



Collaboration in Clinical Research: A Critical Ingredient

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Clinical Research

- **The definition of Clinical Research is very broad**
- **Encompasses – clinical trials, outcomes, health delivery, epidemiological and psychosocial research**
- **Translational clinical research focuses on bench-to-bedside interface and requires investigators schooled in necessary skills**

Translational Clinical Investigators

- **At least two flavors:**
- **Patient Oriented Research (POR)**
 - Actively interact with patients who may enable them to uncover secrets of complex diseases
 - Care for those patients
 - Develop new therapeutic or diagnostic approaches
- **Disease Oriented Researchers (DOR)**
 - Interested in disease mechanisms
 - Do not interact patients/subjects in their research
 - Study tissue samples, cell lines, model systems such as mice, fish, yeast

Several Cs of Translational Clinical Research

David G. Nathan, M.D.

J Clin Invest 115:795-797, 2005

- **C**linical Focus
- **C**ollaboration
- **C**ourage – to learn new techniques/approaches
- **C**ritical Awareness of literature
- **C**onstructive Infrastructure
- **C**onsent from Patients
- **C**onflict of Interest
- **C**aring Mentors

One Route to Successful Patient Oriented Research: **Collaboration**

- “It is increasingly difficult for a single individual simultaneously to fill the roles of physician and scientist. One sure way to cover the spectrum: **Collaboration.**”
- “We are referring to an intimate collaboration between two individuals that allows them jointly to cover a range that neither could cover alone”

Joseph L. Goldstein, M.D. and Michael S. Brown, M.D.

