PLATELET INTERACTIONS WITH LEUKOCYTES AND ENDOTHELIAL CELLS IN VASCULAR INFLAMMATION

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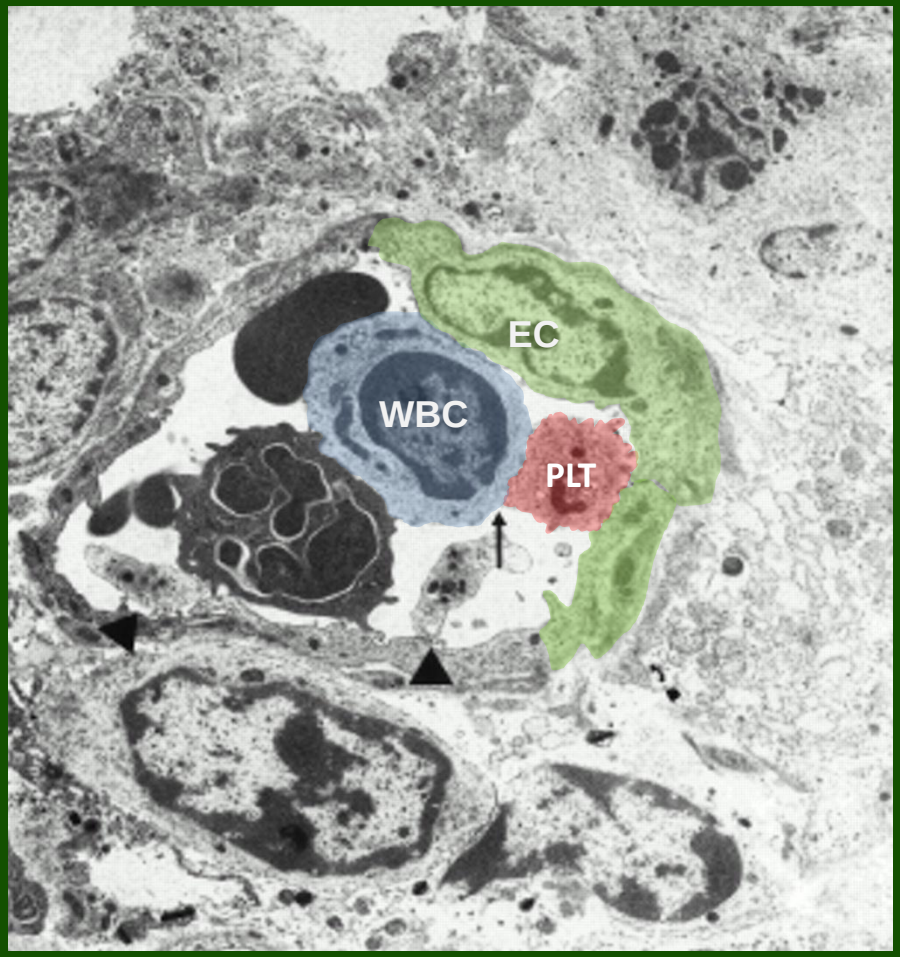
www.Cardioalex.gr



Platelets and the endothelium

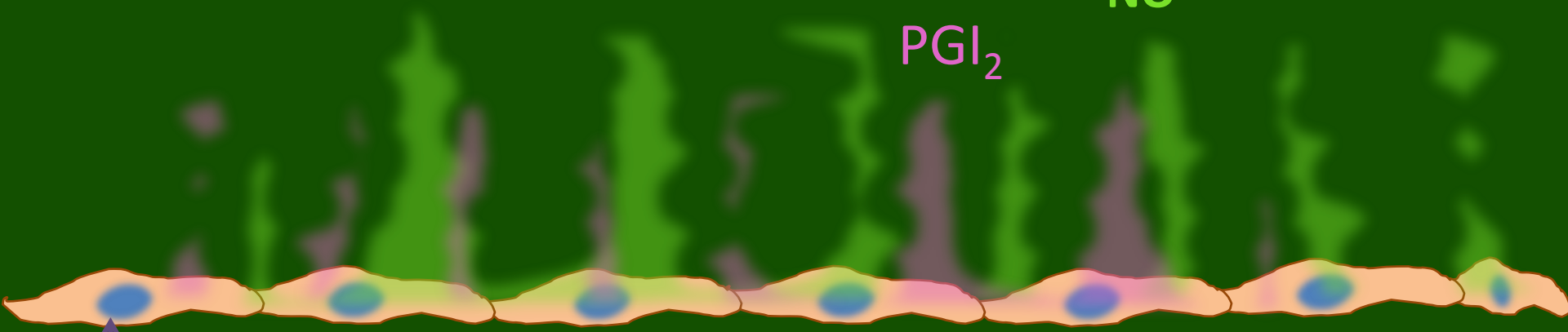
10,000 trips around the circulatory loop during their week lifetime

Homotypic and heterotypic interactions

By paracrine signaling
via transient interactions (“give-and-go” mechanism),
or through receptor-mediated cell–cell adhesion.

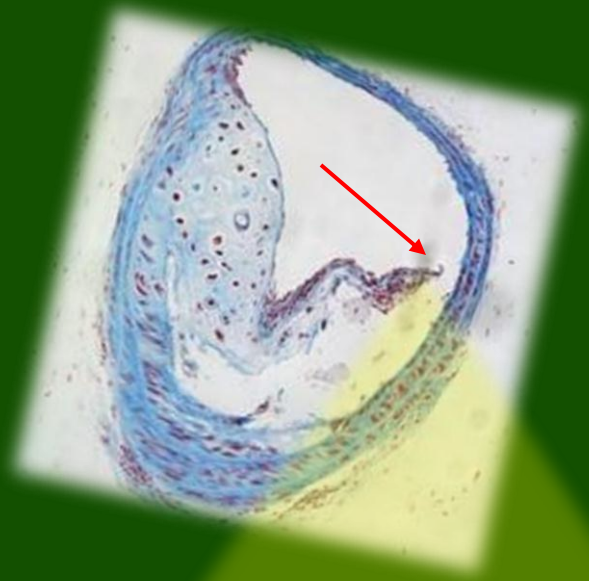
However, most PLTs never undergo firm adhesion to the endothelium

This kind of interactions hampered by ..



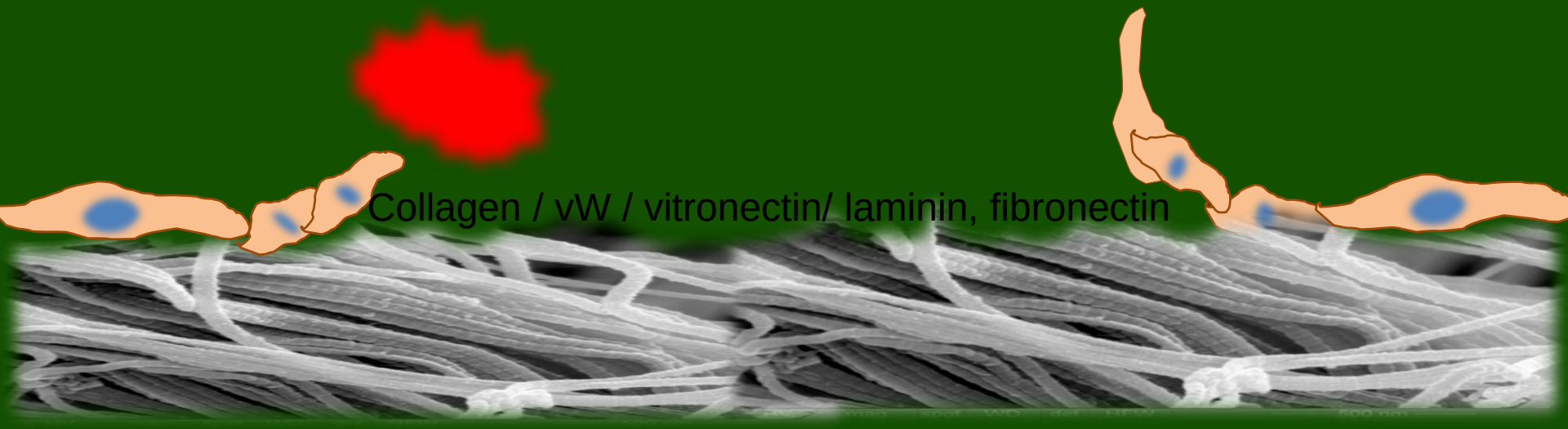
cAMP and cGMP; CD39 ectoNTPDase, heparan sulfate, tissue plasminogen activator

