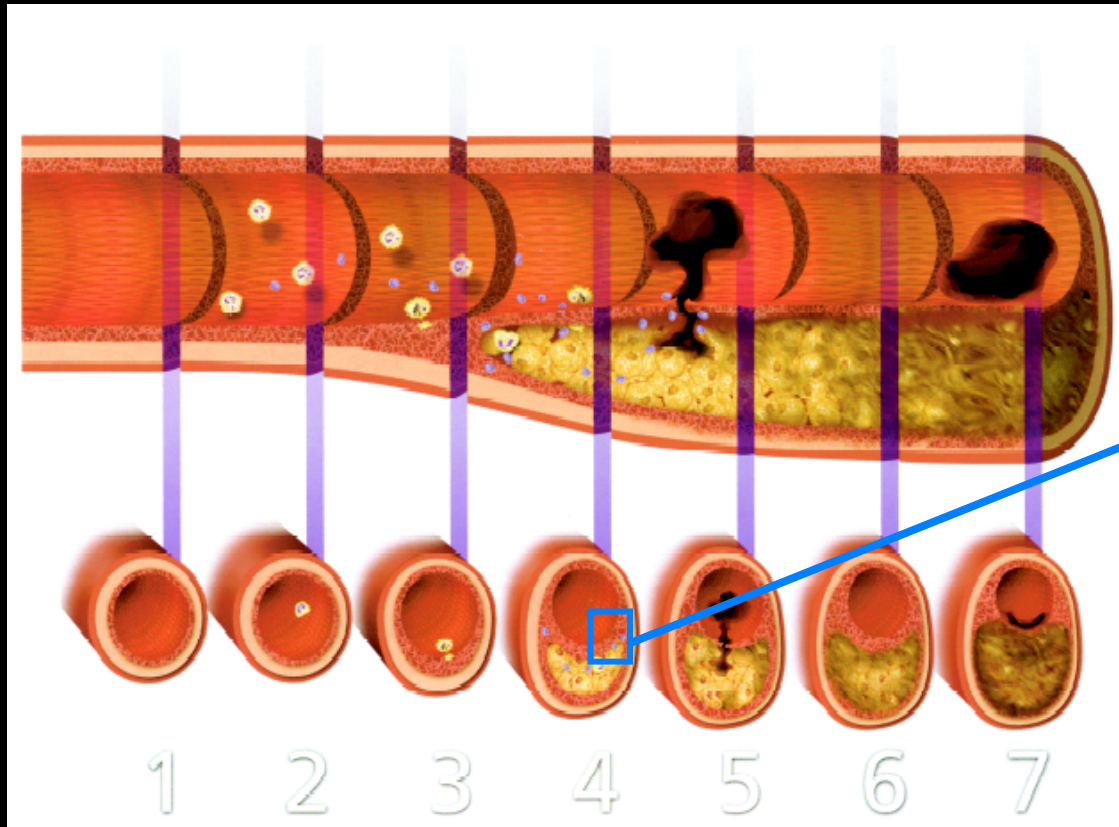
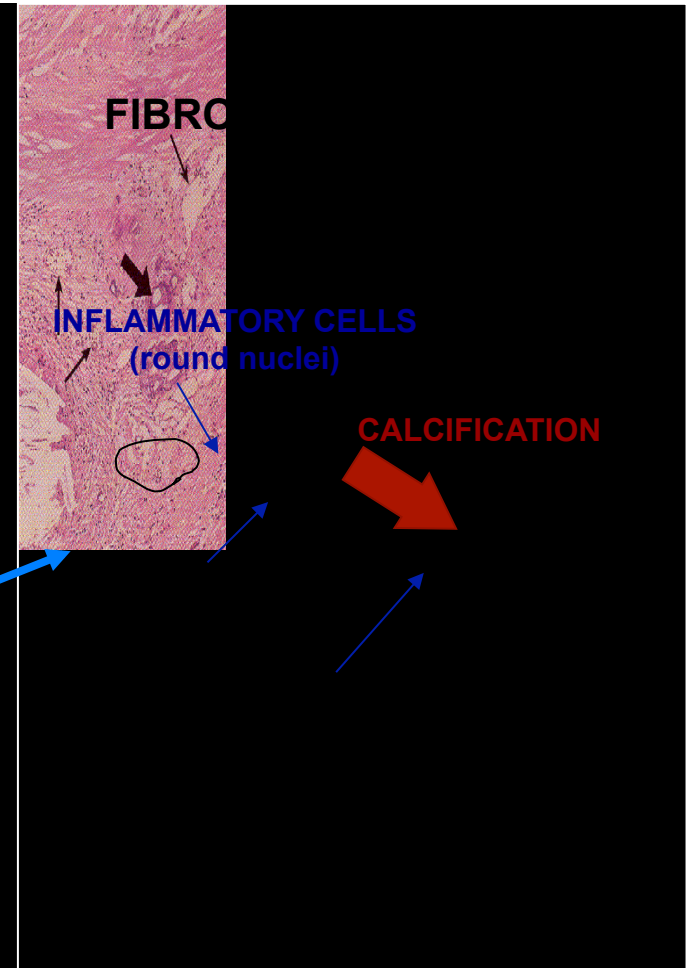


# Atherosclerosis as a Model for Aging:



Libby P. *Circ* 104:365-72, 2001

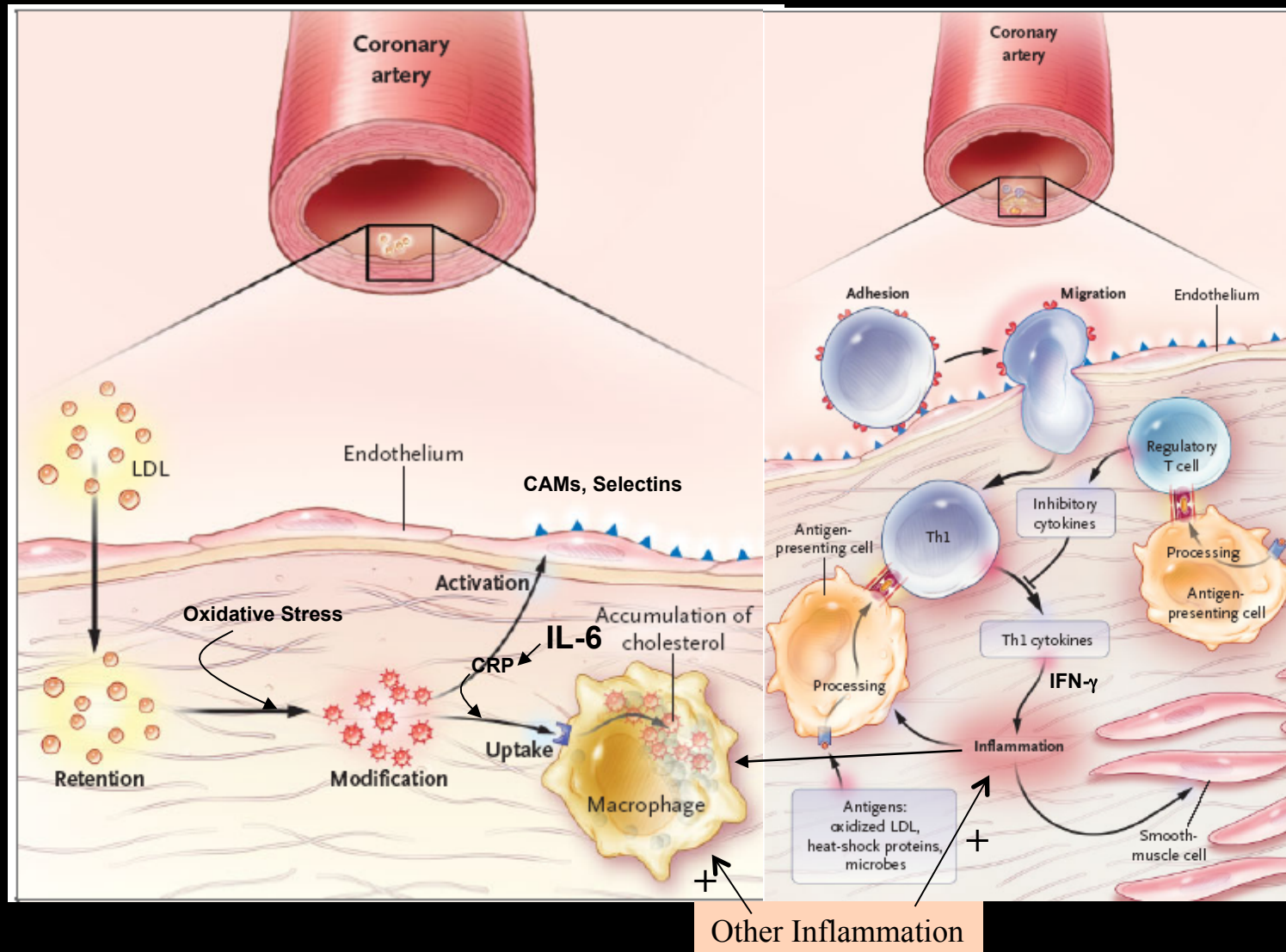


Kotran, Kumar, Collins. *Robbins Pathologic Basis of Disease*. 6th edition. Saunders, 1999