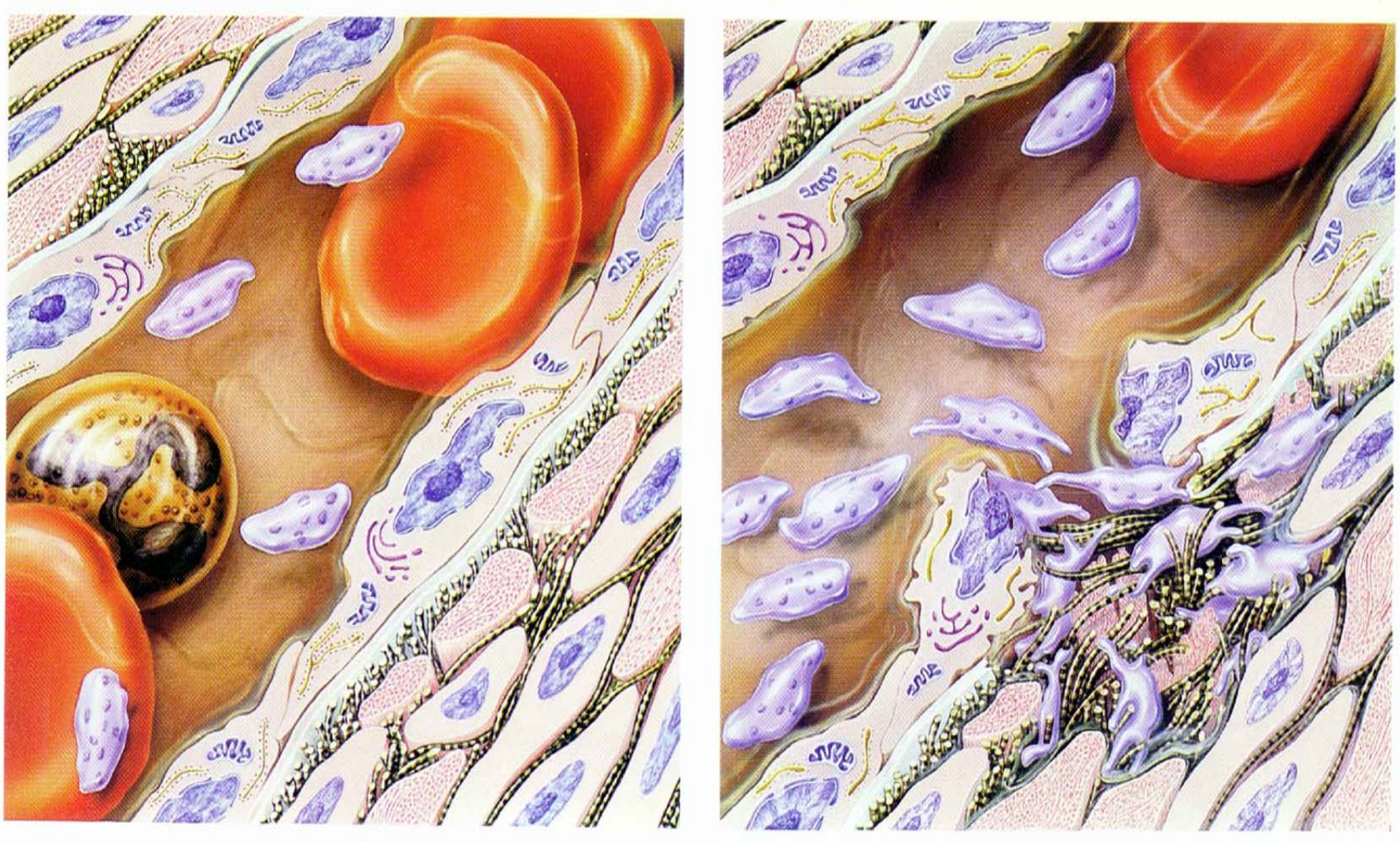
J Clin Invest 1997, 99: 2803-2812

Nobel Laureates

Research Interests: Rao Lab

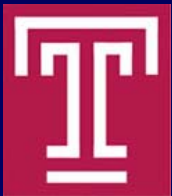
- ♦ **Molecular mechanisms of inherited platelet function.**
- ♦ **Alterations in blood coagulation mechanisms in health and disease.**
- ♦ **Impact of antithrombotic agents on platelets and coagulation systems.**

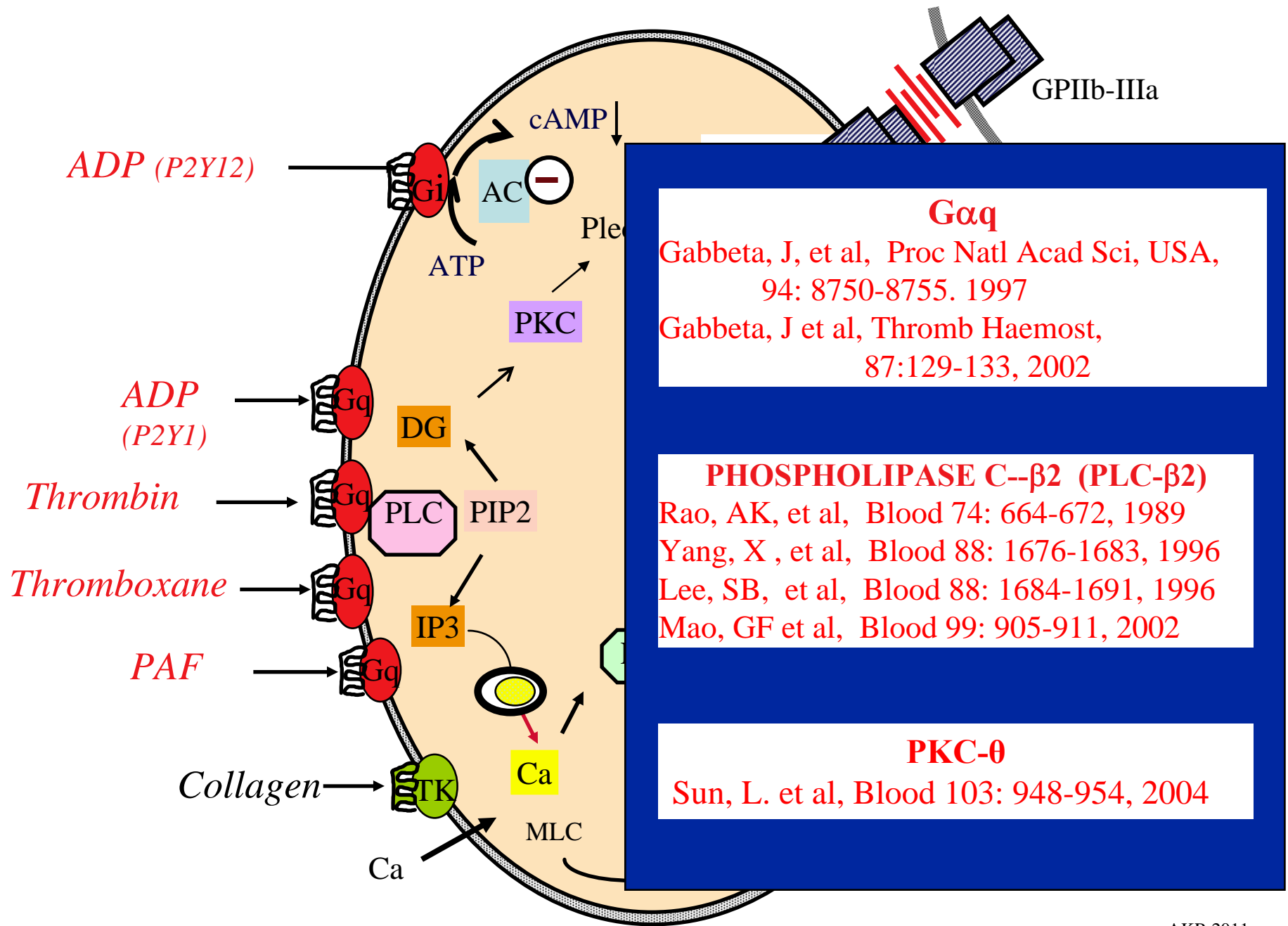
Basic and Clinical Research



Akkerman JW, Bouma BN, Sixma JJ. Atlas of Hemostasis, 1979.