Platelet adhesion to ECM after denudation



Sub-endothelial layers are exposed

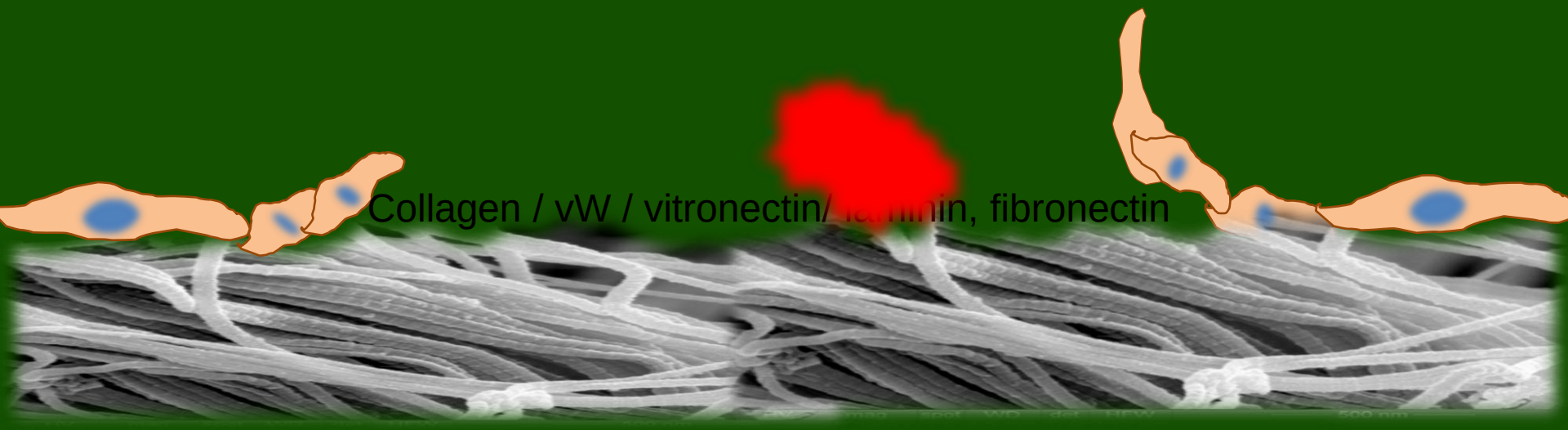
Rolling at high shear


Collagen / vW / vitronectin/ laminin, fibronectin


Collagen / IV / vitronectin/ laminin, fibronectin


Collagen / vW / vitronectin/ laminin, fibronectin

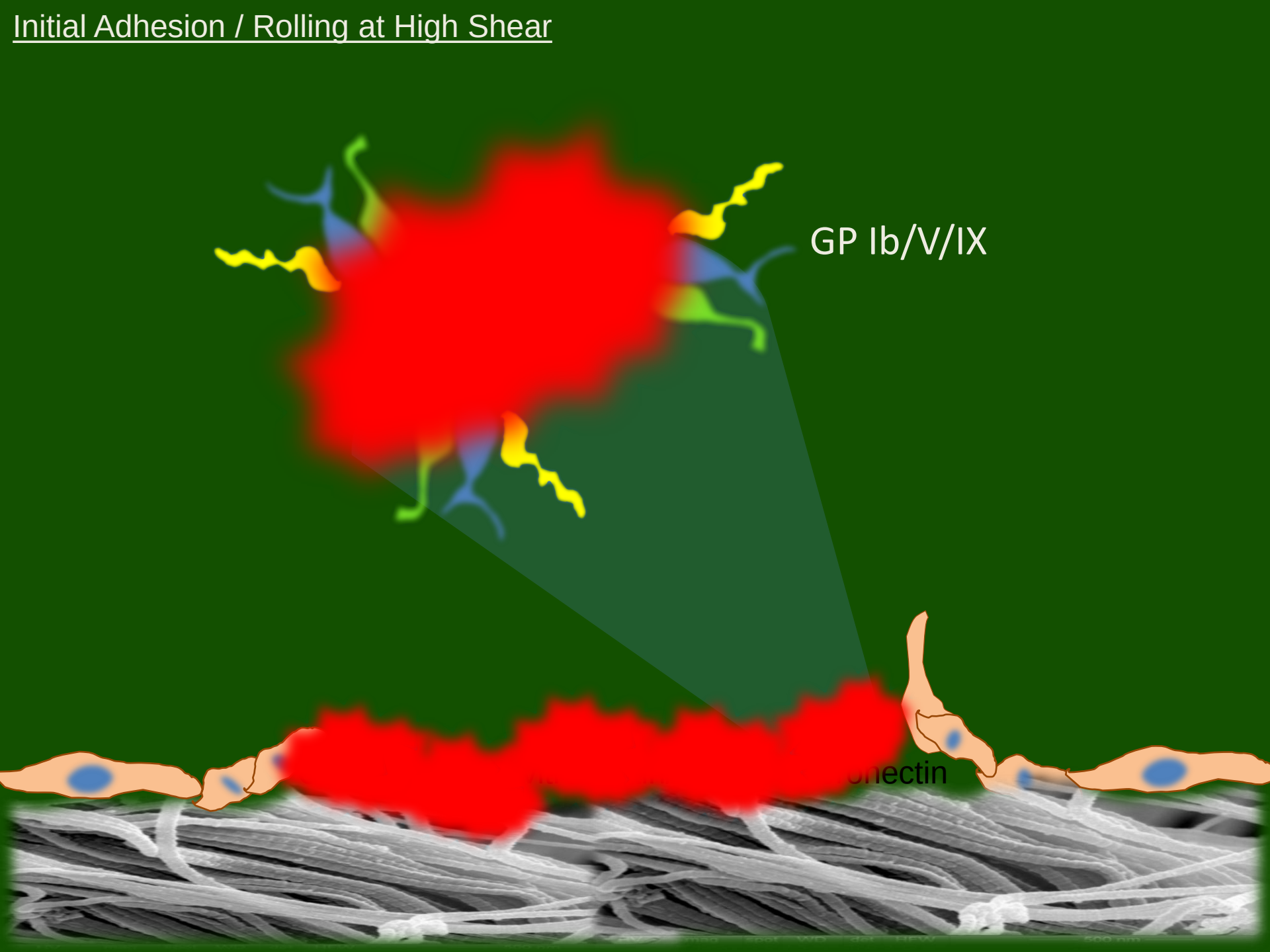

Collagen / vW / vitronectin/ laminin, fibronectin


Collagen / vW / vitronectin/ [illegible]in, fibronectin

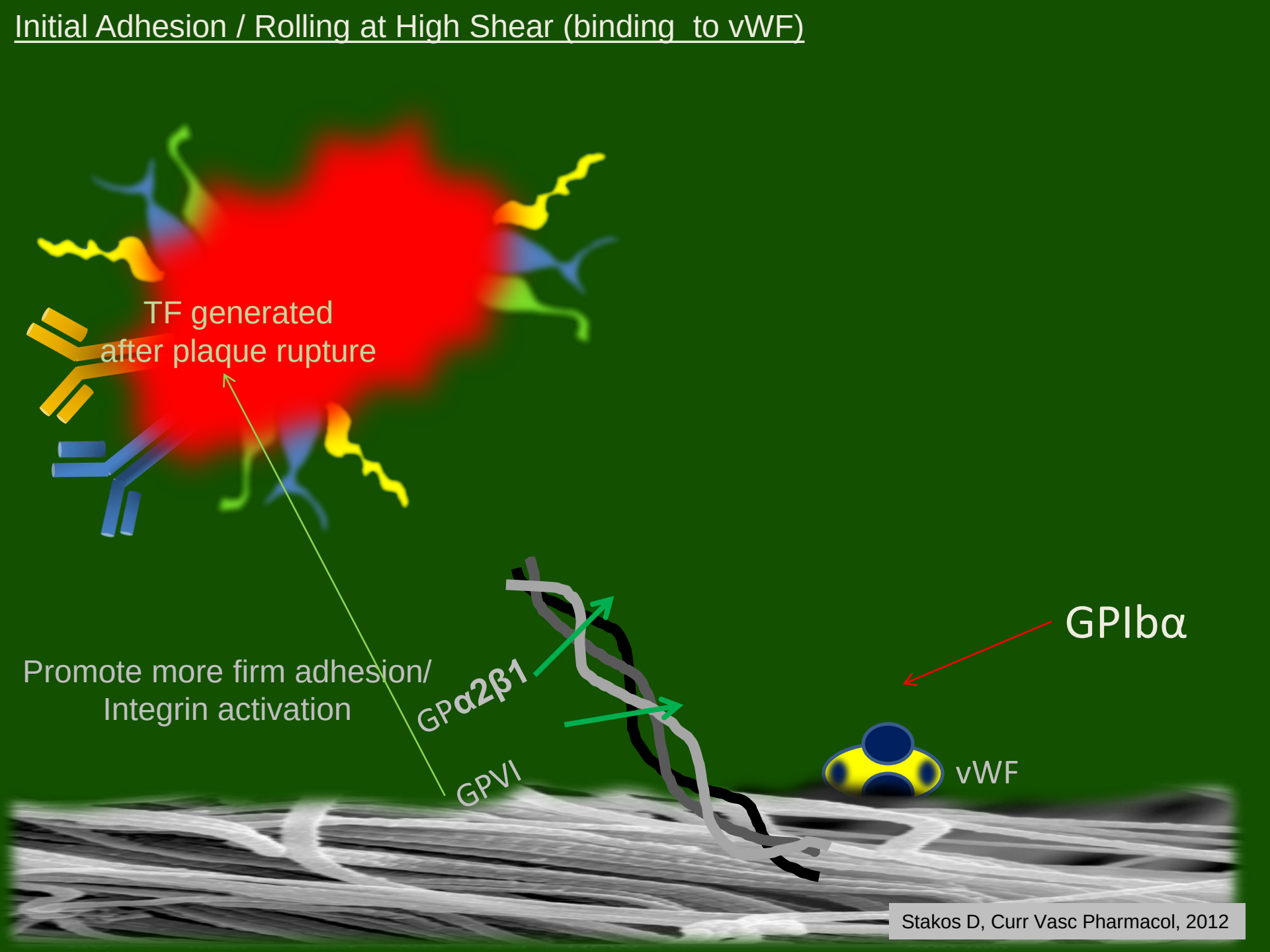

Collagen / vW / vitronectin/ lam
ibronectin


Collagen / vW / vitronectin/ laminin

Initial Adhesion / Rolling at High Shear

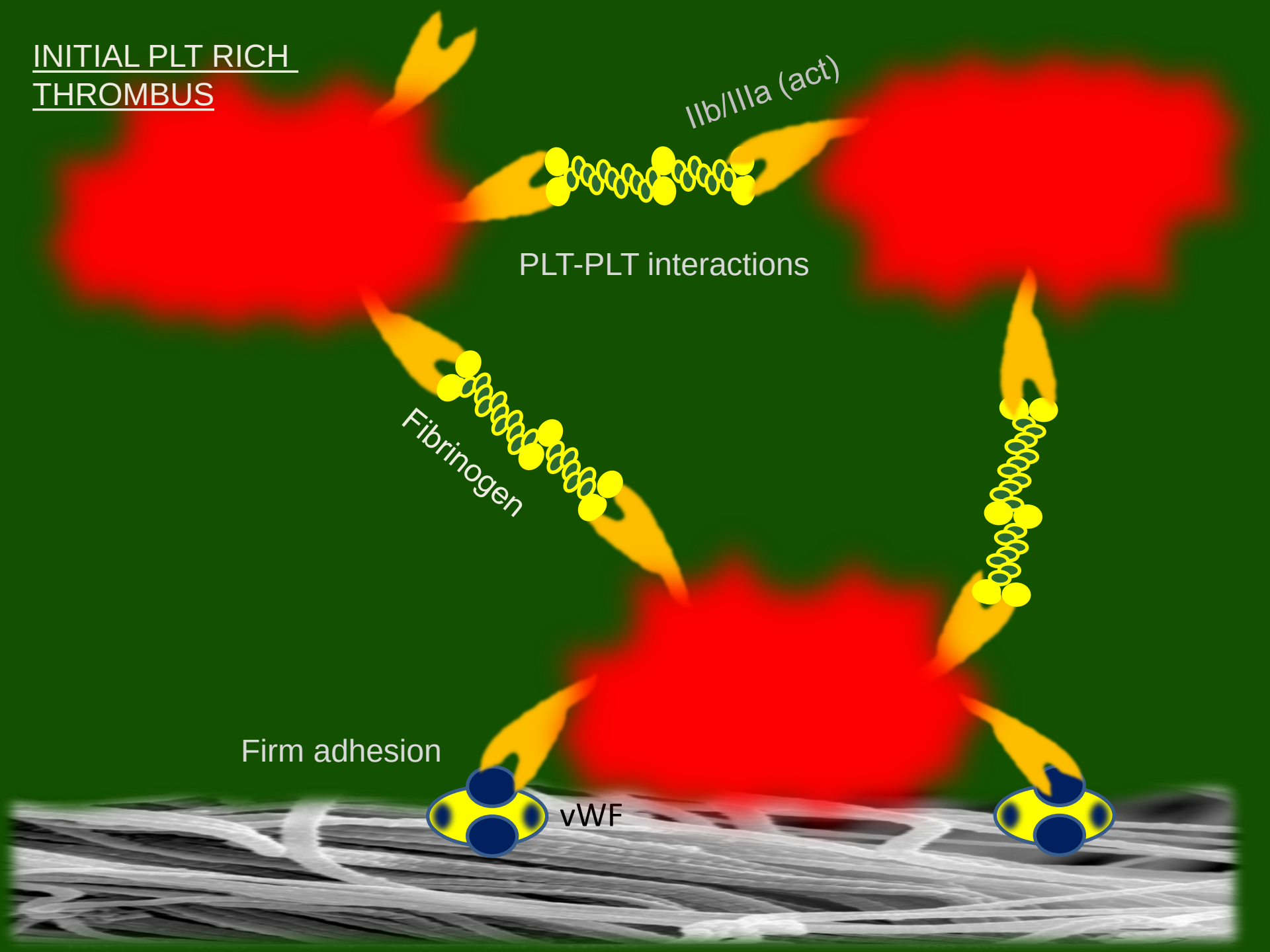


Initial Adhesion / Rolling at High Shear (binding to vWF)



Stakos D, Curr Vasc Pharmacol, 2012

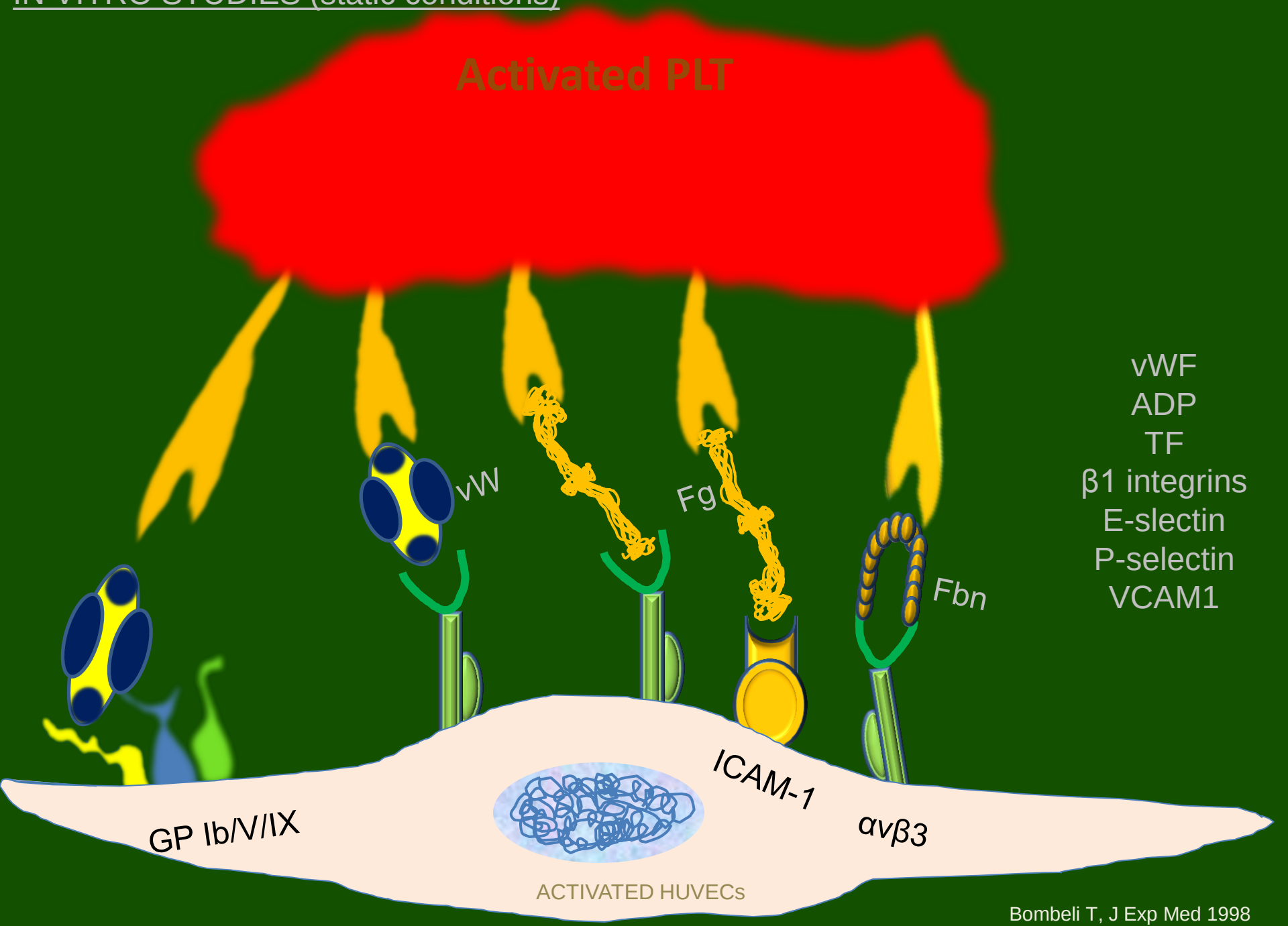
GP IIb/IIIa


INITIAL PLT RICH THROMBUS
IIb/IIIa (act)
PLT-PLT interactions
Fibrinogen
Firm adhesion
vWF