Constant lipid infiltration “mimics” constant tissue damage and cell death → chronic inflammation & fibrosis → loss of arterial function;

Vulnerable plaque: a specialized version mediated by “explosive” atherosclerosis → rupture and thrombosis

# Both Innate and Adaptive Immunity are Involved:

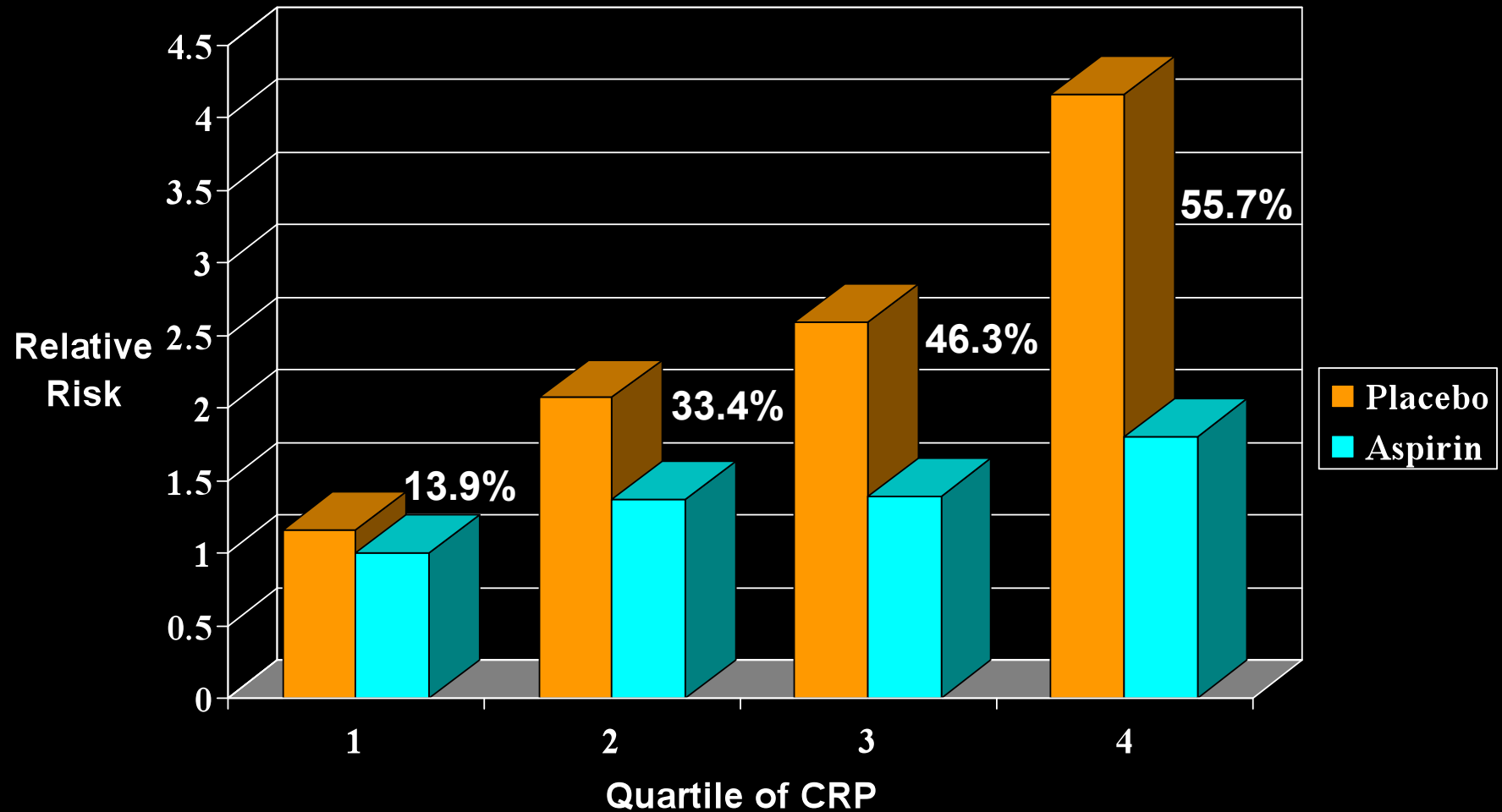


Plus other components of the Innate Immune System such as:

- Complement
- Pentraxins
  - \* CRP
  - \* SAP
  - \* PTX-3
- MØ TF → IIa

# CRP in the Physicians Health Study

People with increased biomarkers of inflammation have increased risk of heart attack



Ridker, Stampfer, Cushman, Tracy, Hennekens.  
N Engl J Med, 336:973-979, 1997

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# Atherosclerosis as a Model for Age-Related Functional Decline: Key aspects

---

Lipid Translocation to media

Driven at least in part by Mass Action;  
mechanism(s) uncertain



Lipid retention

Driven at least in part by GAGs;



Lipid modification

Driven at least in part by oxidative stress;



Activation of innate immunity

Macs, CRP, etc



System in balance?

Yes: no atherosclerosis

No: progression to activation of  
adaptive immunity and  
atherosclerosis

If very rapid: explosive development  
of atheroma → **vulnerable plaque**  
**rupture → clot & MI**

If less rapid: chronic development of  
“sclerosis” → **heart failure**

# T Helper Bias is associated with Coronary Calcification and Carotid IMT

multivariate linear regression

Ln-Agatston Score (LnCAC): %Th1, %Th2, age, gender, ethnicity, blood pressure, diabetes, smoking, lipids.

| <u>LnCAC</u> | <u>Coefficient</u> | <u>p-value</u> |
|--------------|--------------------|----------------|
| %Th1         | 0.030              | <b>0.011</b>   |
| %Th2         | 0.136              | 0.309          |

Common Intima Media Thickening (IMT): %Th1, %Th2, age, gender, ethnicity, blood pressure, diabetes, smoking, lipids

| <u>IMT</u> | <u>Coefficient</u> | <u>p-value</u> |
|------------|--------------------|----------------|
| %Th1       | 0.002              | <b>0.015</b>   |
| %Th2       | -0.025             | <b>0.002</b>   |

# Preliminary Data from the Multi-Ethnic Study of Atherosclerosis

## Correlation of %Th1 cells with serologies:

| Serology   | R        |
|------------|----------|
| HSp60      | -0.014   |
| CMV        | 0.360**  |
| c. pneum.  | 0.000    |
| Hep A      | -0.148** |
| h. pylorii | 0.086*   |
| HSV1       | 0.080    |

Possible aging-related effects of CMV:

- Taking over too much “immune landscape”;
- Driving immunosenescence;
- Driving a Th1 bias;

# HIV & Atherosclerosis

- WHAT DO WE KNOW ABOUT  
ATHEROSCLEROSIS IN THE CONTEXT  
OF HIV?