Molecular Basis of Inherited Platelet Function Disorders





Gαq
 Gabbeta, J, et al, Proc Natl Acad Sci, USA, 94: 8750-8755. 1997
 Gabbeta, J et al, Thromb Haemost, 87:129-133, 2002

PHOSPHOLIPASE C--β2 (PLC-β2)
 Rao, AK, et al, Blood 74: 664-672, 1989
 Yang, X, et al, Blood 88: 1676-1683, 1996
 Lee, SB, et al, Blood 88: 1684-1691, 1996
 Mao, GF et al, Blood 99: 905-911, 2002

PKC-θ
 Sun, L. et al, Blood 103: 948-954, 2004

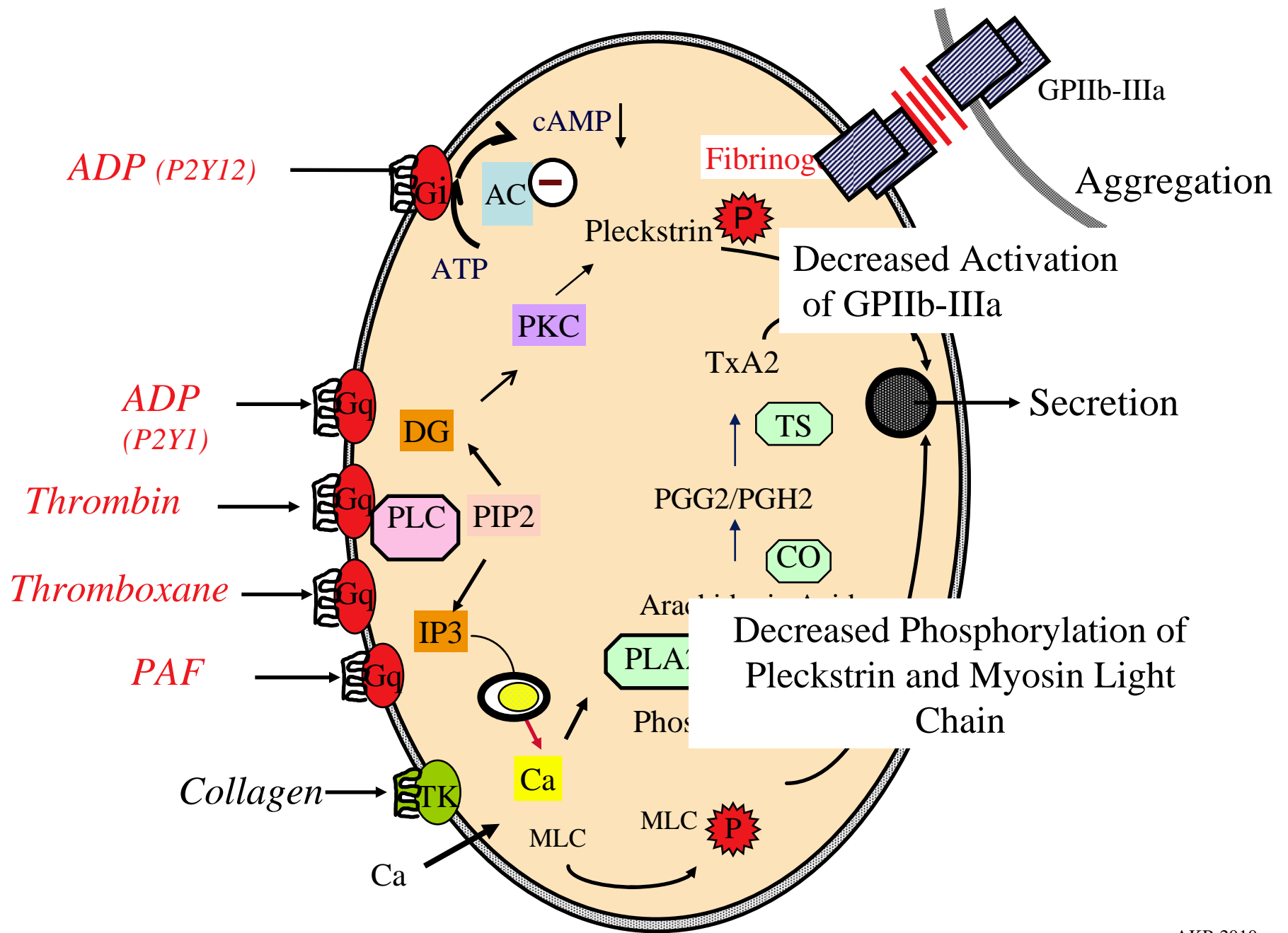
The Long Story of studies in one patient ...In Short