.. not really a PLT – EC interaction

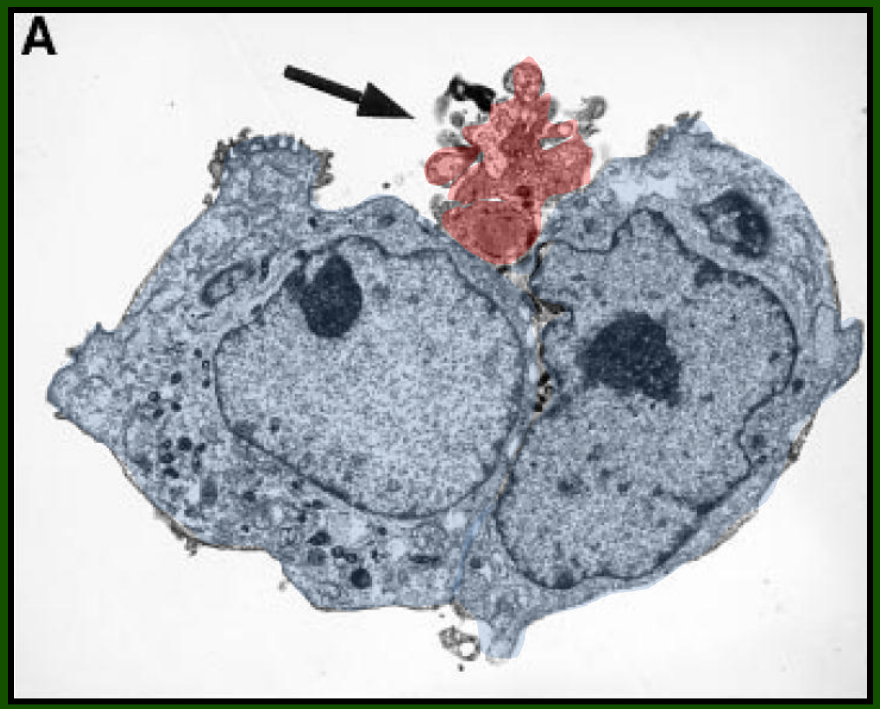
Platelet adhesion to “intact” endothelium

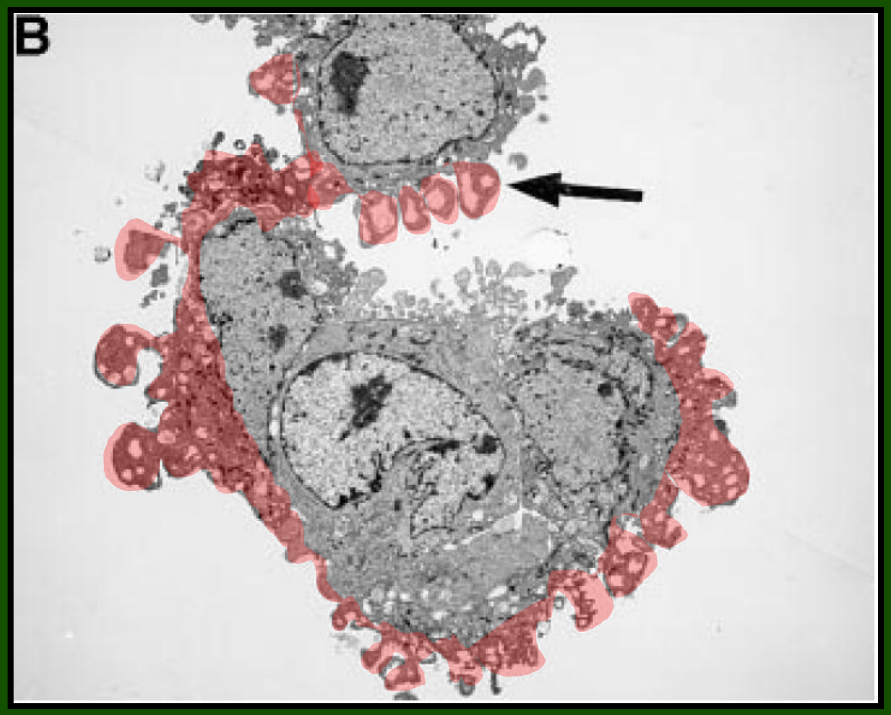
IN VITRO STUDIES (static conditions)



Bombeli T, J Exp Med 1998

1. PLTs adhere to sbECM
2. PLTs form aggregates
3. PLTs adhere to activated endothelium in vitro, but also..





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1. PLTs adhere to sbECM
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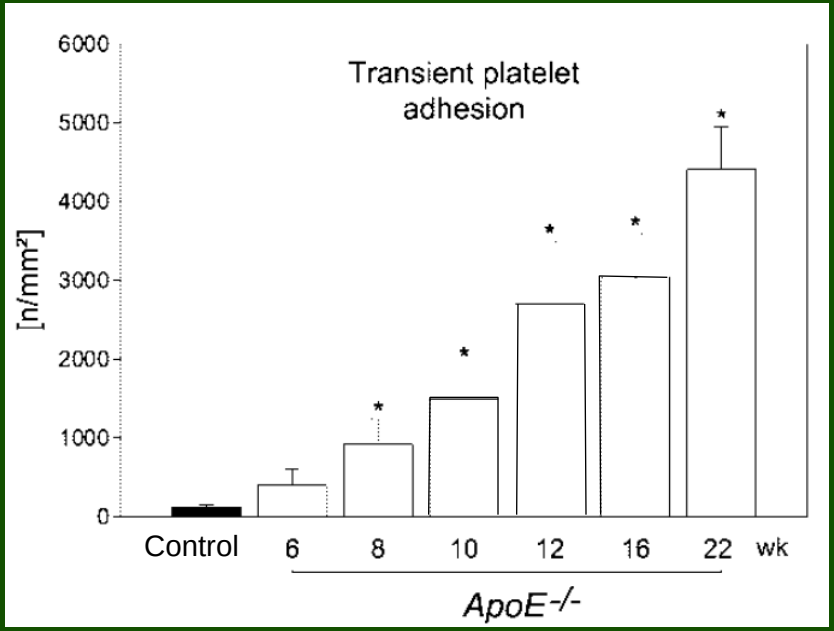
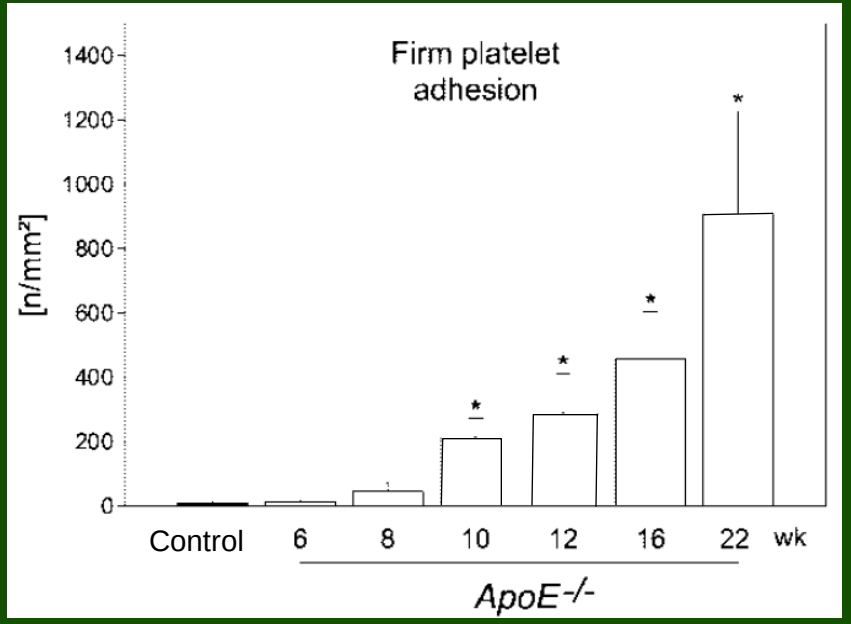
4. PLTs adhere to endothelium under flow

(a. tethering b. rolling c. firm adhesion)





Massberg S, J Exp Med, 2002

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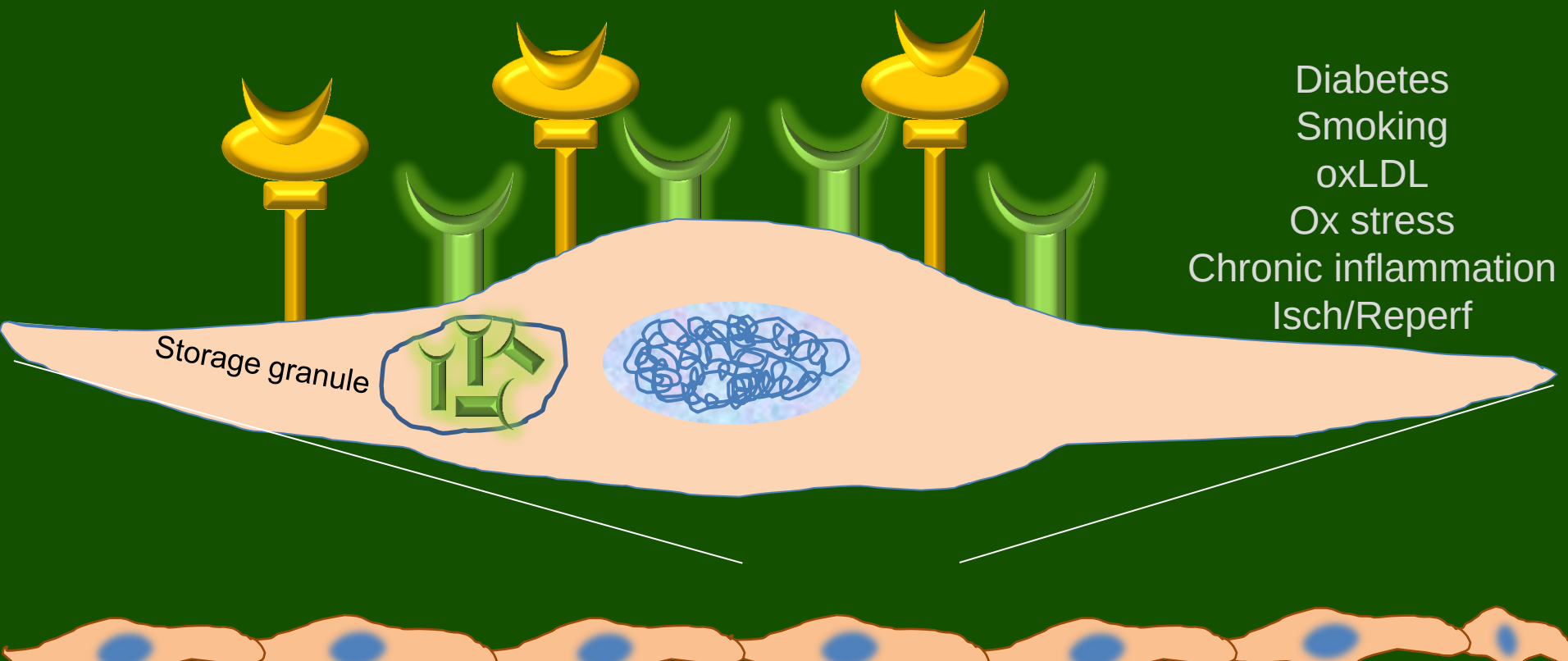
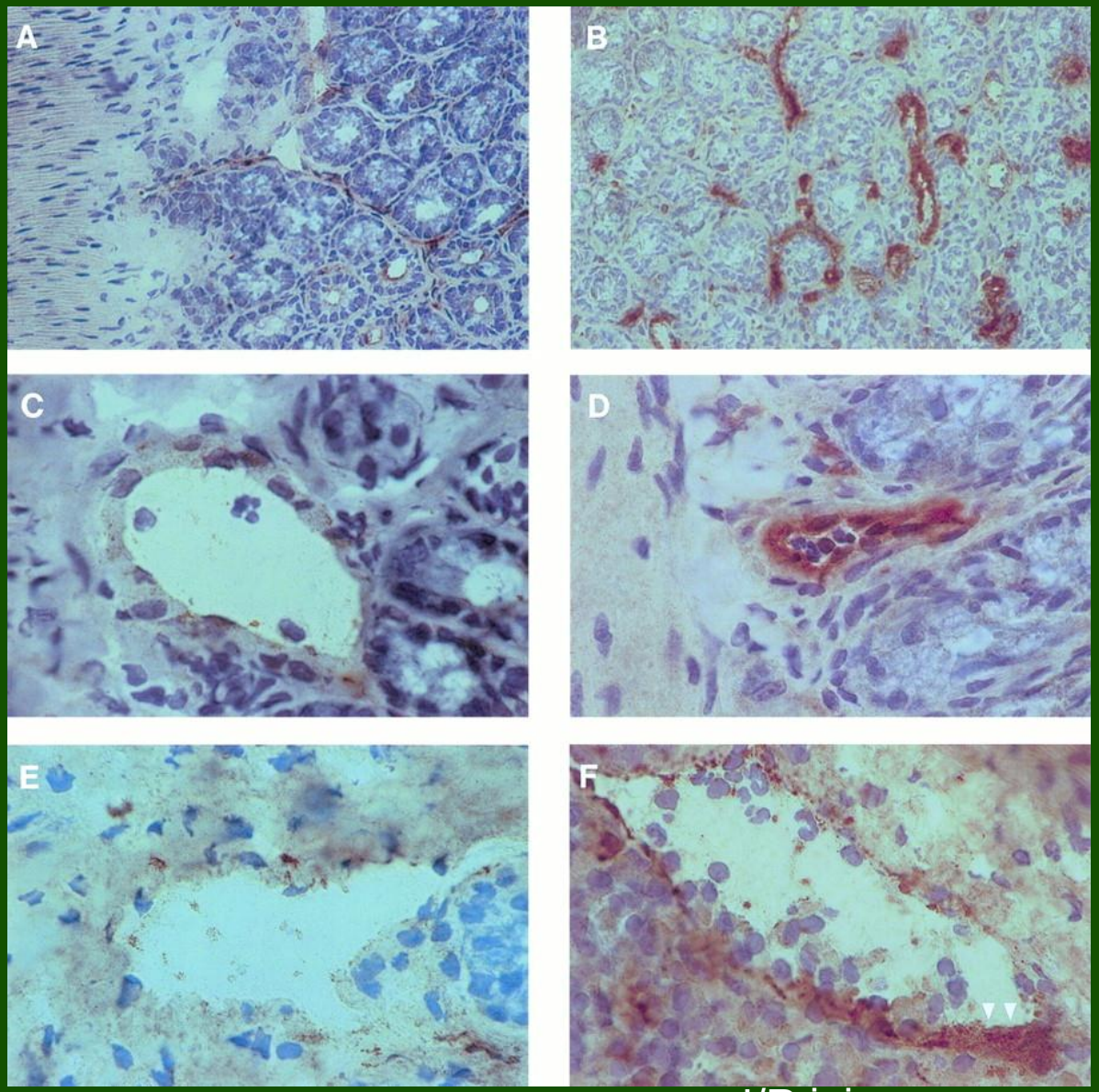
P-selectin (CD62P) and E-selectin expression in inflamed endothelium