# There appears to be more of it:

## Progression of Atherosclerosis as Assessed by Carotid Intima-Media Thickness in Patients With HIV Infection

Priscilla Y. Hsue, MD; Joan C. Lo, MD; Arlana Franklin, RDMS; Ann F. Bolger, MD;  
Jeffrey N. Martin, MD; Steven G. Deeks, MD; David D. Waters, MD

**Conclusions**—Carotid IMT is higher in HIV patients than in age-matched control subjects and progresses much more rapidly than previously reported rates in non-HIV cohorts. In HIV patients, carotid IMT is associated with classic coronary risk factors and with nadir CD4 count  $\leq 200$ , suggesting that immunodeficiency and traditional coronary risk factors may contribute to atherosclerosis. (*Circulation*. 2004;109:1603-1608.)

# This is true for other (all?) conditions associated with increased inflammation

# HIV athero is associated with CMC co-infection

## Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses

Priscilla Y. Hsue<sup>a</sup>, Peter W. Hunt<sup>b</sup>, Elizabeth Sinclair<sup>c</sup>, Barry Bredt<sup>c</sup>,  
Arlana Franklin<sup>a</sup>, Maudi Killian<sup>c</sup>, Rebecca Hoh<sup>b</sup>, Jeffrey N. Martin<sup>b,d</sup>,  
Joseph M. McCune<sup>c</sup>, David D. Waters<sup>a</sup> and Steven G. Deeks<sup>b</sup>

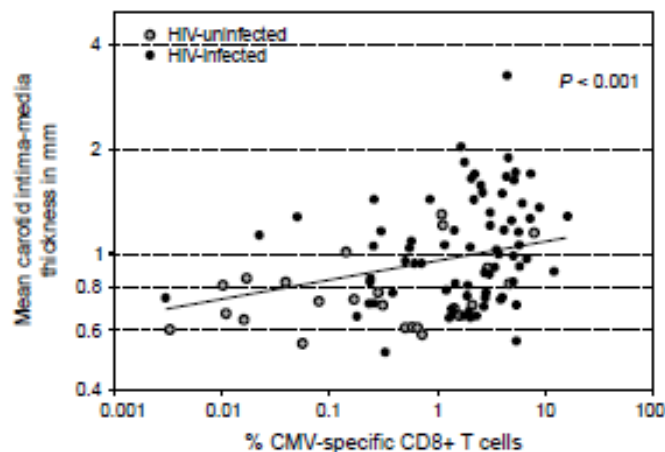


**Fig. 2. Carotid IMT and CMV-specific T-cell responses in HIV-infected subjects.** Carotid IMT is plotted against the percentage of CMV-specific CD8 T cells for HIV-infected subjects (black circles) and uninfected controls (gray circles). The black line represents the linear prediction from an unadjusted linear regression model. Among all participants, for every 10-fold increase in the percentage of CMV-specific T cells, there was a 14% increase in carotid IMT ( $P < 0.001$ ).

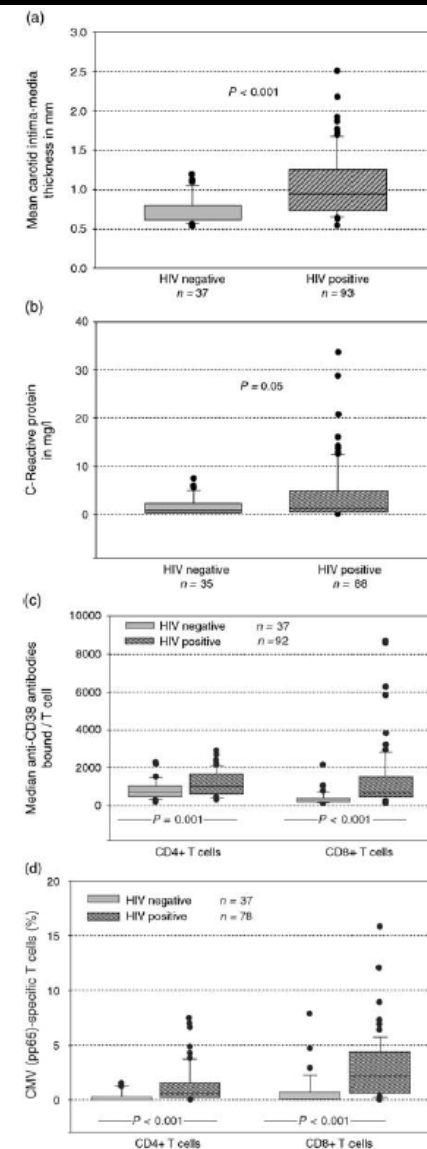
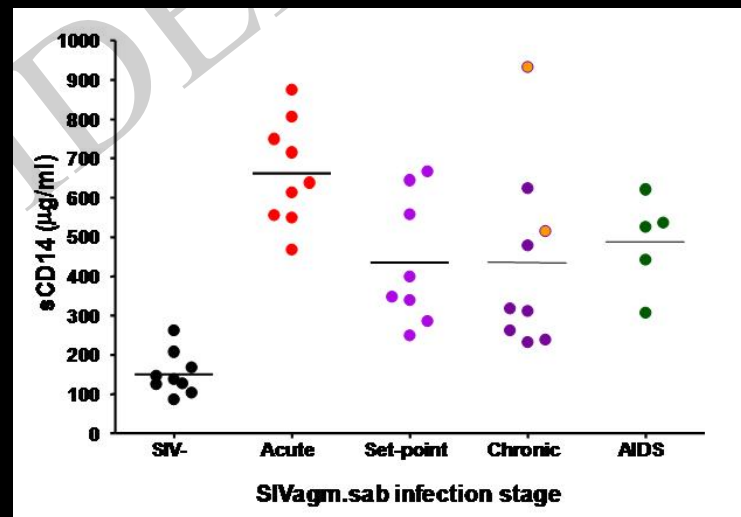
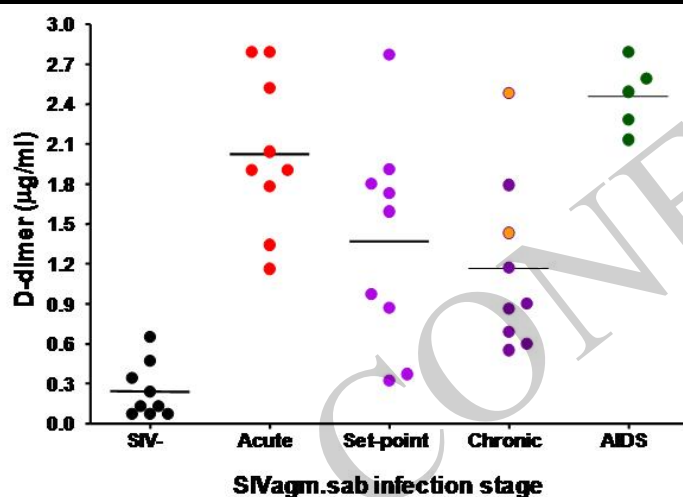
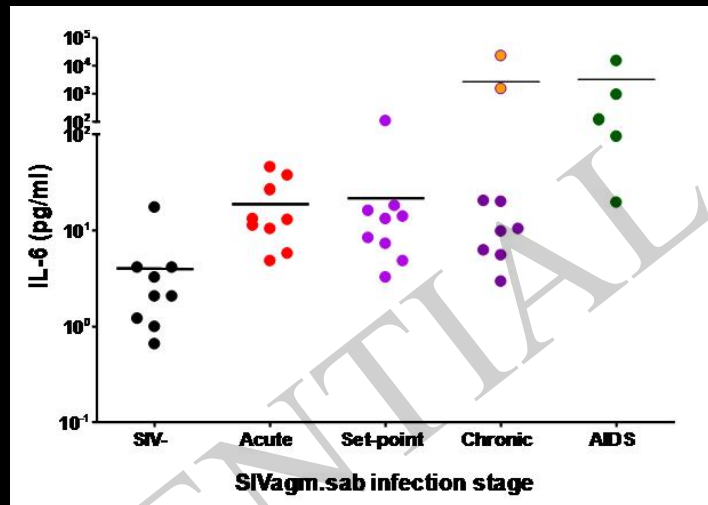
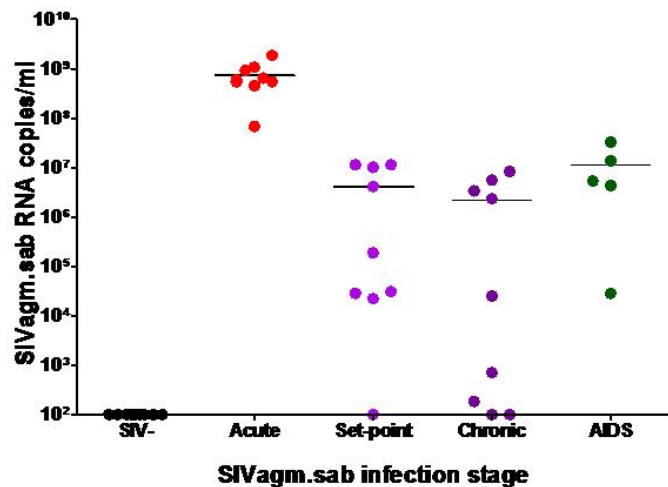