3 years old white male

Thrombocytopenia

Platelet Function Defect

Father had died of Acute Leukemia



RUNX1

Exon 3

Intron 3

Exon 4

WT 5'-GCTAGgtgcc...gttta^g GTGGTGGCCCTAGGG

|||||

Patient 5'-GCTAGgtgcc...gttta^g GTGGTGGCCCTAGGG

cryptic splice
acceptor

Protein Sequence:

WT: KKVVALGDVPDGT¹²⁸LV.....AFNPQPQSQMQDT..

Patient: KK: GMFQMALWSL*

Antibody

RUNX1 Immunoblot:

P N N N



Sun et al, *BLOOD*, 103: 948-954, 2004

What are Transcription Factors??



- **Each cell contains a complete copy of organism's genome**
- **What makes cells different?**
 - **Each cell utilizes and expresses only a subset of genes**
 - **Differential expression is regulated at transcriptional level**

Questions

- **What are the genes regulated by transcription factor RUNX1 in platelets/megakaryocytes?**
- **This would help us understand the mechanisms leading to the platelet dysfunction and the thrombocytopenia**

ACT 3
Approach: Genome-Wide
*Platelet Expression
Profiling*



Platelet Expression Profiling

- Expression profiling permits assessment of gene expression (mRNA)
- Established a collaboration: Children's National Medical Center, Washington, DC
- Affymetrix U133 human genomic chip sets (A and B). ~ 44,000 probe sets.
- Platelet total RNA isolated ~350 ml blood

Sun et al, J Thromb Haemost, 5:146-154, 2007

Platelet Expression Profiling: Findings

- **70 genes down-regulated**
- **Most down-regulated gene was *MYL9* (*myosin light chain*)**
- **Revealed alterations in expression of genes we could not have predicted**
- **Genes whose function not even known in platelets**

Sun et al, J Thromb Haemost, 5:146-154, 2007

Are these regulated by RUNX1 at Transcriptional level?

Myosin Light Chain (MYL9)

Jalagadugula et al *Blood* 2010;116:6037-6045

12-Lipoxygenase (ALOX12)

Kaur et al *Blood* 2010;115:3128-3135

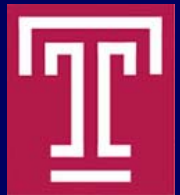
Platelet factor-4 (PF4)

Aneja et al *J Thromb Haemost*

DOI:10.1111/j.1538-7836.2010.04154.x

Protein kinase C- θ (PKC- θ)

Jalagadugula et al *Athero Thromb Vasc Biol*
2011 (in press)