Diabetes
Smoking
oxLDL
Ox stress
Chronic inflammation
Isch/Reperf

P-selectin expression in mouse inflamed endothelium (Isc/Repf)



Arteriole

Venule

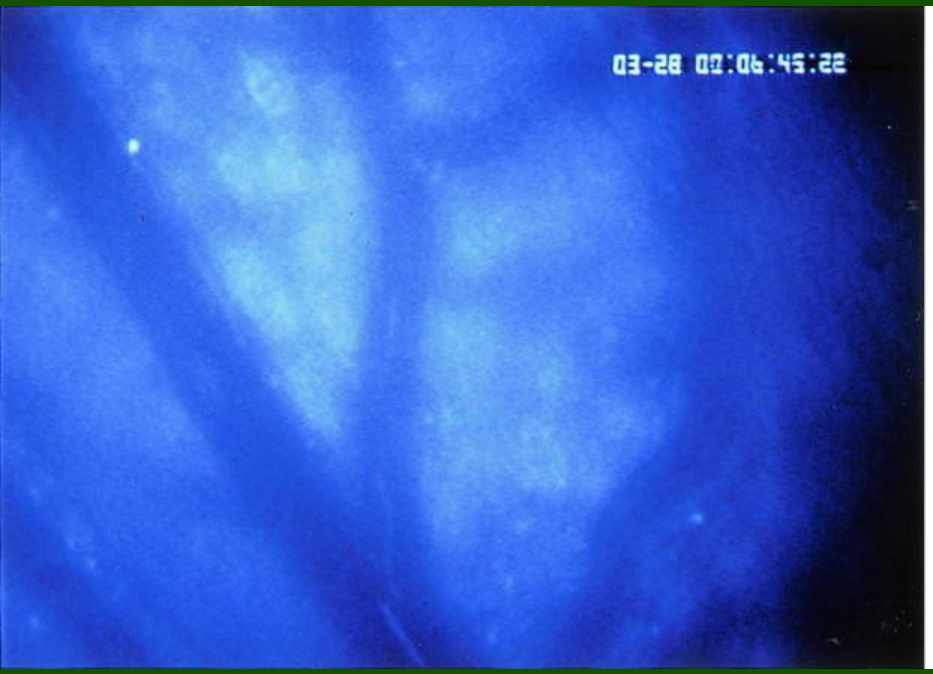
Control

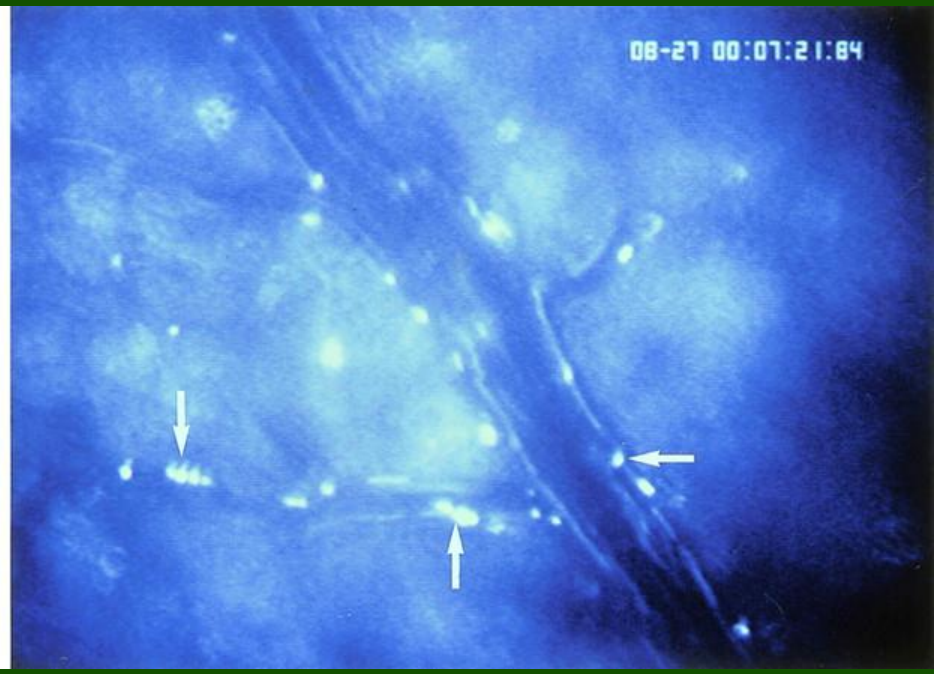
I/R injury

Massberg S, Blood, 1998

fluorescence microscopy (PLT – EC interactions 5 min after reperfusion)

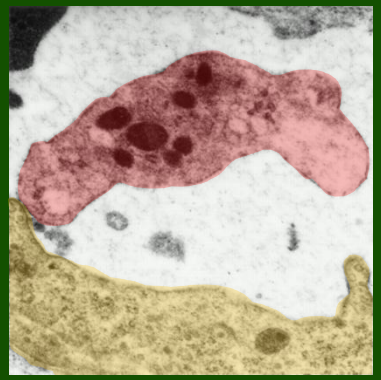
Rhodamine-6G-labeled platelets





Control animals

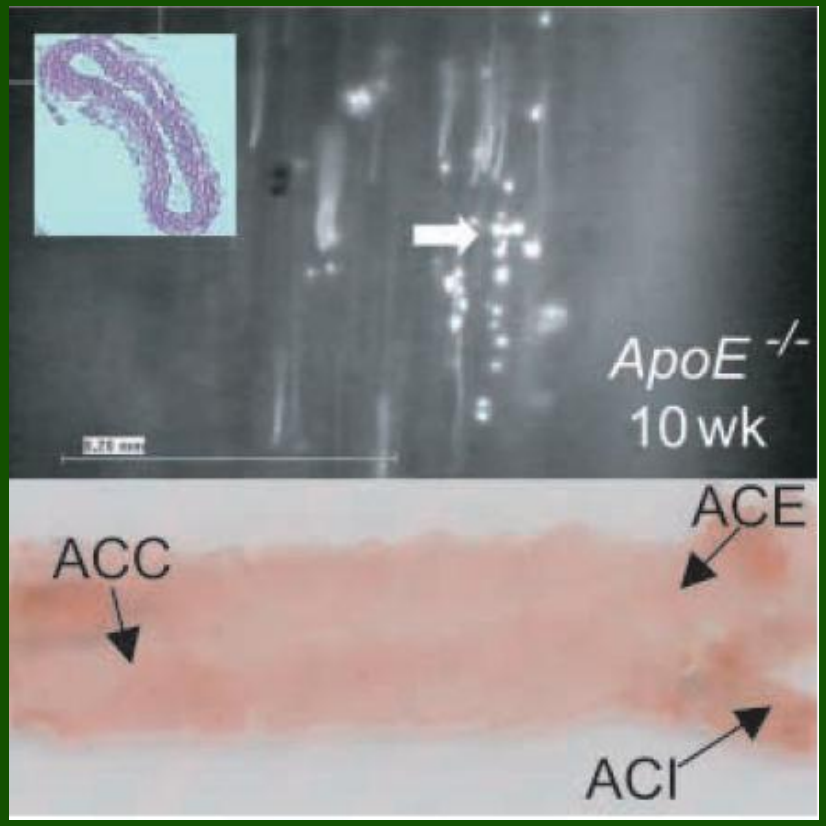
I/R 5 minutes after reoxygenation

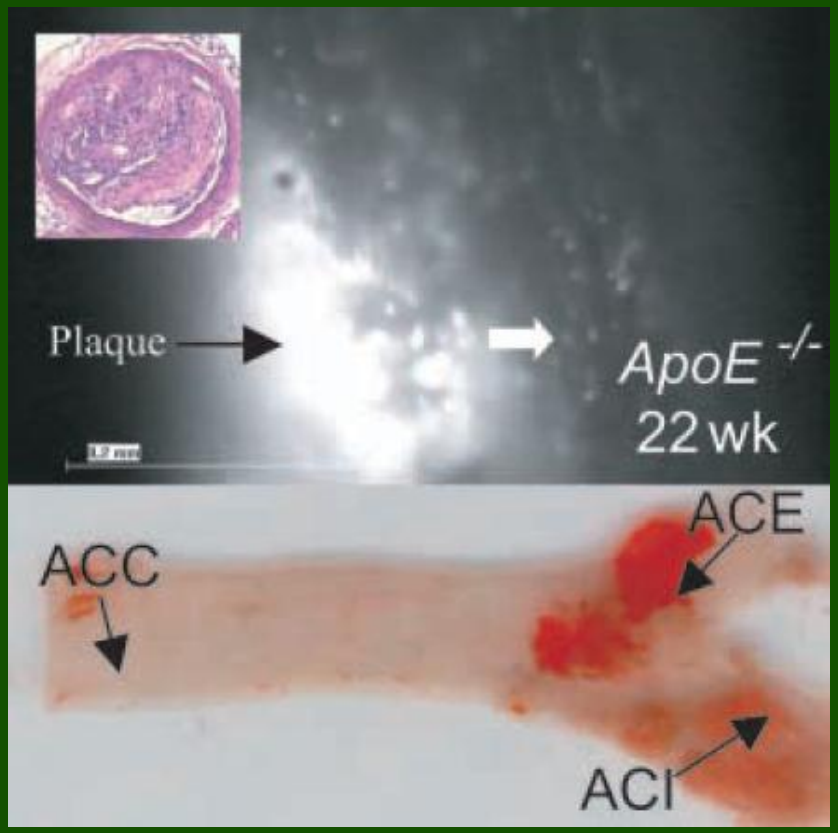


Massberg S, Blood, 1998

Not only at small arterioles..