**Fig. 1.** (a) Carotid IMT in HIV-infected and -uninfected subjects. Carotid IMT was measured in 12 predefined segments by a single vascular technician blinded to the subject's clinical features, including HIV status. A total of 93 HIV-infected subjects and 37 HIV-uninfected controls were

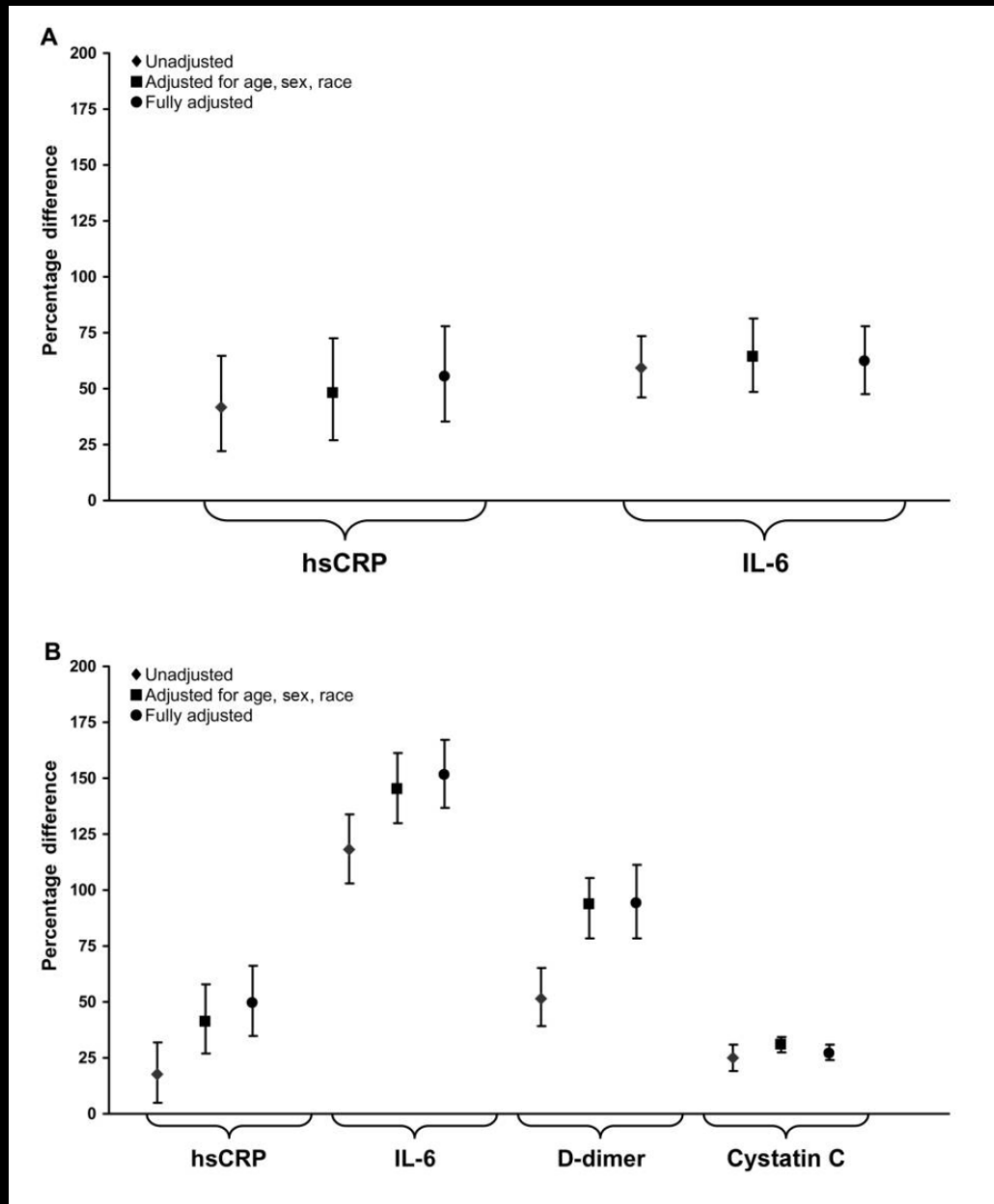
## Inflammation & HIV/AIDS – four points:

- Despite being an “immunodeficiency” disease HIV like most viral infections is inflammatory;
- Inflammation is associated with risk of death from all causes not just AIDS-related;
- Inflammation is associated with decreased lymphoid organ function (chronic low-level “wound repair”);
- Co-morbidities are critical to understanding biomarkers and risk factors in HIV/AIDS

# HIV & Aging: lessons from Pig-Tailed Macaques



# HIV & Aging: Biomarker data from SMART & MESA



- A) Percentage difference in the levels of hsCRP and IL-6 in HIV-infected study participants **33–44 years** of age vs. the general population.
- B) Percentage difference in the levels of hsCRP, IL-6, D-dimer, and cystatin C in HIV-infected study participants **45–76 years** of age vs. the general population

Neuhaus J et al., Markers of Inflammation, Coagulation, and Renal Function Are Elevated in Adults with HIV Infection. *Journal of Infectious Diseases* 2010; 201(12):1788–1795

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## Inflammation & HIV/AIDS – four points:

- Despite being an “immunodeficiency” disease HIV/AIDS is an inflammatory disorder;
- Inflammation in HIV is associated with risk of death from all causes not just AIDS-related;
- Inflammation is associated with decreased lymphoid organ function (chronic low-level “wound repair”);
- Co-morbidities are critical to understanding biomarkers and risk factors in HIV/AIDS