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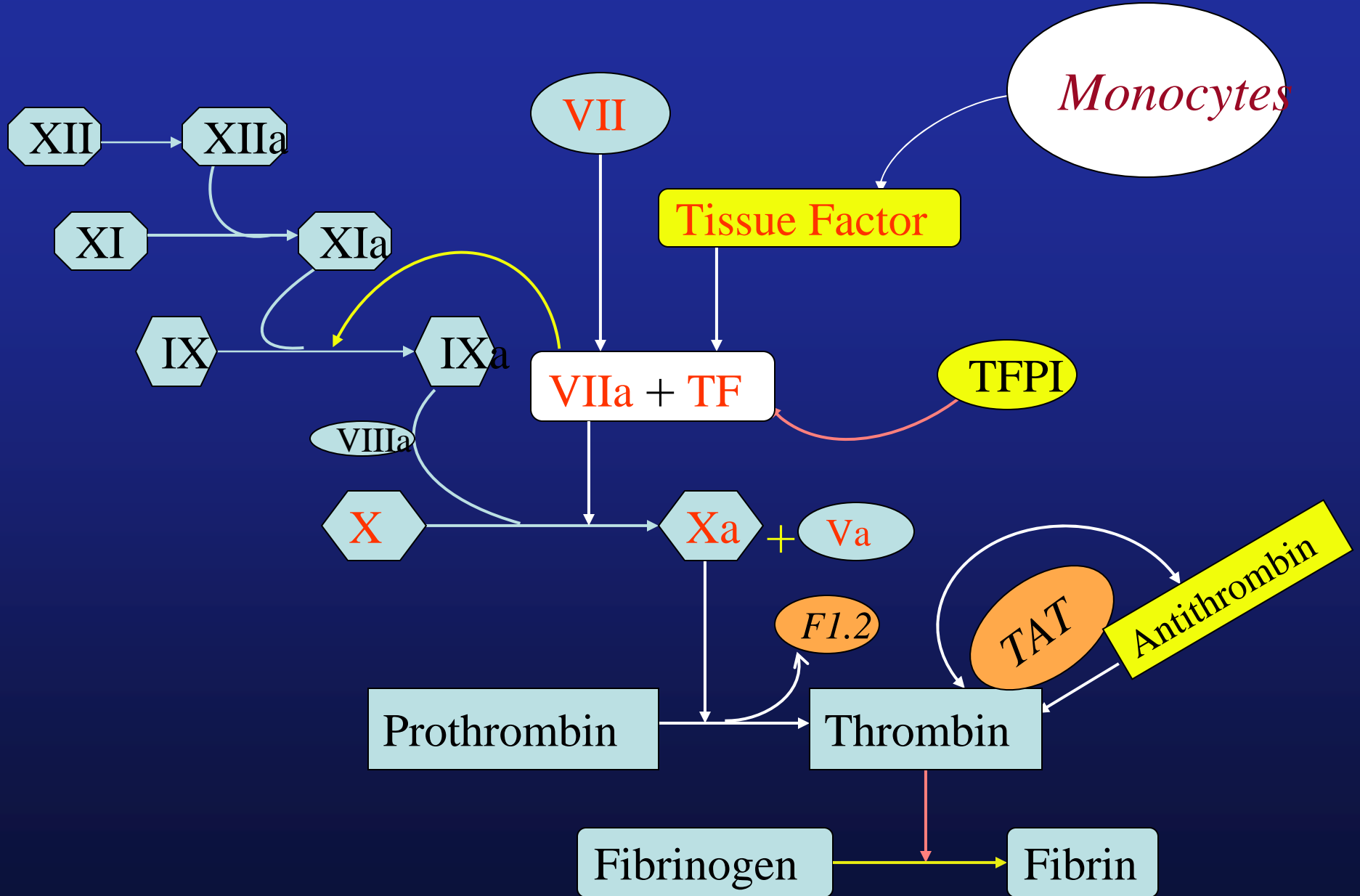
Diabetes Mellitus, Vascular Disease, Blood Coagulation and Platelets

**HYPERGLYCEMIA AND HYPERINSULINEMIA
INDUCED PROCOAGULANT STATE IN
DIABETES MELLITUS**

BACKGROUND

- **Patients with DM have increased atherosclerotic and acute vascular events.**
- **The rupture of the atherosclerotic plaque is a major cause of sudden death in humans.**
- **Diabetes mellitus is a procoagulant state.**
- **Both *high glucose* and *high insulin* levels are independently associated with increased mortality.**
- **The effects of hyperglycemia and hyperinsulinemia on blood coagulation are ill-defined.**

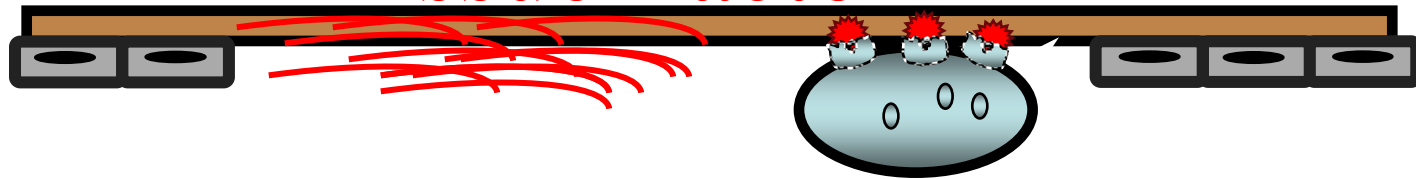
COAGULATION MECHANISMS



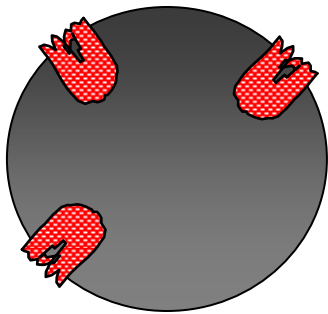
STUDY DESIGN

- ❖ **Hyperglycemia and Hyperinsulinemia (HG+ HI):**
10 healthy individuals
 - **Infused with glucose (~ 200 mg/dl) for 24 hrs;**
Endogenous insulin ~ 1000 pM.
- ❖ **Euglycemia and Hyperinsulinemia (EG+HI):**
7 individuals
 - **Infused with regular insulin (~ 1000 pM), and glucose (100 mg/dl) for 24 hrs.**
- ❖ **Selective Hyperglycemia and Euinsulinemia (HG+EI):**
6 individuals
 - **Infused with high glucose (200 mg/dl); and Somatostatin to inhibit endogenous insulin release.**
- ❖ **Euglycemia and Euinsulinemia (EG+EI) Placebo:**
5 individuals: infused with saline.