Platelets adhere at lesion-prone sites before the development of atherosclerotic plaque

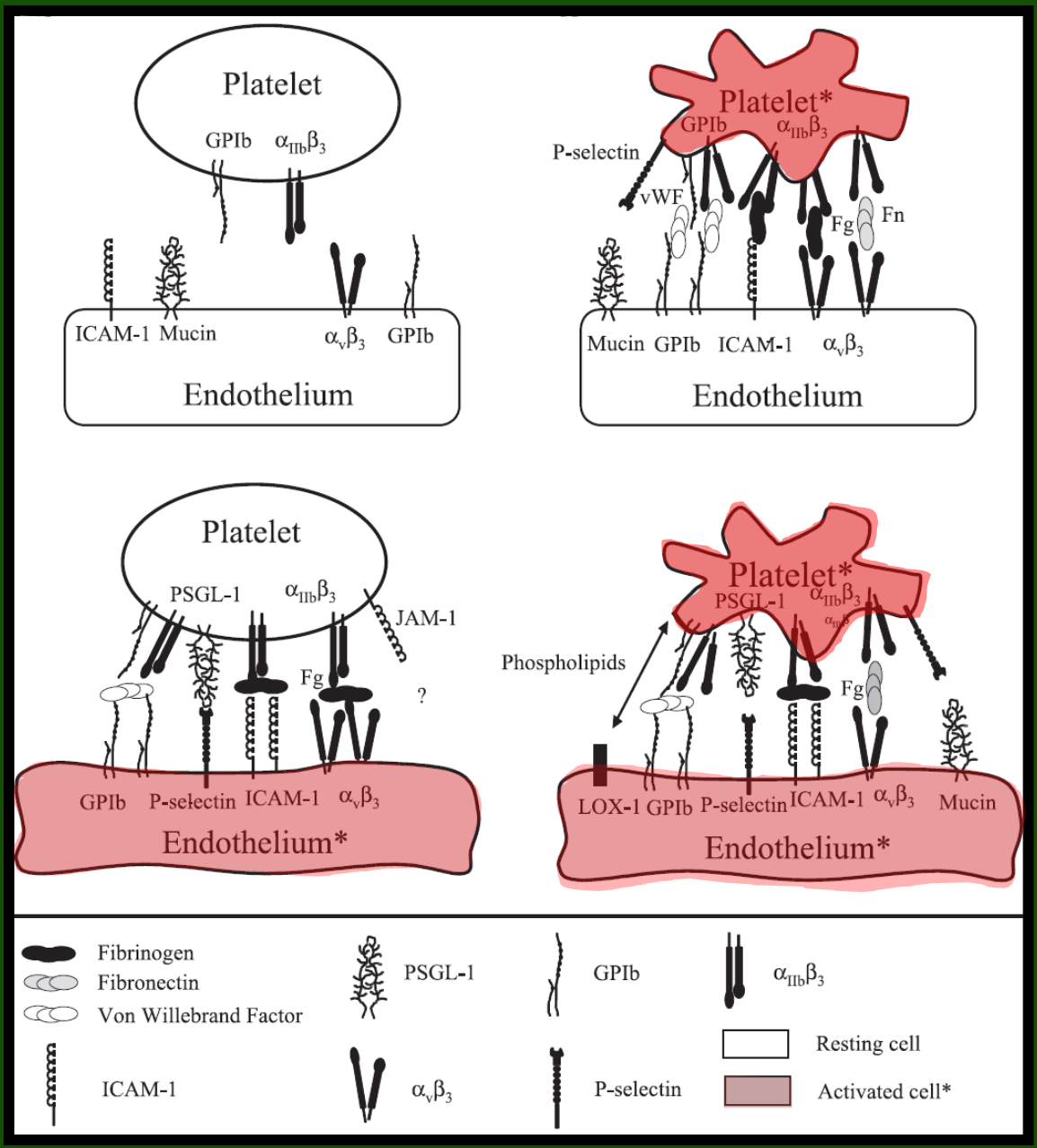




Massberg S, J Exp Med, 2002

Requirements for these interactions

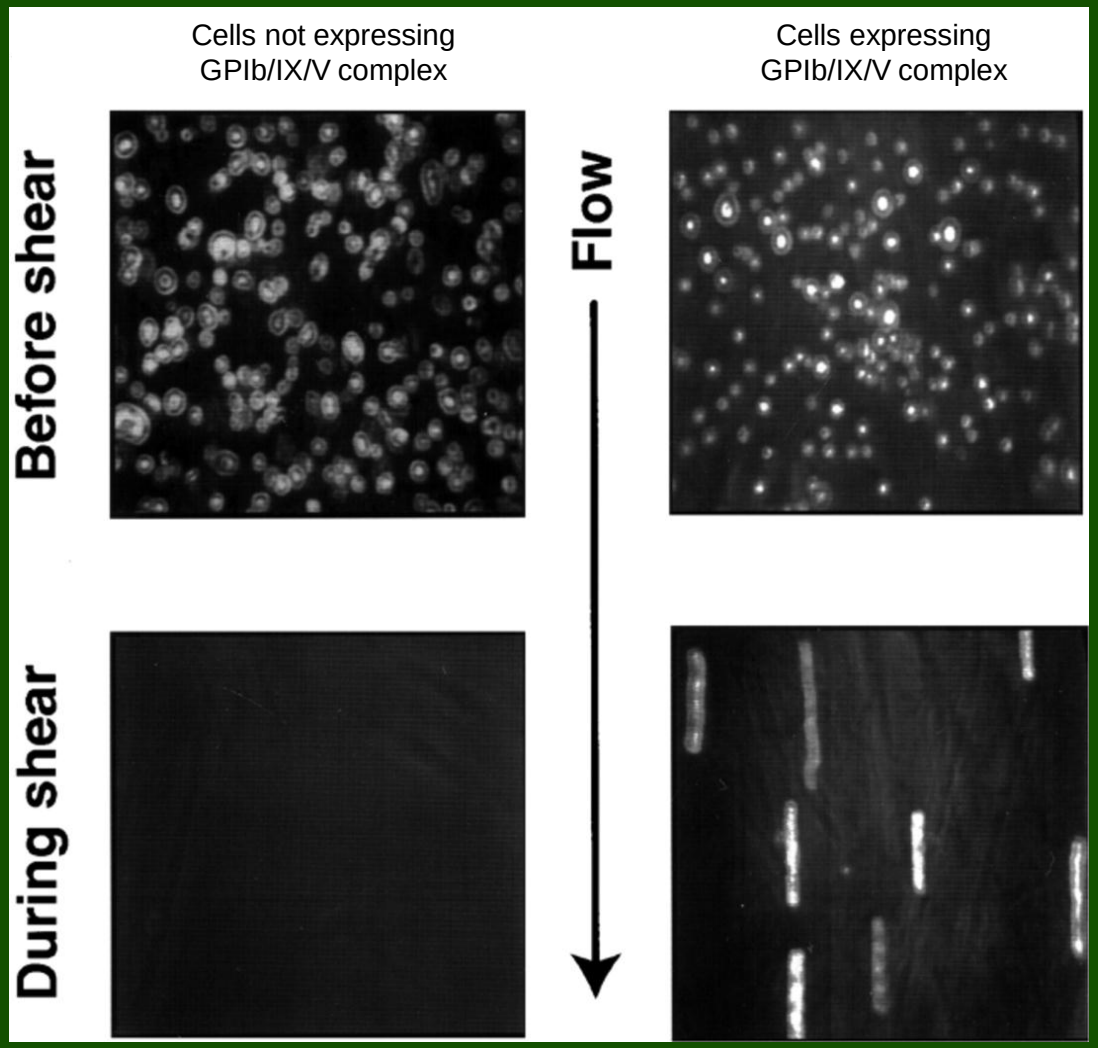
Non activated PLT – non activated EC
Non activated PLT – activated EC
Activated PLT – non activated EC
Activated PLT – activated EC



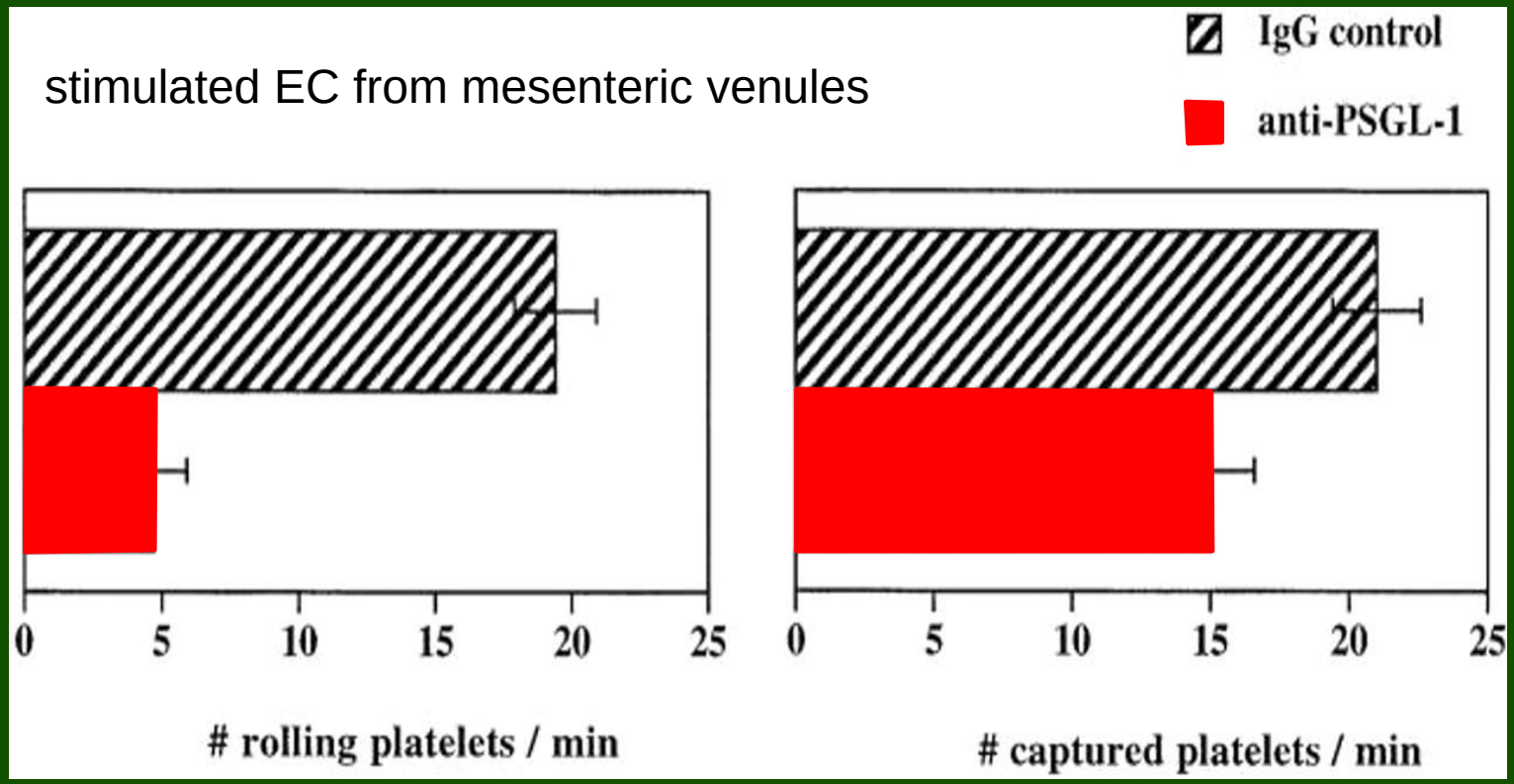
We know that activated ECs express P-selectin.

How do PLTs roll on activated ECs ? (P-selectin ligands)

GPIb/IX/V complex as PLT ligand to EC P-selectin

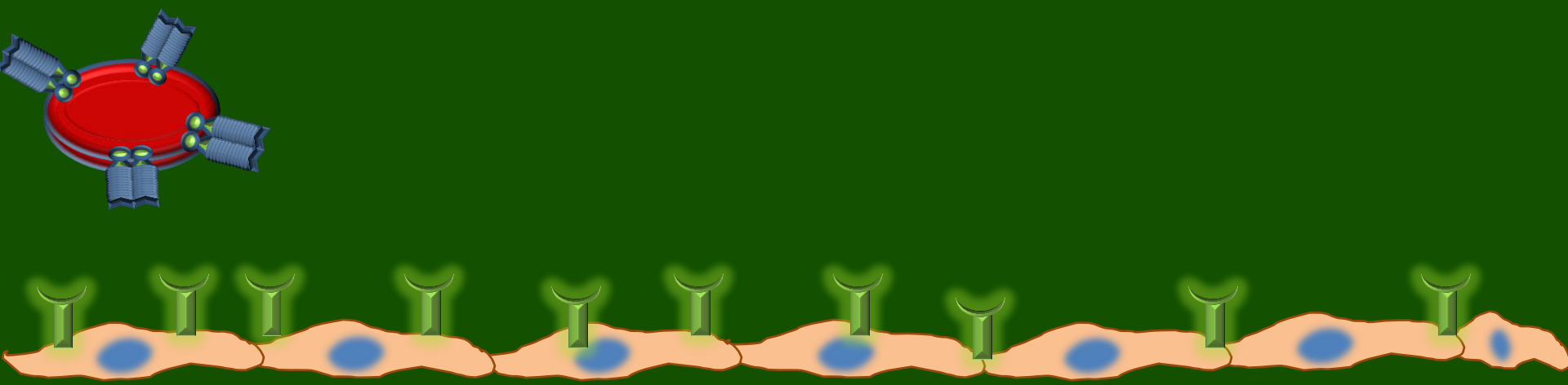


P selectin glycoprotein ligand1 (PSGL-1) as PLT ligand to EC P-selectin



Frenette PS, J Exp Med, 2000

P selectin – PSGL-1 or GPIb/IX/V lingation insuficient for stable adhesion



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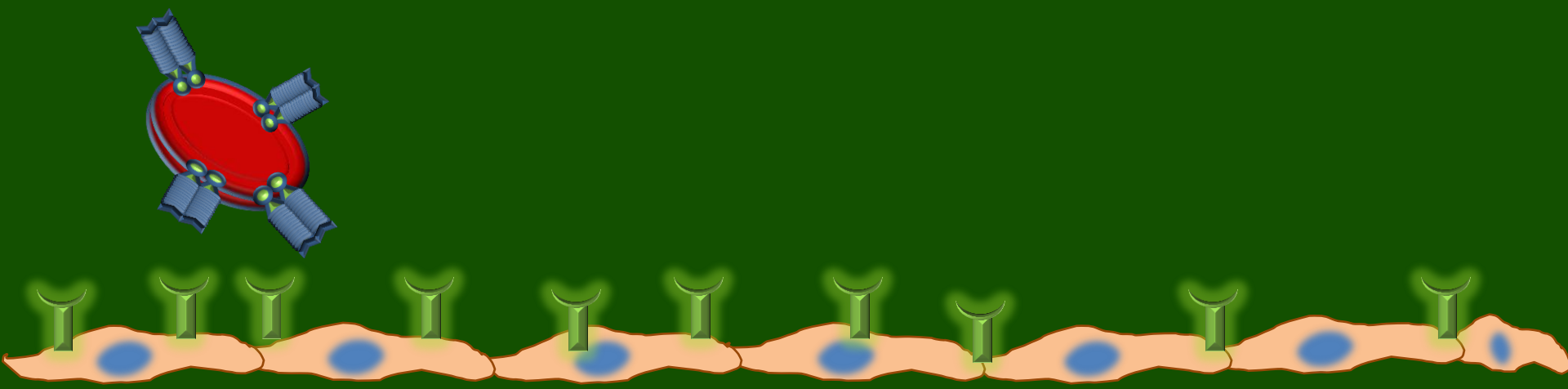