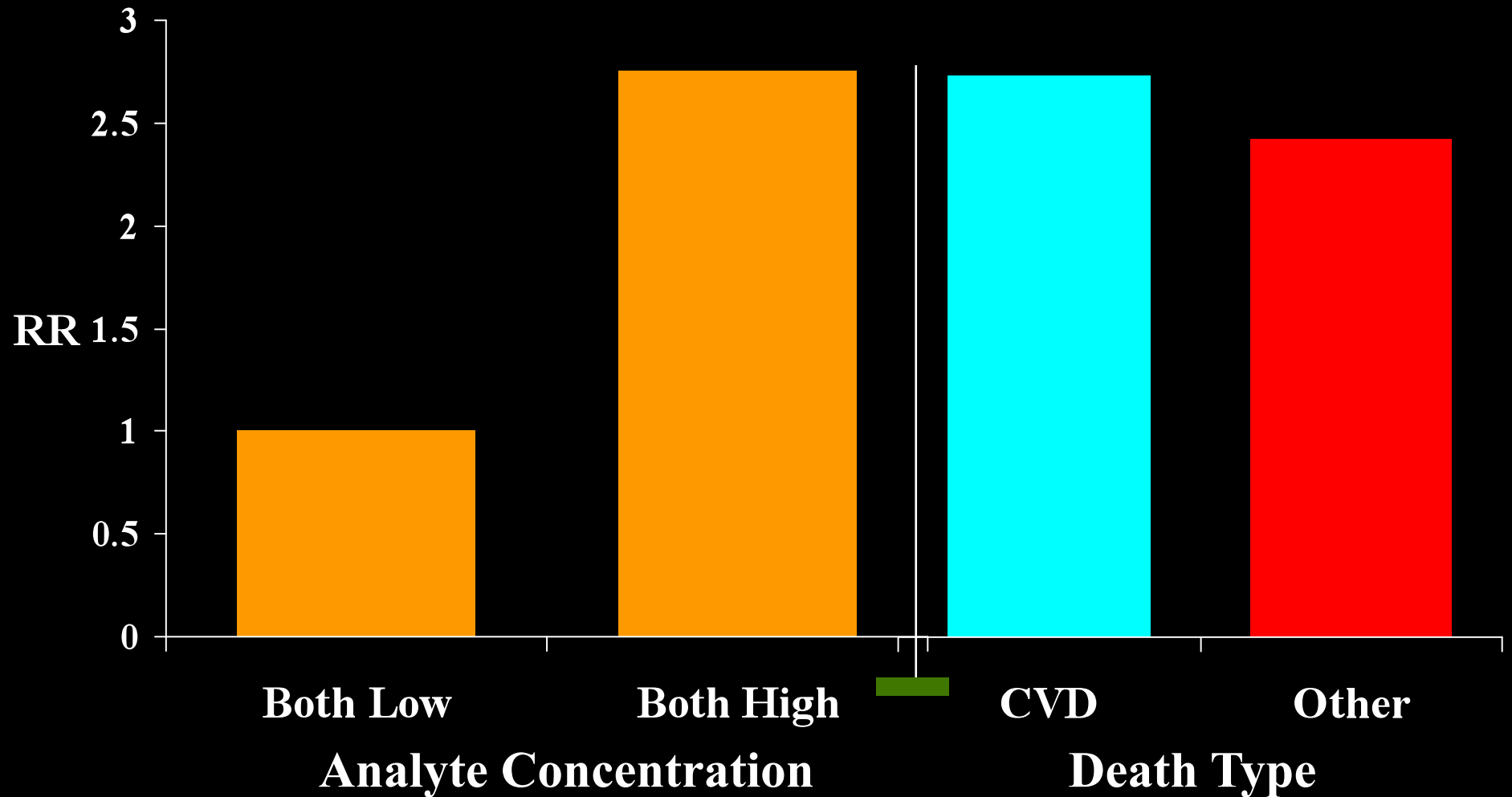
# SMART: Risk of death associated with biomarker at study entrance

| Biomarker         | Type of Analysis | <25 <sup>th</sup> Percentile (Reference) | 25th–49th Percentile |         | 50th–74th Percentile |         | ≥75 <sup>th</sup> Percentile |         | OR associated with One IQR Higher Biomarker Level after Log <sub>10</sub> Transformation |         |
|-------------------|------------------|------------------------------------------|----------------------|---------|----------------------|---------|------------------------------|---------|------------------------------------------------------------------------------------------|---------|
|                   |                  |                                          | OR (95% CI)          | p-Value | OR (95% CI)          | p-Value | OR (95% CI)                  | p-Value | OR (95% CI)                                                                              | p-Value |
| hsCRP (µg/ml)     | No. <sup>a</sup> | 16/45                                    | 9/42                 |         | 20/28                |         | 40/55                        |         |                                                                                          |         |
|                   | Univariate       | 1.0 (ref.)                               | 0.6 (0.2–1.5)        | 0.29    | 2.0 (0.9–4.6)        | 0.09    | 2.0 (1.0–4.1)                | 0.05    | 1.7 (1.2–2.4)                                                                            | 0.005   |
|                   | Adjusted         | 1.0 (ref.)                               | 0.7 (0.2–2.1)        | 0.50    | 2.5 (0.9–7.2)        | 0.08    | 3.1 (1.2–8.0)                | 0.02    | 2.3 (1.4–3.7)                                                                            | 0.001   |
| Amyloid A (mg/l)  | No.              | 11/46                                    | 17/35                |         | 28/33                |         | 29/56                        |         |                                                                                          |         |
|                   | Univariate       | 1.0 (ref.)                               | 2.0 (0.8–5.2)        | 0.13    | 3.4 (1.5–7.7)        | 0.005   | 2.2 (0.9–5.2)                | 0.07    | 1.3 (0.9–1.7)                                                                            | 0.11    |
|                   | Adjusted         | 1.0 (ref.)                               | 3.4 (1.0–11.1)       | 0.04    | 3.5 (1.2–10.2)       | 0.02    | 3.1 (1.0–9.5)                | 0.05    | 1.3 (0.9–1.9)                                                                            | 0.12    |
| Amyloid P (µg/ml) | No.              | 25/44                                    | 20/25                |         | 16/43                |         | 24/56                        |         |                                                                                          |         |
|                   | Univariate       | 1.0 (ref.)                               | 1.5 (0.7–3.1)        | 0.32    | 0.7 (0.3–1.4)        | 0.32    | 0.7 (0.4–1.5)                | 0.39    | 0.7 (0.6–0.9)                                                                            | 0.009   |
|                   | Adjusted         | 1.0 (ref.)                               | 1.5 (0.6–4.0)        | 0.42    | 0.8 (0.3–2.0)        | 0.65    | 1.1 (0.4–3.0)                | 0.78    | 0.7 (0.5–1.0)                                                                            | 0.06    |
| IL-6 (pg/ml)      | No.              | 8/48                                     | 10/41                |         | 26/48                |         | 40/29                        |         |                                                                                          |         |
|                   | Univariate       | 1.0 (ref.)                               | 1.3 (0.5–3.6)        | 0.62    | 3.2 (1.3–7.9)        | 0.01    | 8.3 (3.3–20.8)               | <0.0001 | 3.4 (2.2–5.4)                                                                            | <0.0001 |
|                   | Adjusted         | 1.0 (ref.)                               | 1.0 (0.3–3.6)        | 0.98    | 4.5 (1.4–14.2)       | 0.01    | 12.4 (3.6–42.0)              | <0.0001 | 4.1 (2.3–7.3)                                                                            | <0.0001 |
| D-dimer (µg/ml)   | No.              | 8/51                                     | 22/54                |         | 18/40                |         | 37/25                        |         |                                                                                          |         |
|                   | Univariate       | 1.0 (ref.)                               | 3.2 (1.1–9.0)        | 0.03    | 4.0 (1.3–12.3)       | 0.02    | 12.4 (4.2–37.0)              | <0.0001 | 3.9 (2.3–6.6)                                                                            | <0.0001 |
|                   | Adjusted         | 1.0 (ref.)                               | 8.3 (1.9–36.8)       | 0.005   | 12.6 (2.4–65.1)      | 0.003   | 41.2 (7.5–225.6)             | <0.0001 | 5.3 (2.6–10.9)                                                                           | <0.0001 |
| F1.2 (pmol/l)     | No.              | 17/29                                    | 21/43                |         | 15/43                |         | 31/53                        |         |                                                                                          |         |
|                   | Univariate       | 1.0 (ref.)                               | 0.8 (0.4–1.9)        | 0.64    | 0.6 (0.3–1.5)        | 0.28    | 1.0 (0.5–2.1)                | 0.92    | 1.0 (0.8–1.4)                                                                            | 0.81    |
|                   | Adjusted         | 1.0 (ref.)                               | 1.0 (0.4–2.9)        | 0.94    | 0.9 (0.3–2.6)        | 0.82    | 1.3 (0.5–3.4)                | 0.64    | 1.1 (0.7–1.6)                                                                            | 0.71    |

Adj: age, race, use of ART and HIV-RNA level, CD4+cell count, smoking status, BMI, prior CVD, diabetes, use of BP medication, use of lipid-lowering medication, total/HDL cholesterol, co-infection with hepatitis B or C, and treatment group.