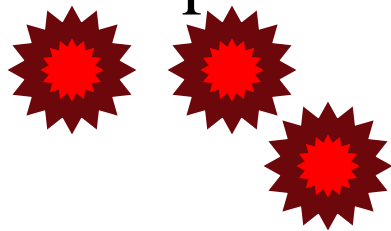
Tissue Factor



Neutrophil



Microparticles

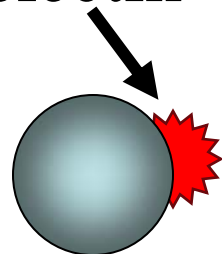


TF + Other Proteins

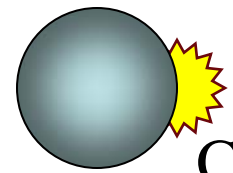
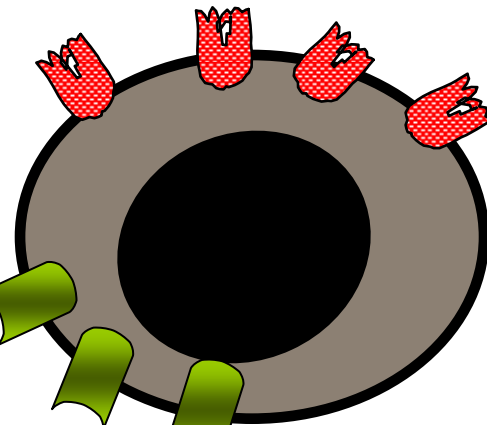
TF