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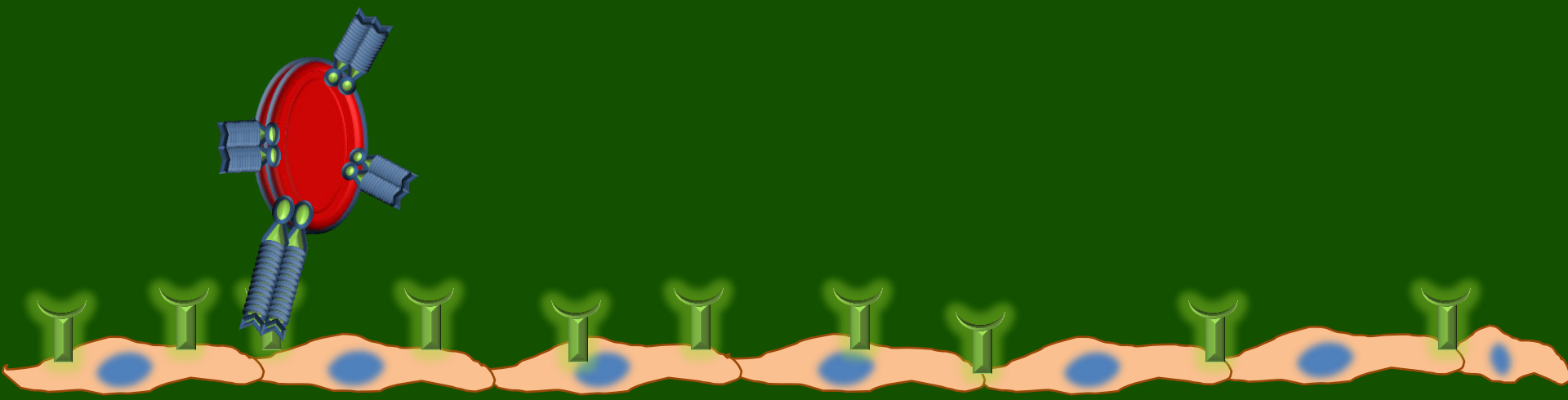

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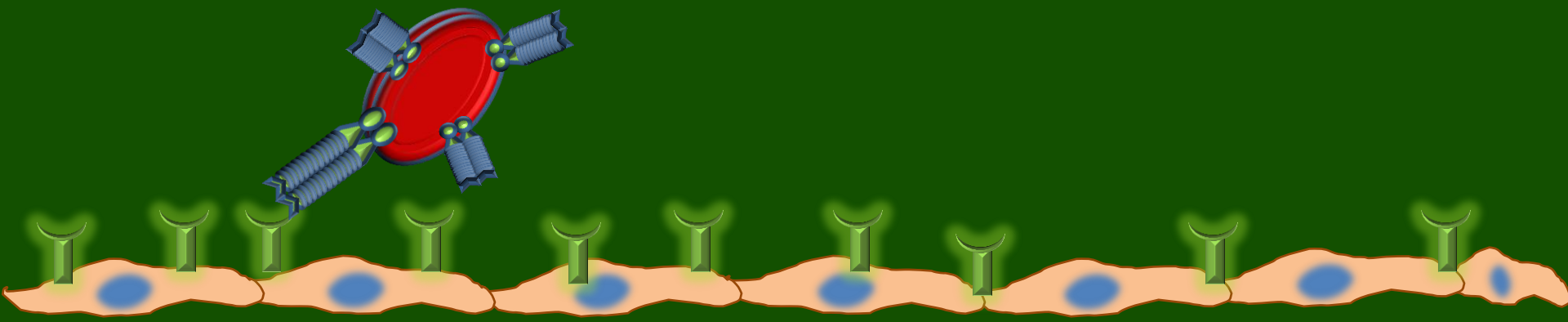

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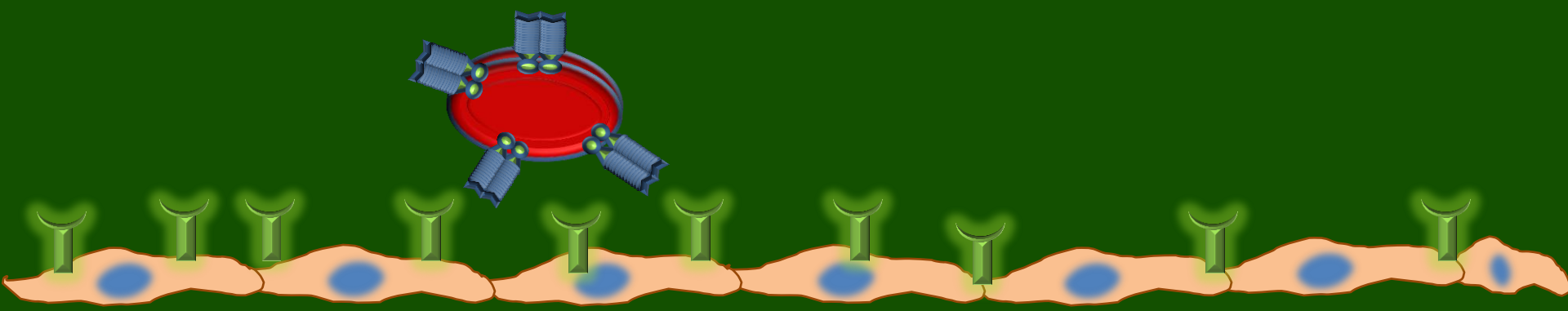

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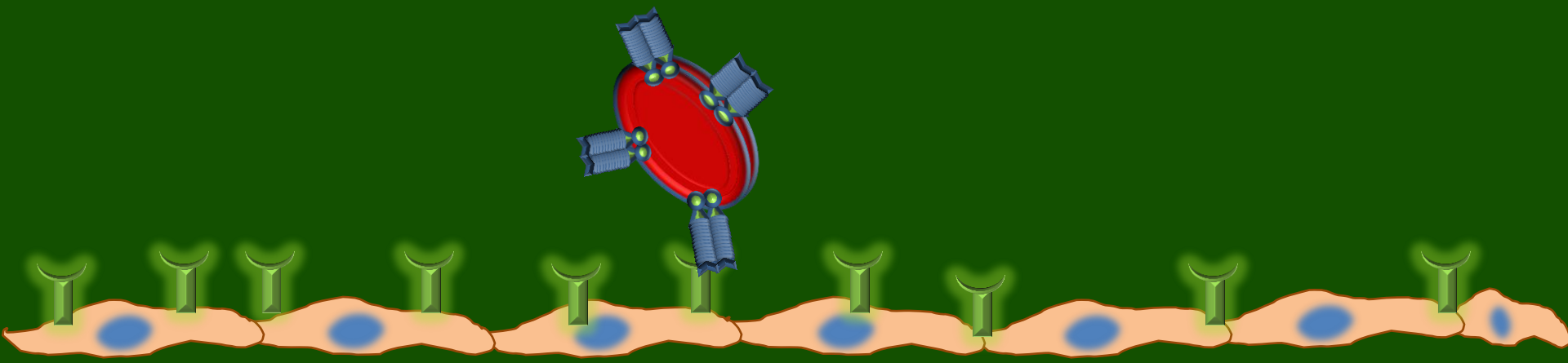

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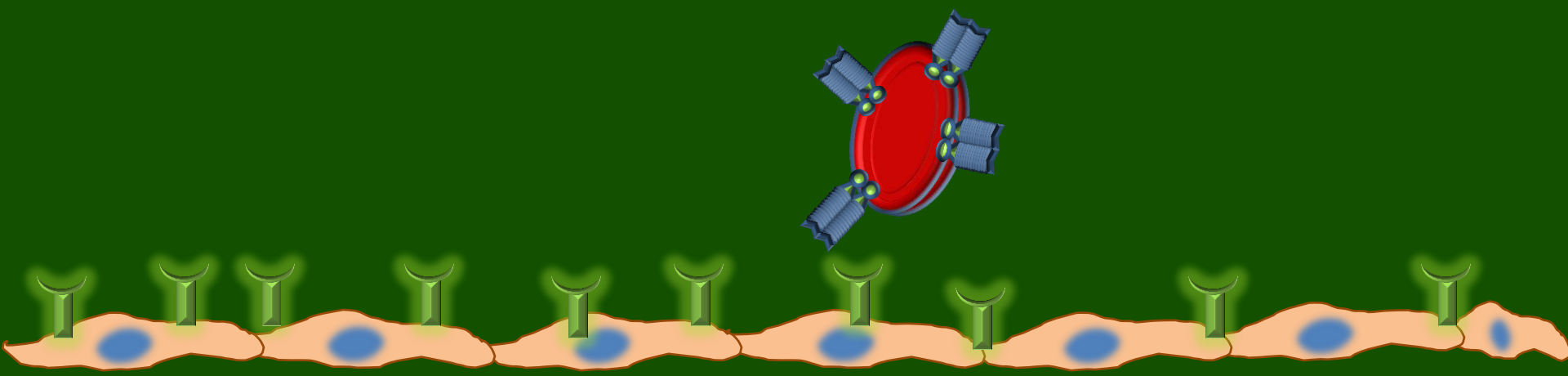

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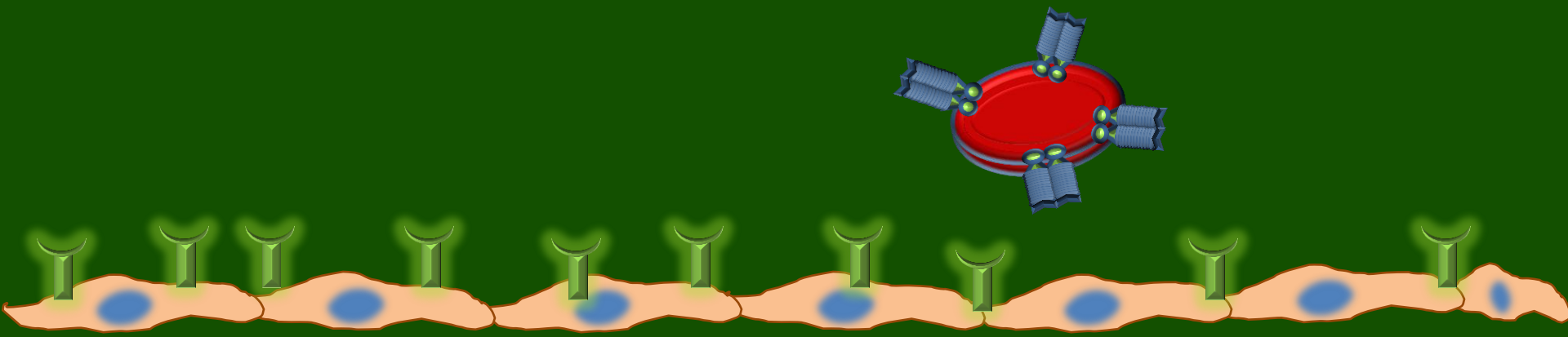

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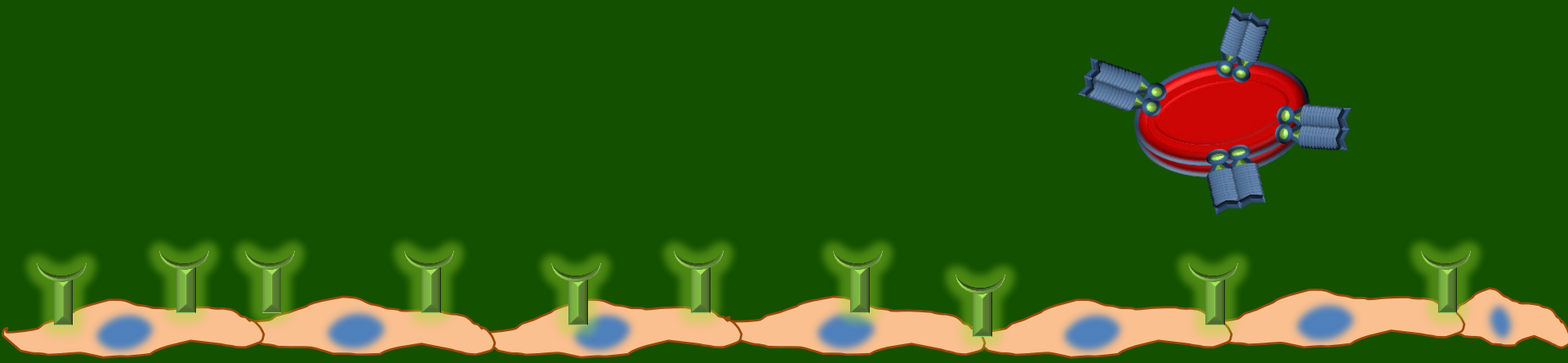