No significant interactions based on treatment

# CRP, IL-6 and Mortality: Iowa 65+ Rural Health Study



Harris, Tracy et al Am J Med 1999;106:506

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# Inflammation, Atherosclerosis, and Aging

**Supplementary Table 1: Underlying Cause of Death for Participants in SMART**

| Cause of Death                          | Treatment Group |       |         |             |
|-----------------------------------------|-----------------|-------|---------|-------------|
|                                         | DC              | VS    | Total   |             |
|                                         | No.             | No.   | No.     | Percent     |
| AIDS                                    | 4               | 3     | 7       | 8.2         |
| Cancer, excluding AIDS-defining cancers | 11              | 5     | 16      | 18.8 ✓      |
| Cardiovascular disease                  | 7               | 4     | 11      | 12.9 ✓      |
| Infection                               | 3               | 1     | 4       | 4.7         |
| Hepatic disease                         | 1               | 2     | 3       | 3.5         |
| Renal disease                           | 3               | 0     | 3       | 3.5         |
| Chronic obstructive lung disease        | 1               | 1     | 2       | 2.3         |
| Hematological disease                   | 2               | 0     | 2       | 2.3         |
| Digestive system disease                | 1               | 1     | 2       | 2.3         |
| CNS disease                             | 1               | 0     | 1       | 1.2         |
| Respiratory disease                     | 0               | 1     | 1       | 1.2         |
| Substance abuse                         | 3               | 5     | 8       | 9.4         |
| Accident/violent/suicide                | 3               | 4     | 7       | 8.2         |
| Cause unknown (unwitnessed)             | 15 (8)          | 3 (2) | 18 (10) | 21.2 (11.7) |
| Total                                   | 55              | 30    | 85      | 100.0       |

\* Deaths occurring through January 11, 2006

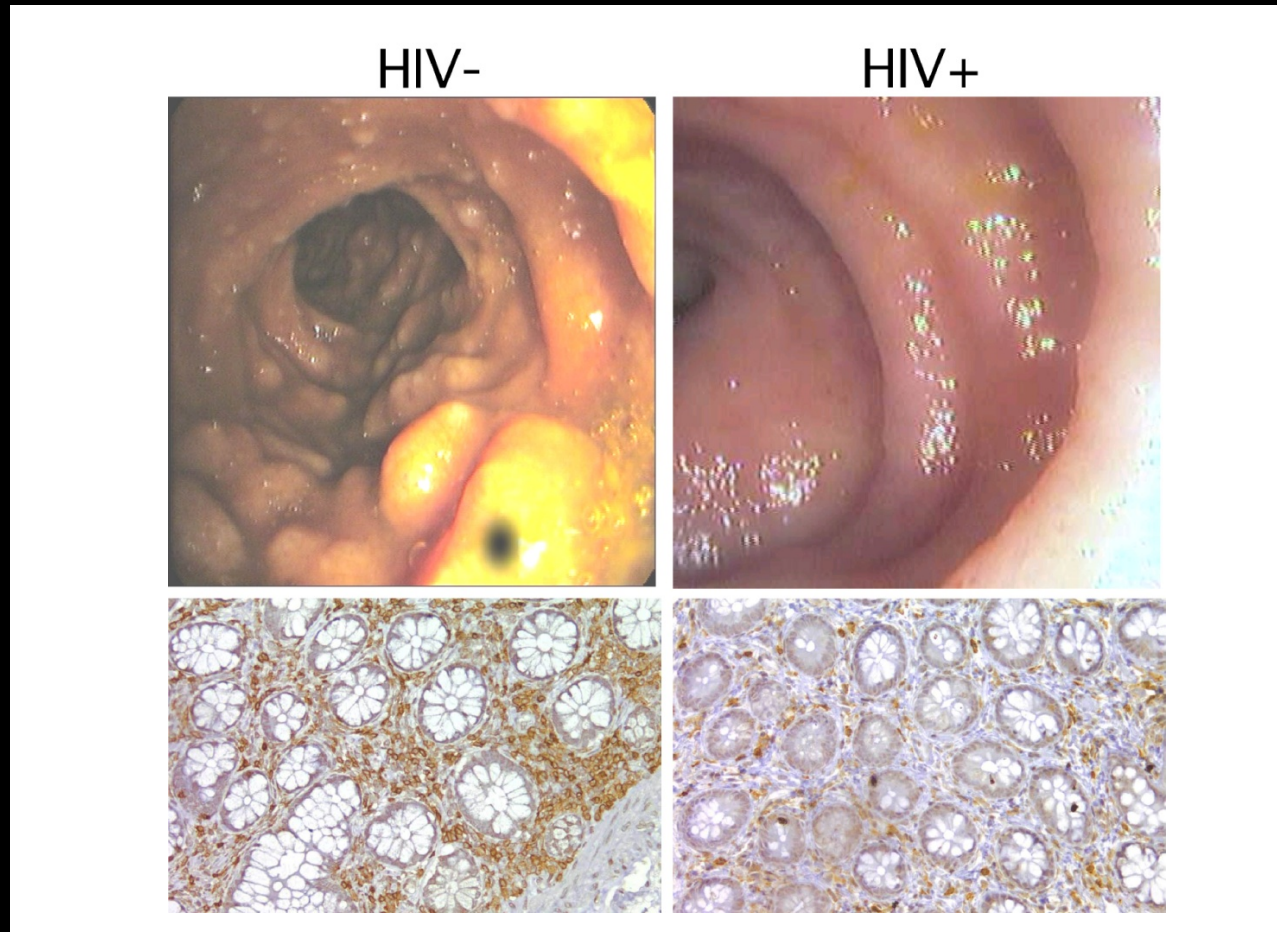
DC = Drug Conservation

VS = Viral Suppression

## Inflammation & HIV/AIDS – four points:

- Despite being an “immunodeficiency” disease HIV/AIDS is an inflammatory disorder;
- Inflammation is associated with risk of death from all causes not just AIDS-related;
- Inflammation is associated with decreased lymphoid organ function (chronic low-level “wound repair”);
- Co-morbidities are critical to understanding biomarkers and risk factors in HIV/AIDS

# Absence of Peyer's Patches in HIV Infection

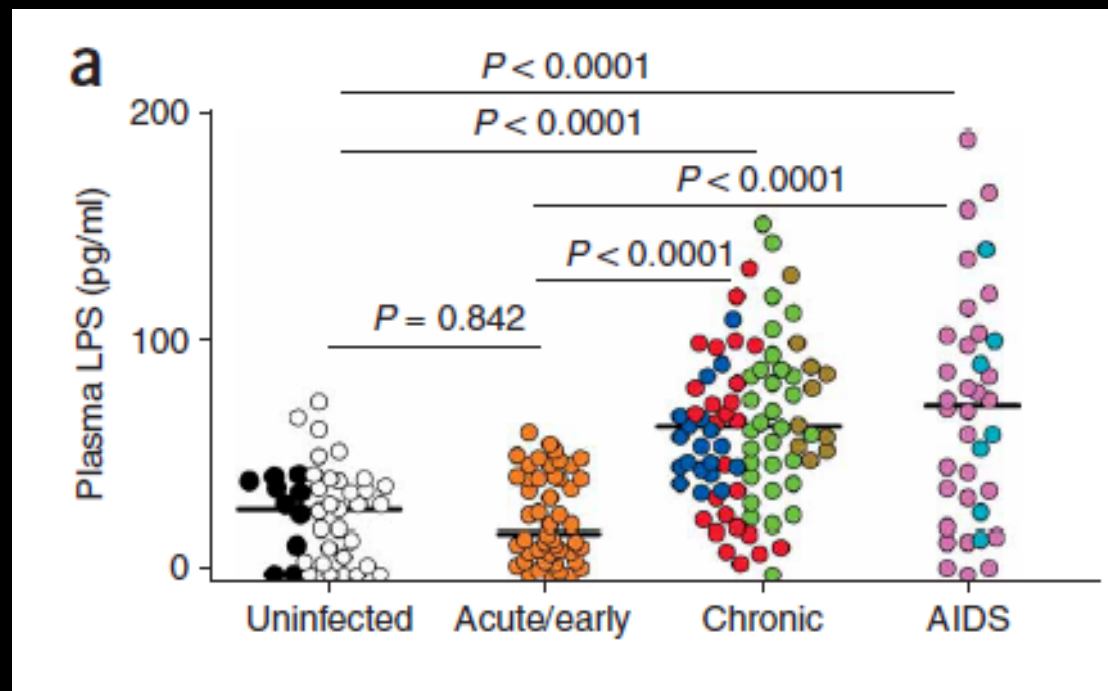


slide courtesy of Timothy Schacker, UM

# Inflammation, Atherosclerosis, and Aging

## Microbial translocation is a cause of systemic immune activation in chronic HIV infection

Jason M Brenchley<sup>1</sup>, David A Price<sup>1</sup>, Timothy W Schacker<sup>2</sup>, Tedi E Asher<sup>1</sup>, Guido Silvestri<sup>3</sup>, Srinivas Rao<sup>4</sup>, Zachary Kazzaz<sup>1</sup>, Ethan Bornstein<sup>1</sup>, Olivier Lambotte<sup>5</sup>, Daniel Altmann<sup>6</sup>, Bruce R Blazar<sup>7</sup>, Benigno Rodriguez<sup>8</sup>, Leila Teixeira-Johnson<sup>8</sup>, Alan Landay<sup>9</sup>, Jeffrey N Martin<sup>10</sup>, Frederick M Hecht<sup>10</sup>, Louis J Picker<sup>11</sup>, Michael M Lederman<sup>8</sup>, Steven G Deeks<sup>10</sup> & Daniel C Douek<sup>1</sup>