Tissue Factor

P-selectin



Platelet



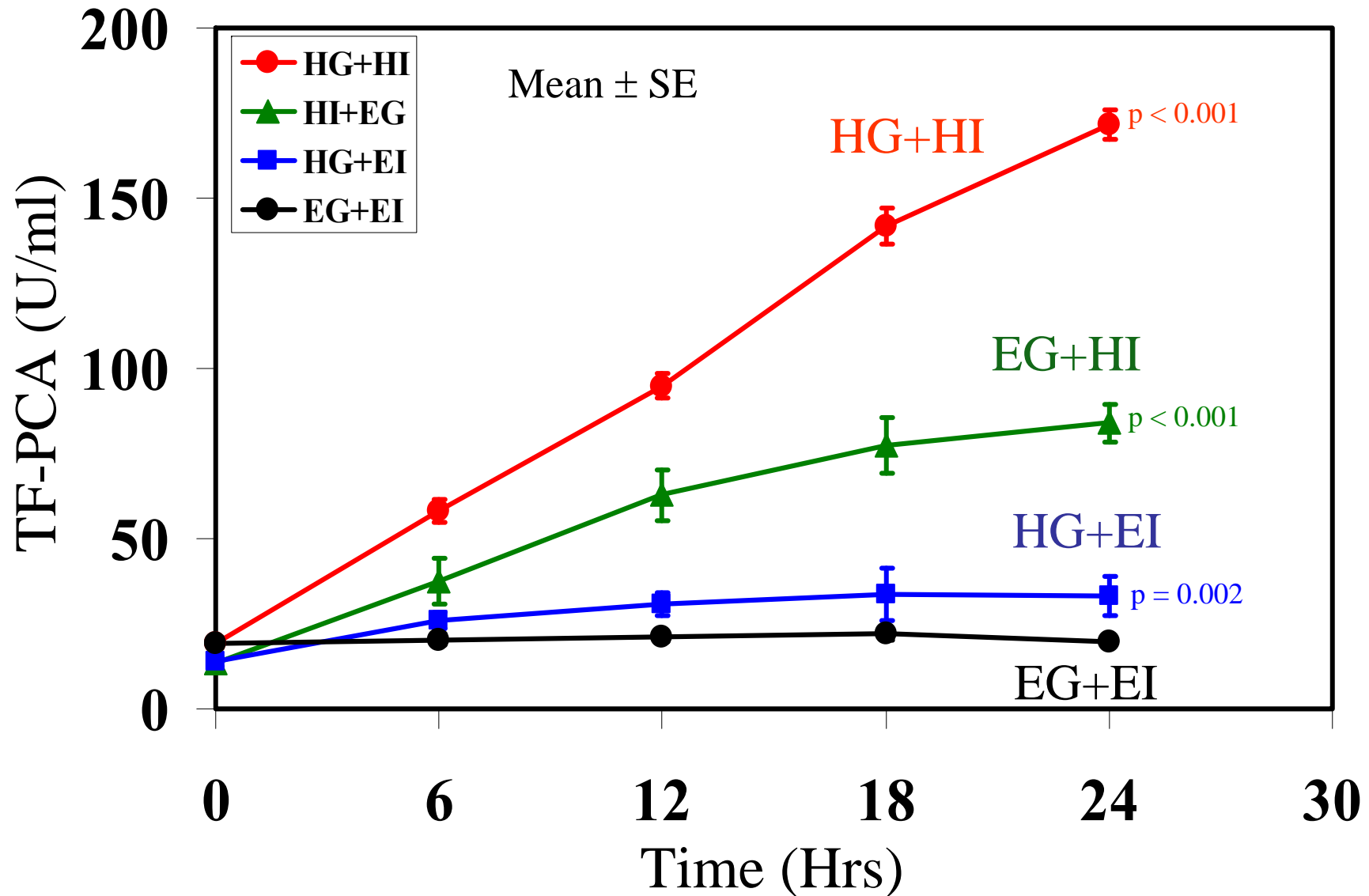
CD40L

PSGL-1

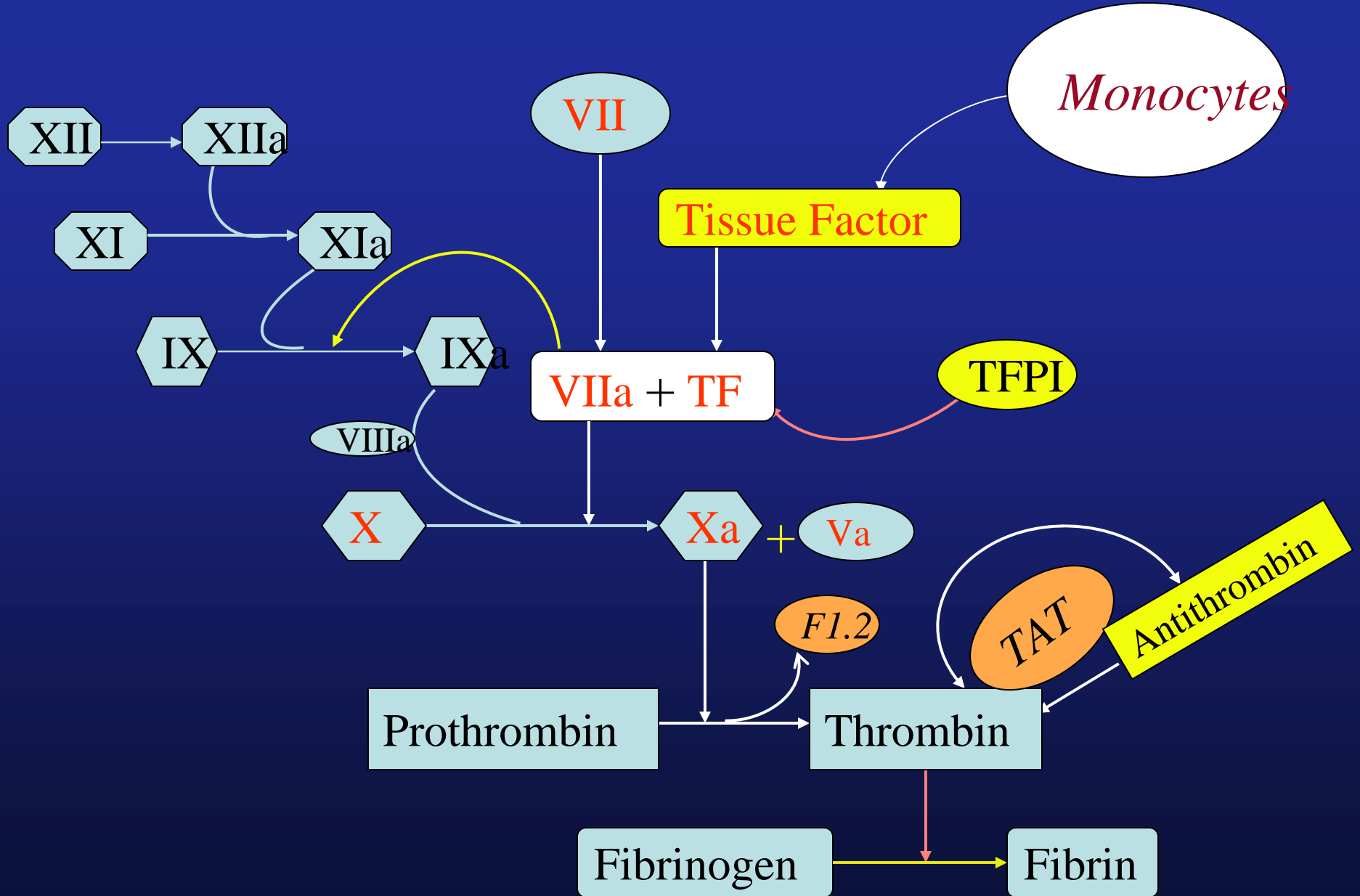
Monocyte

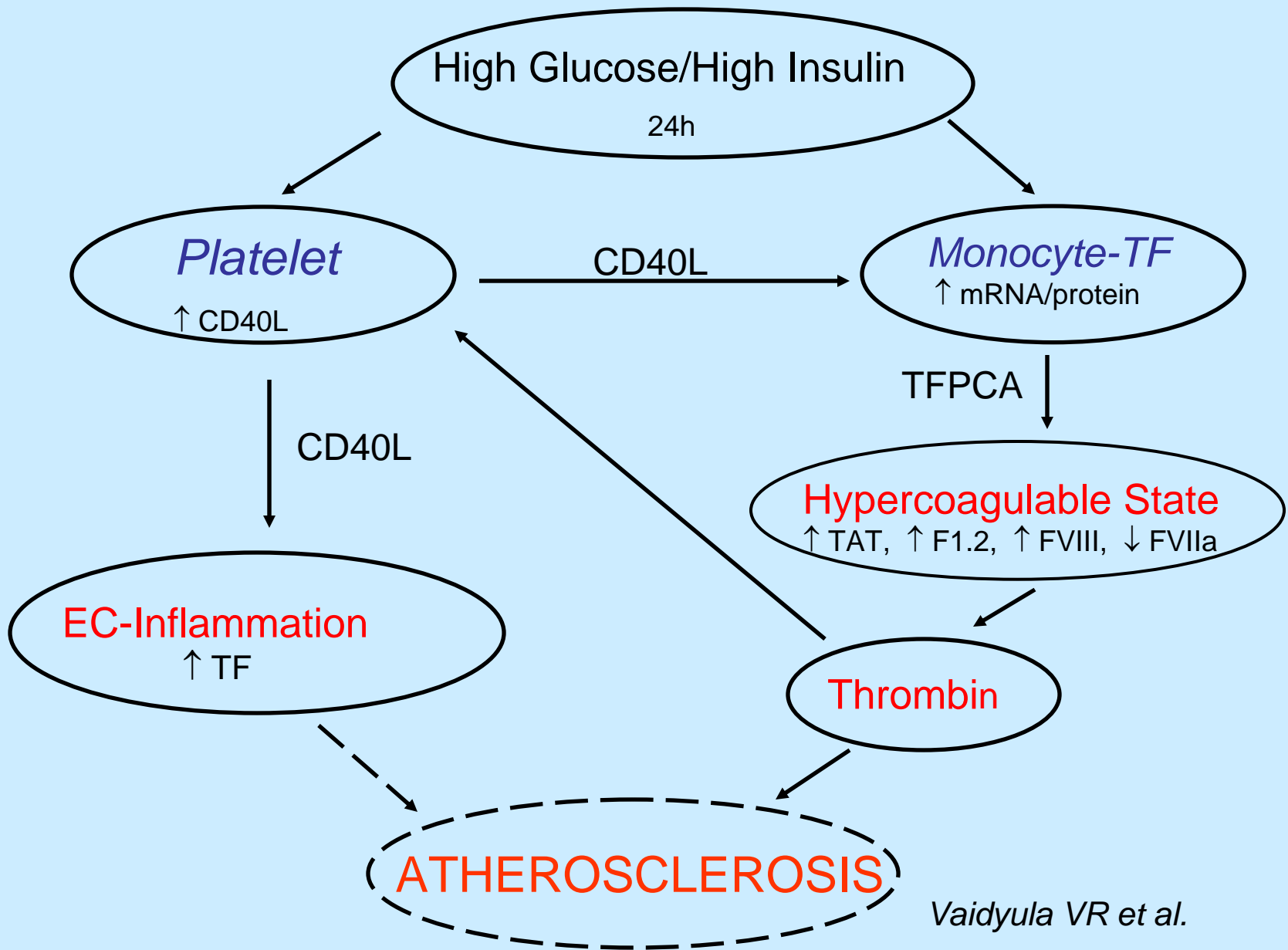
AKR

Tissue Factor Procoagulant Activity In Whole Blood



COAGULATION MECHANISMS





Vaidyula VR et al.

Diabetes 55:202-208, 2006

Translational Clinical Research: Power of Collaboration

- Collaborations are essential
- Essential for moving forward in the world of exploding information and technology
- “It is crucial that they interact as equals, each contributing ideas...Partnerships based on subservience are doomed to failure” (Goldstein, J and Brown, M, *J Clin Invest* 1997, 99: 2803-2812)
- Not all collaborations work out



AKR 2005

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**The future of medicine
absolutely depends on clinical
investigators**

A tropical sunset scene with palm trees and gazebos. The sun is low on the horizon, casting a warm orange glow across the sky. The foreground is dominated by the dark silhouettes of palm trees and several gazebos. The ocean is visible in the background, with a few small waves. The overall mood is peaceful and serene.

Thank you

Hawaii 2009