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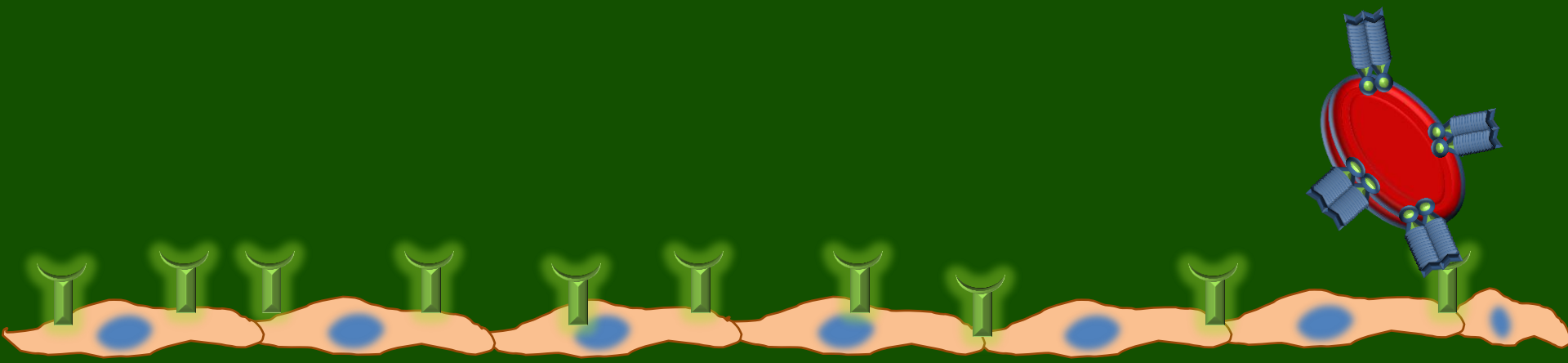

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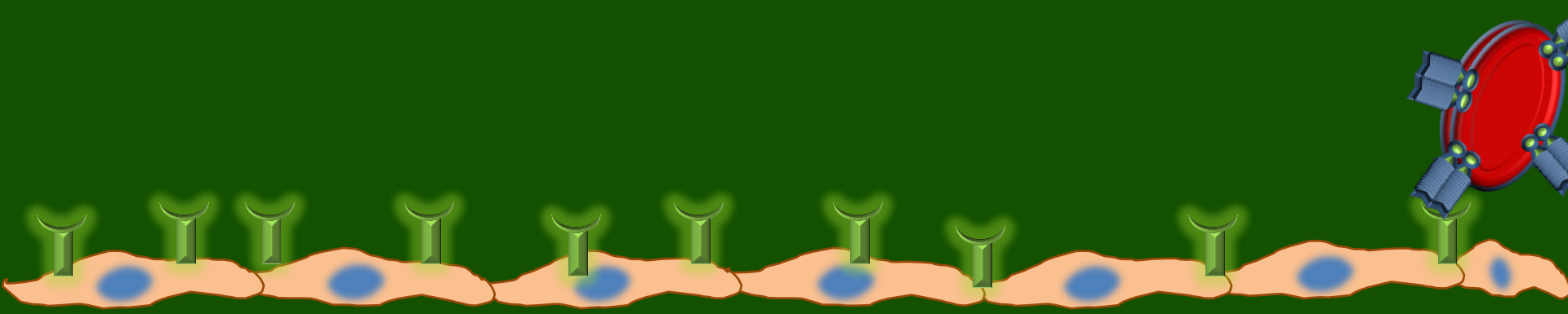
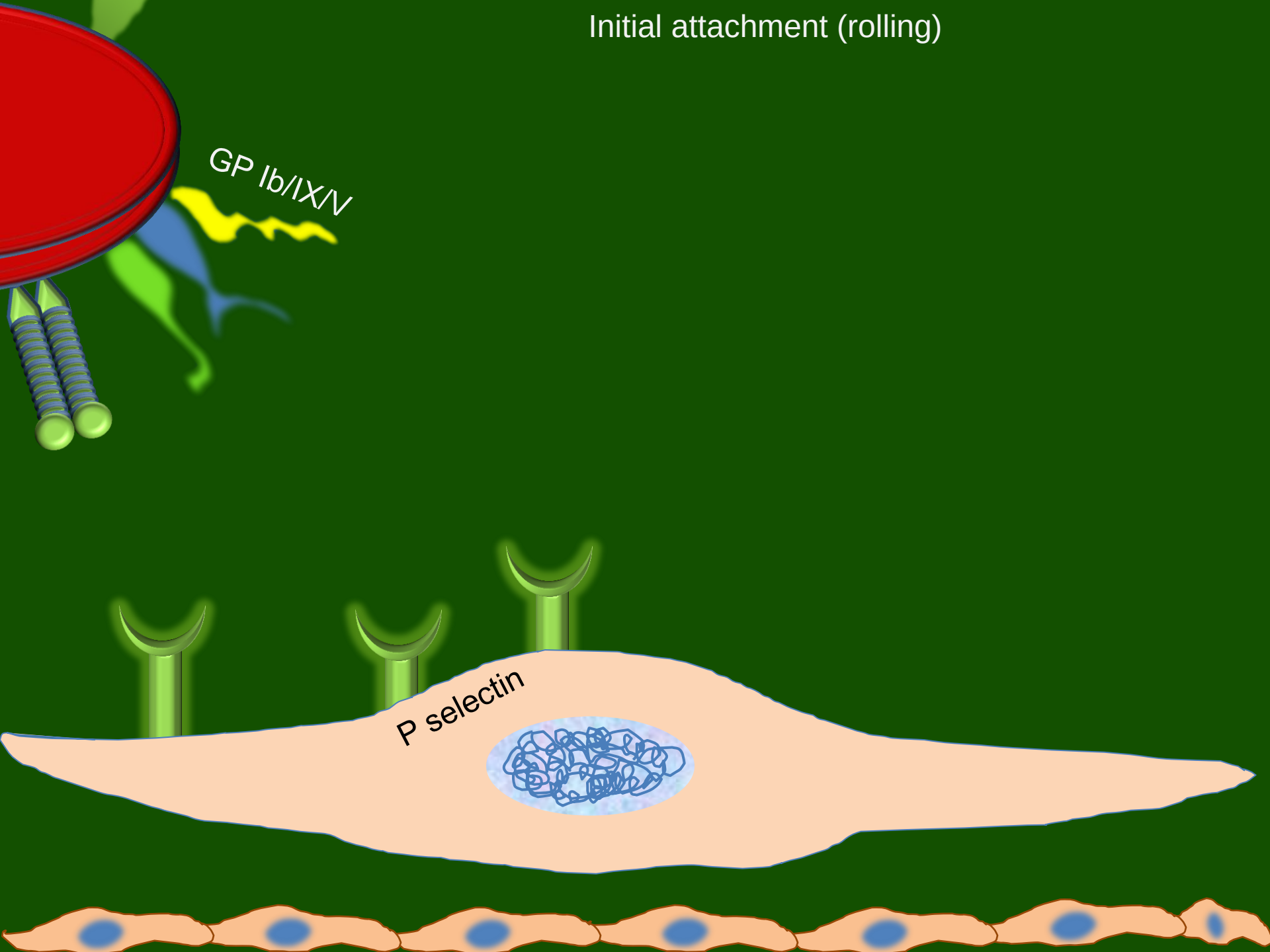
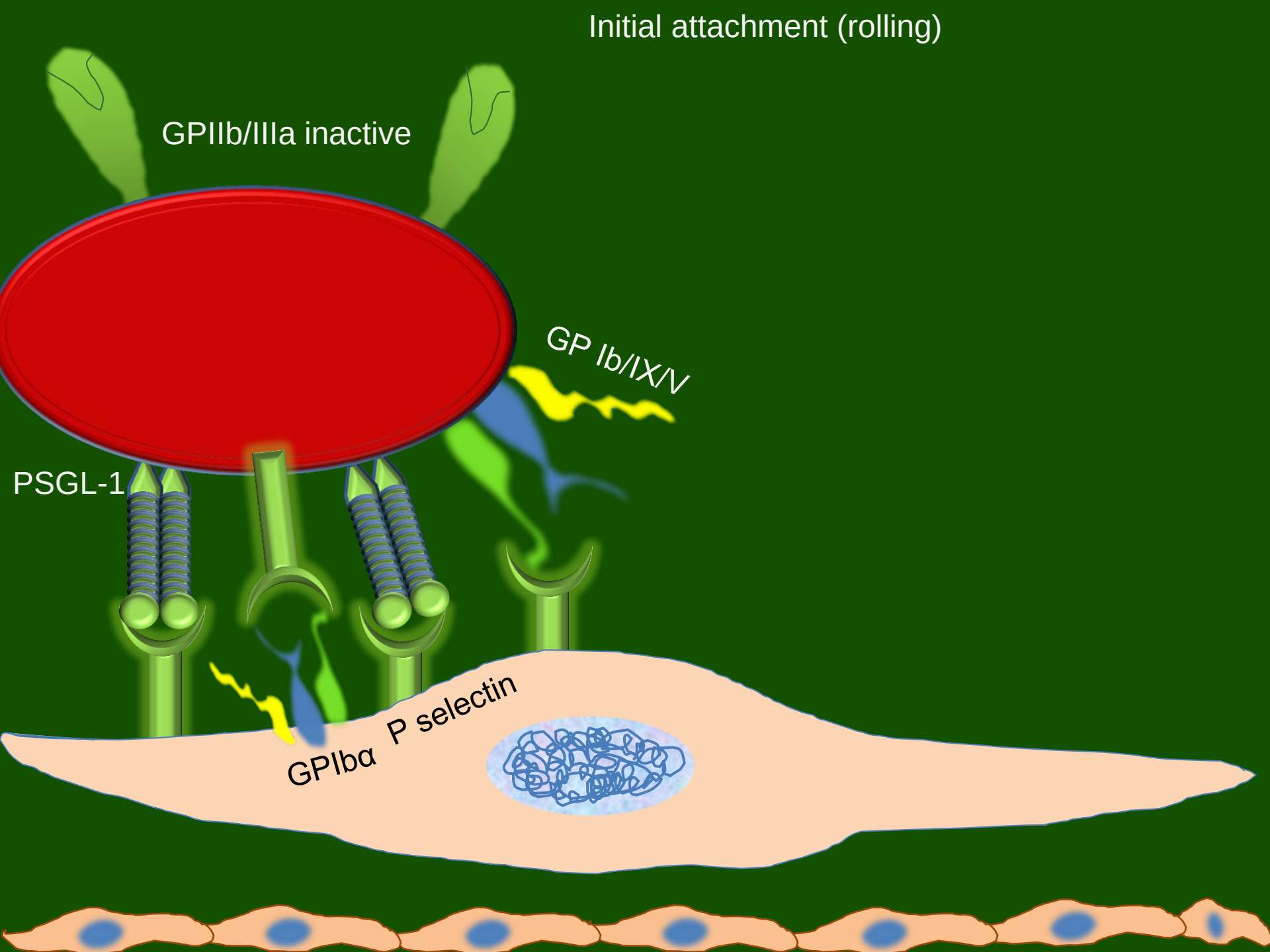
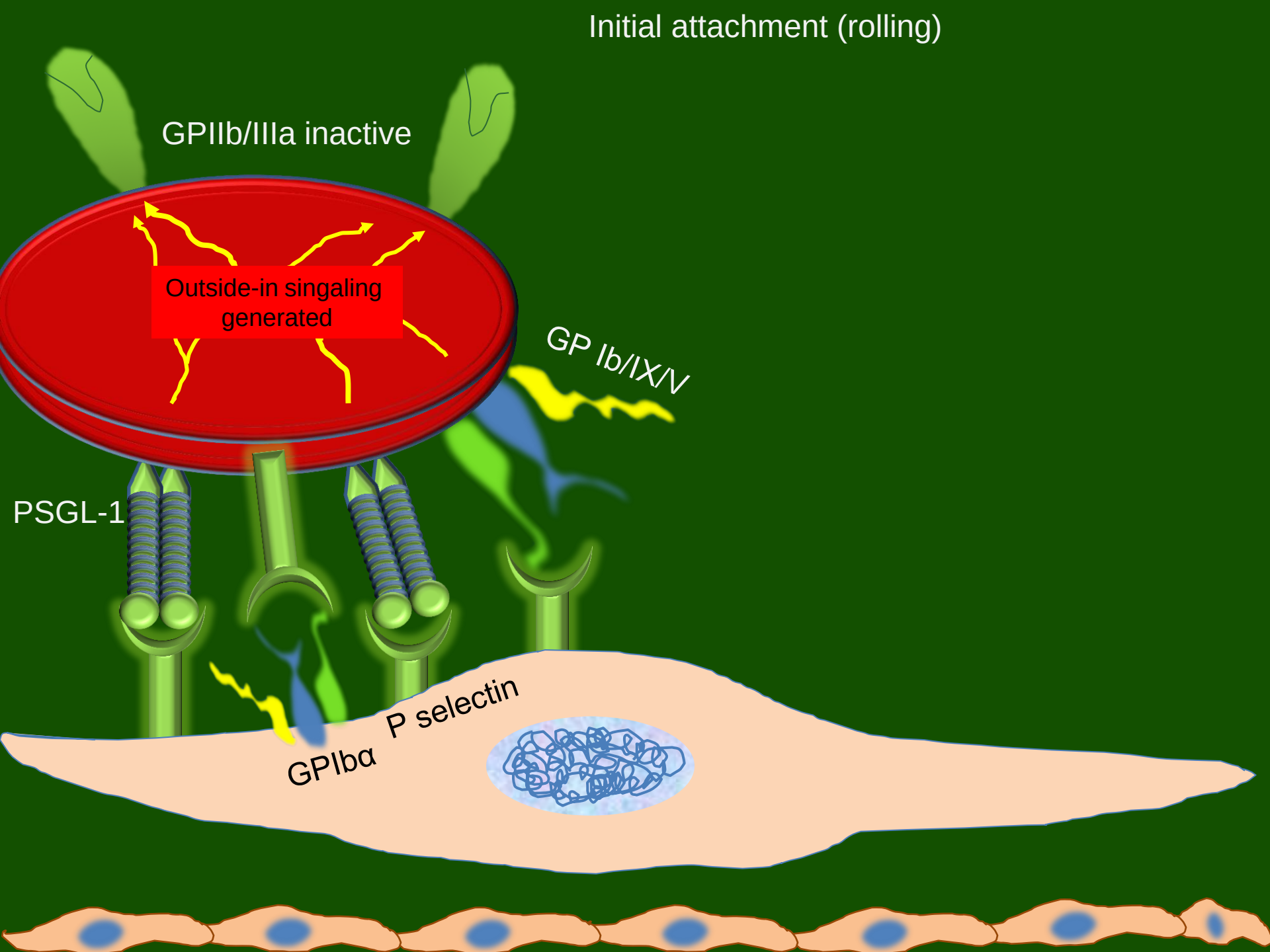
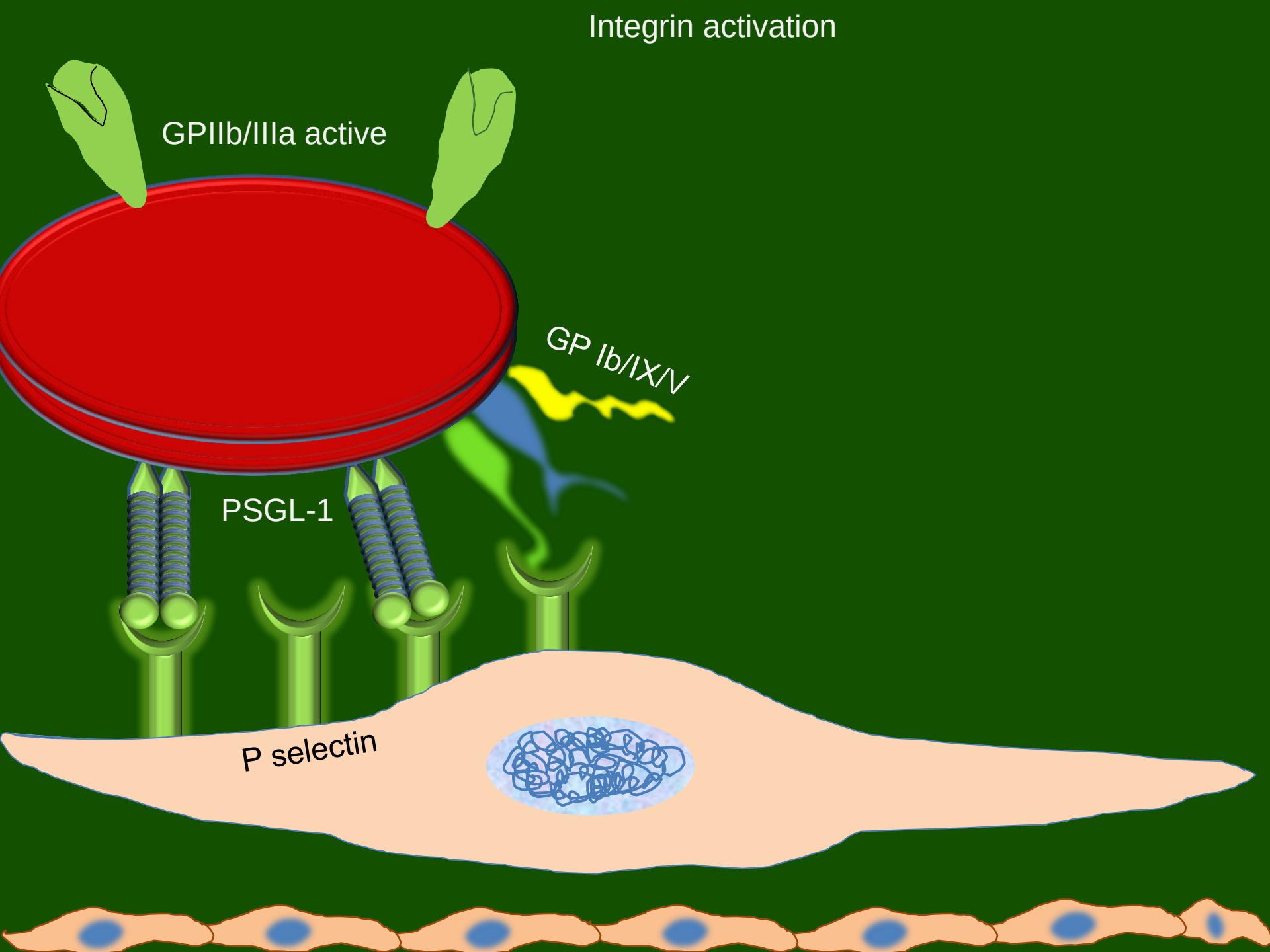
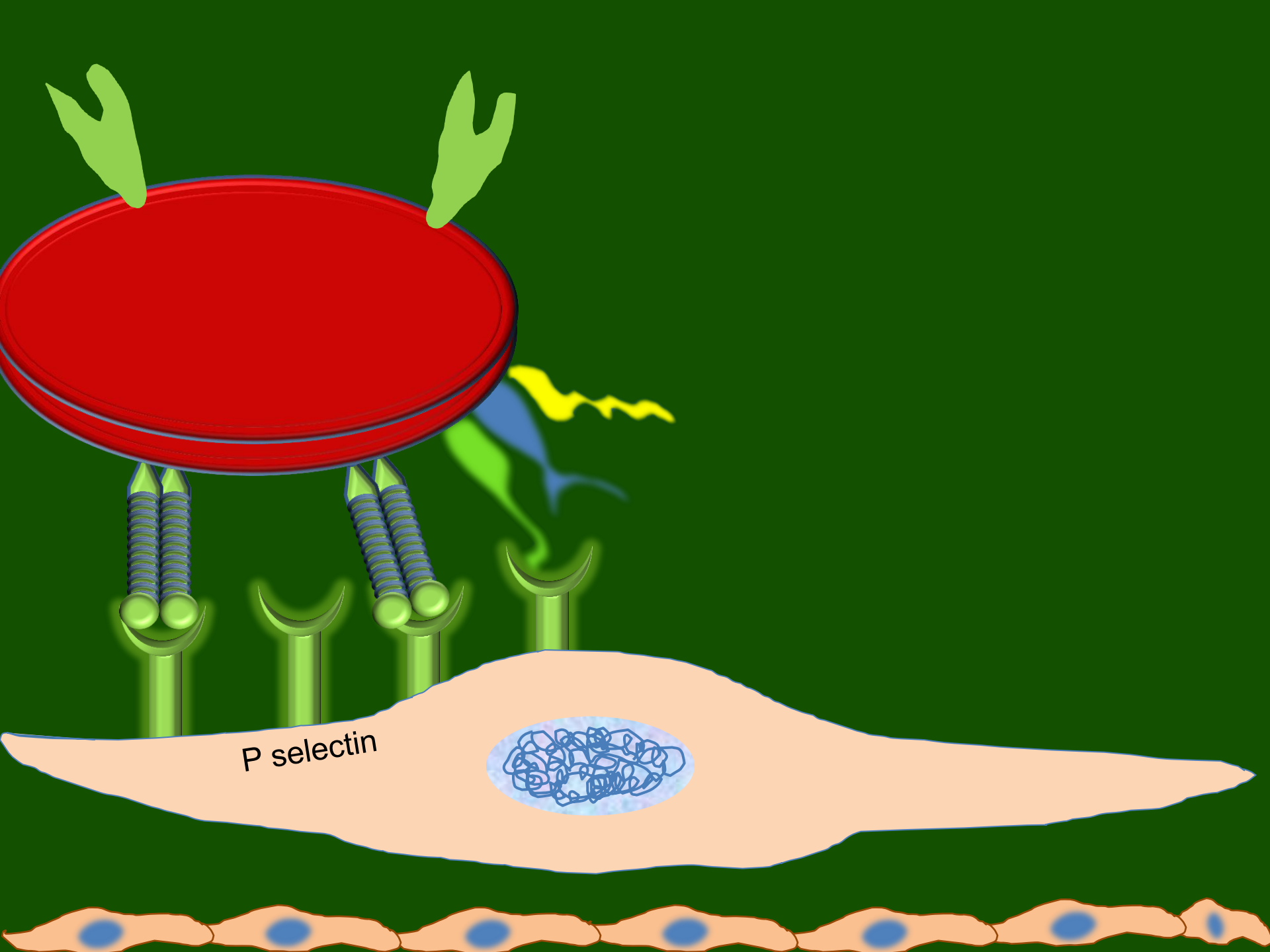