## Inflammation & HIV/AIDS – four points:

- Despite being an “immunodeficiency” disease HIV/AIDS is an inflammatory disorder;
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- Inflammation is associated with decreased lymphoid organ function (chronic low-level “wound repair”);
- Co-morbidities are critical to understanding biomarkers and risk factors in HIV/AIDS

## FRAM: CRP is high with HIV infection, but normal/low with HIV/HCV co-infection

### Association of HIV Infection and HIV/HCV Coinfection With C-Reactive Protein Levels

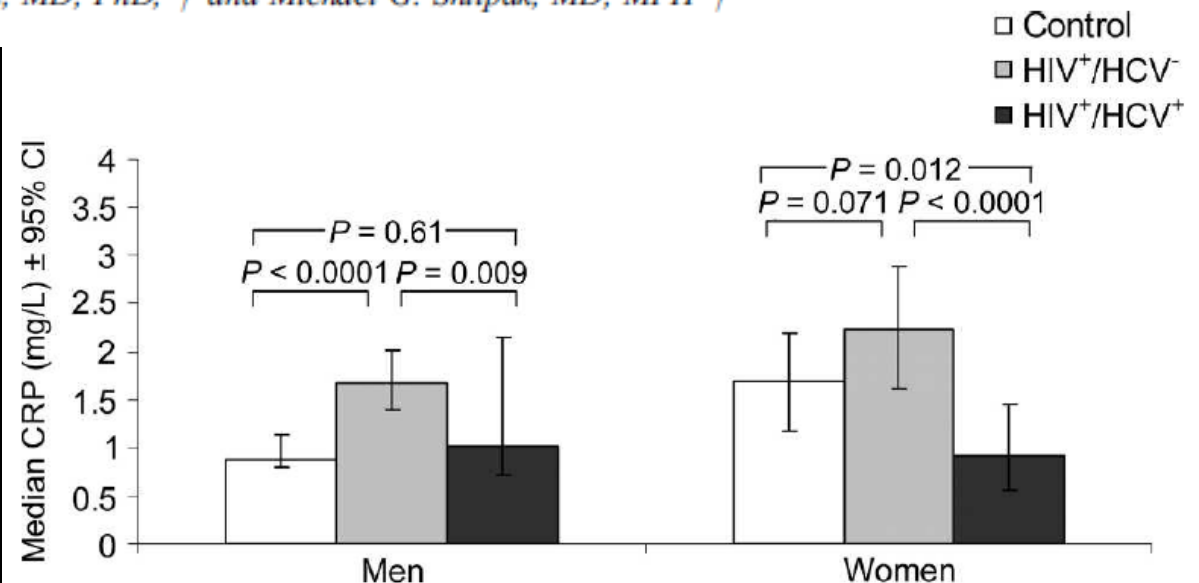
#### *The Fat Redistribution and Metabolic Change in HIV Infection (FRAM) Study*

Jason S. Reingold, MD,\*† Christine Wanke, MD,‡ Donald P. Kotler, MD,§ Cora E. Lewis, MD, MPH,||  
Russell Tracy, PhD,¶ Steven Heymsfield, MD,# Phyllis C. Tien, MD,\*† Peter Bacchetti, PhD,\*  
Rebecca Scherzer, PhD,† Carl Grunfeld, MD, PhD,\*† and Michael G. Shlipak, MD, MPH\*†

The health of the liver may be critical to our understanding of a liver-mediated biomarker such as CRP

A “return to health” process might simultaneously:

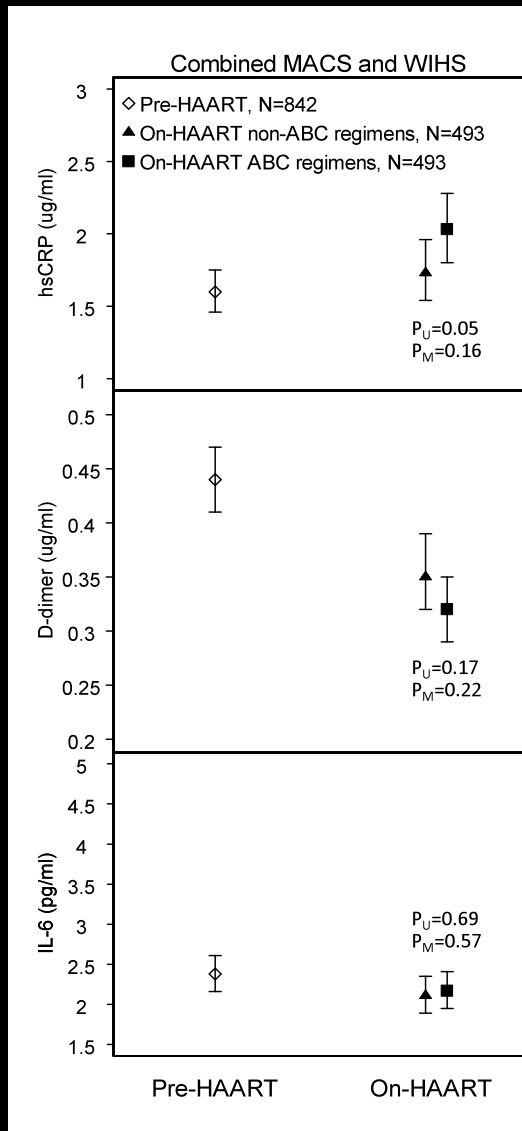
- Lower inflammation and thereby **lower** the biomarker;
- But also return the liver to health and **raise** the production of the biomarker



Participants were age restricted and those with recent opportunistic infections were excluded

FIGURE 1. Median CRP levels by gender and HIV/HCV status.

# Inflammation, Atherosclerosis, and Aging



## Inflammatory Biomarkers among Abacavir and non-Abacavir Recipients in the Women's Interagency HIV Study (WIHS) and the Multicenter AIDS Cohort Study (MACS)

Frank J. Palella Jr MD, Stephen J. Gange PhD, Lorie Benning MS, Lisa Jacobson ScD, MS, Robert C. Kaplan PhD, Alan L. Landay PhD, Russell P. Tracy MD, Richard Elion MD

Northwestern University Feinberg School of Medicine, Chicago, IL; Johns Hopkins University, Baltimore, MD; Albert Einstein College of Medicine, New York, NY; Rush University Medical Center, Chicago, IL; University of Vermont, Burlington, VT; George Washington University, Washington, DC.

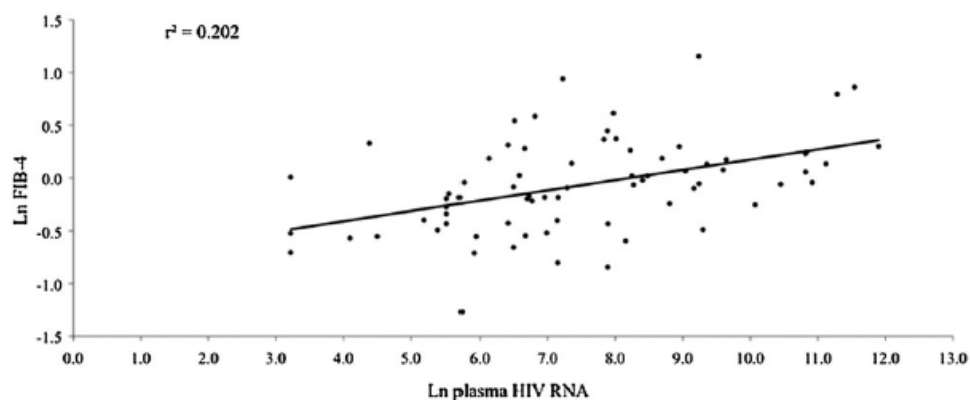
### CRP results: “Return to Health”?

# Liver effect may not require co-infection

HIV/AIDS MAJOR ARTICLE

## HIV Mono-infection Is Associated With FIB-4 – A Noninvasive Index of Liver Fibrosis – in Women

Jason T. Blackard,<sup>1</sup> Jeffrey A. Welge,<sup>2,3</sup> Lynn E. Taylor,<sup>4</sup> Kenneth H. Mayer,<sup>4</sup> Robert S. Klein,<sup>5</sup> David D. Celentano,<sup>6</sup> Denise J. Jamieson,<sup>7</sup> Lytt Gardner,<sup>8</sup> and Kenneth E. Sherman<sup>1</sup>



## Human Immunodeficiency Virus–Induced Apoptosis of Human Hepatocytes via CXCR4

Stacey R. Vlahakis,<sup>1</sup> Angelina Villasis-Keever,<sup>2</sup> Timothy S. Gomez,<sup>2</sup> Gary D. Bren,<sup>2</sup> and Carlos V. Paya<sup>1,2,3</sup>

<sup>1</sup>Division of Infectious Diseases, <sup>2</sup>Department of Immunology, and <sup>3</sup>Division of Experimental Pathology, Mayo Clinic, Rochester, Minnesota

Blackard et al., CID 2011:52  
Vlahakis et al., JID 2003:188

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# Summary: Biomarker Effects of HIV and relation to “aging”

## Increases

- Triglyceride
  - Factor VIIc\*
  - vWF\*
  - D-dimer\*
  - IL-6\*
  - Cystatin C\*
- 

– Not as high as they should be due to liver effects:

- Fibrinogen\*
- CRP\*

## Reduces

- LDLc\*/\*\*
- Factor VIIc\*/\*\*
- HDL\*
- Antithrombin
- Protein S
- Protein C

\* = what we see with aging and frailty

\*\* = Increases with ART;  
a) Return to Health  
b) direct drug effect?

Red = “bad”

Green = “good”

Clearly inflammatory & tipped towards clotting by:

- ?? Factor imbalance??
- ?? External force (e.g., lymphoid fibrosis → endotoxemia → TF expression) ??

# Brief literature review: HIV & Coagulation

- Abdollahi A, et al. 2011 Jan;93(1):53-8. **Factor VIII concentration is greater** in female than male patients with HIV infection.
  - PTT, plasma fibrinogen, antithrombin, protein C and protein S levels were significantly lower, and plasma factor VIII levels were significantly higher in HIV patients vs Controls
- Jong E, ten Cate H, et al. Thromb Haemost. 2010 Dec;104(6):1228-34. The effect of initiating combined antiretroviral therapy on endothelial cell activation and coagulation markers in South African HIV-infected individuals.
  - cART-naïve, HIV+ vs Controls: **elevated von Willebrand factor , D-dimer, activated protein C sensitivity ratio;**
  - cART-naïve, HIV+ vs Controls: decreased total and free protein S, protein C levels;
  - Post-cART, all biomarkers, except APCsr, improved although not to normal levels
- Jong E, et al. AIDS Res Ther. 2010 Apr 16;7:9. Markers of inflammation and coagulation indicate a **prothrombotic state in HIV-infected patients** with long-term use of antiretroviral therapy with or without abacavir.
  - Vs Controls, elevated vWF and F1+2 levels observed in 23% and 37%; increased APCsr was found in 79% ; lower protein C levels in 40%;
- Pontrelli G, et al. AIDS. 2010 May 15;24(8):1145-51. HIV is associated with **thrombophilia and high D-dimer in children and adolescents**.
  - Vs. Controls, protein S and protein C deficiency in 51 and 8%;
  - High viral load vs. low viral load: reduction of protein S, protein C and antithrombin activities, and an increase of D-dimer levels;

# Cytomegalovirus-Specific T Cells Persist at Very High Levels during Long-Term Antiretroviral Treatment of HIV Disease

David M. Naeger<sup>1</sup>, Jeffrey N. Martin<sup>1,2</sup>, Elizabeth Sinclair<sup>1</sup>, Peter W. Hunt<sup>1</sup>, David R. Bangsberg<sup>3</sup>, Frederick Hecht<sup>1</sup>, Priscilla Hsue<sup>1</sup>, Joseph M. McCune<sup>1</sup>, Steven G. Deeks<sup>1\*</sup>

- The CD8<sup>+</sup> T cell response to just two CMV proteins (pp65 and IE) was approximately 6% during long-term therapy, which was over twice that seen in HIV-seronegative persons.
- CMV-specific CD4<sup>+</sup> T cell responses followed the same trends, but the magnitude of the effect was smaller.
- **Conclusions/Significance:** Long-term successfully treated HIV infected patients have remarkably high levels of CMV-specific effector cells. These levels are similar to that observed in the elderly, but occur at much younger ages.

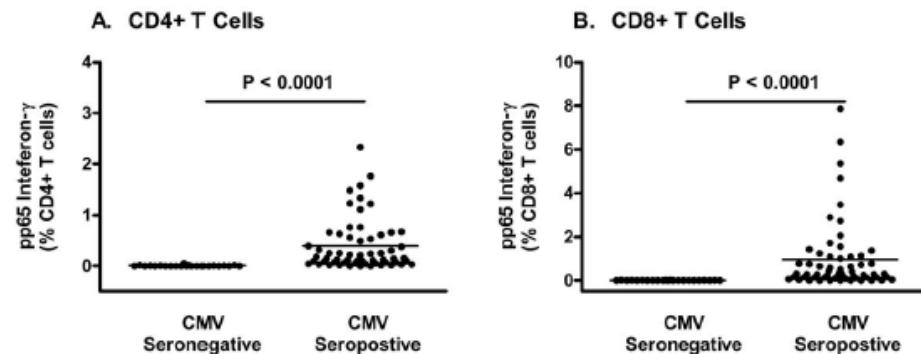


Figure 1. The distribution of percent of CMV-specific T cells in CMV-seronegative and CMV-seropositive, HIV-uninfected subjects. doi:10.1371/journal.pone.0008886.g001

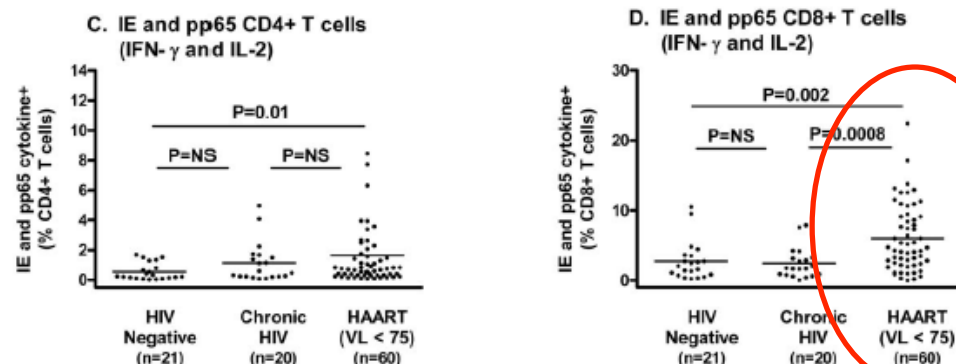


Figure 3. The distribution of the CMV-specific CD4+ and CD8+ T cell responses (background corrected) in three unique groups: (1) HIV-seronegative, CMV-seropositive, (2) established chronic untreated HIV infection, and (3) antiretroviral-treated infection with undetectable plasma HIV RNA levels. The IE IFN- $\gamma$ -bright levels are shown in panel A (CD4+ T cells) and panel B (CD8+ T cells). The combined

# Downward Spirals....