Initial attachment (rolling)
GP Ib/IX/V
P selectin


Initial attachment (rolling)
GPIIb/IIIa inactive
GP Ib/IX/V
PSGL-1
GPIbα
P selectin


Initial attachment (rolling)
GPIIb/IIIa inactive
Outside-in singaling generated
GP Ib/IX/V
PSGL-1
GPIbα
P selectin


Integrin activation
GPIIb/IIIa active
GP Ib/IX/V
PSGL-1
P selectin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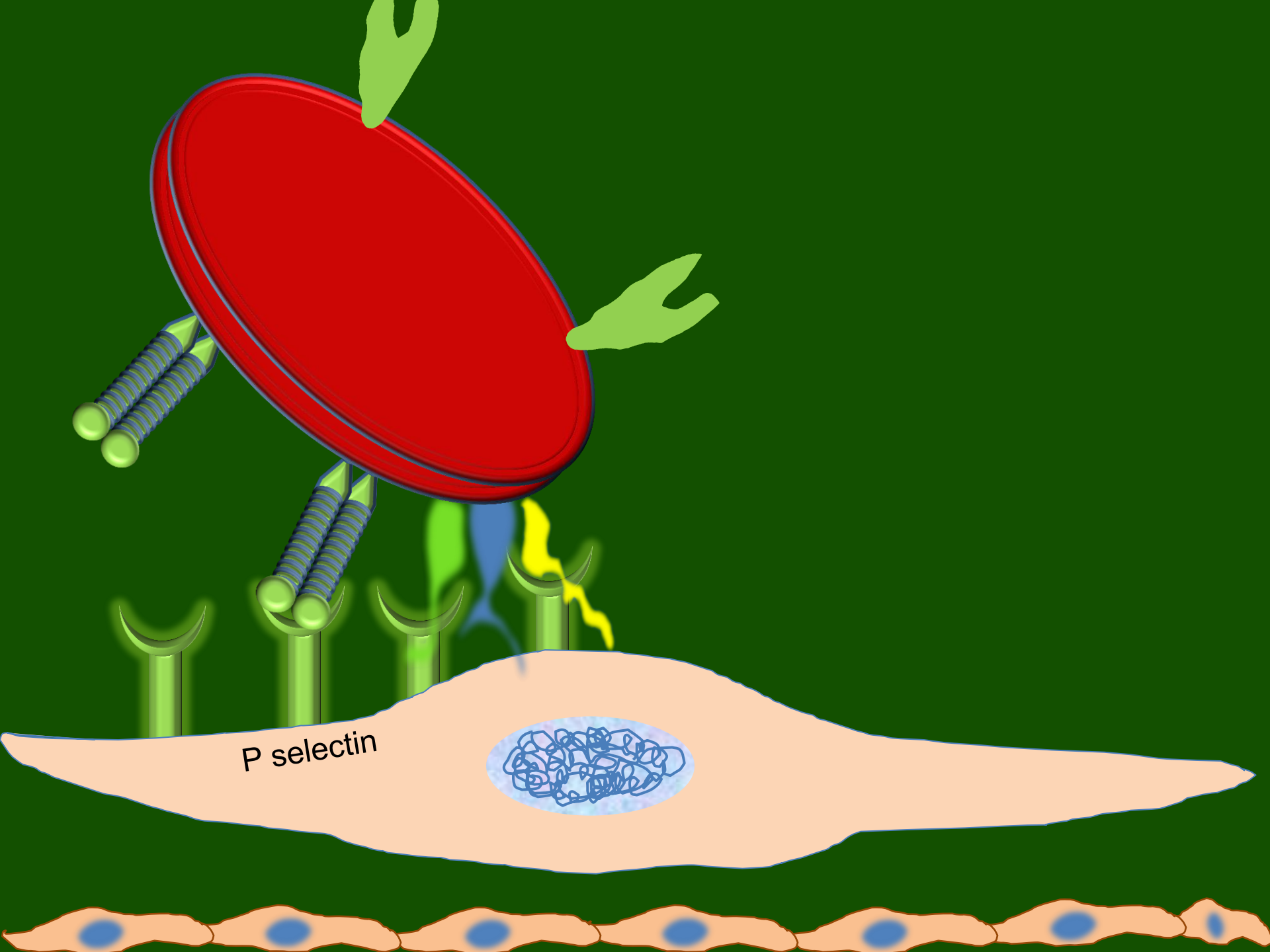
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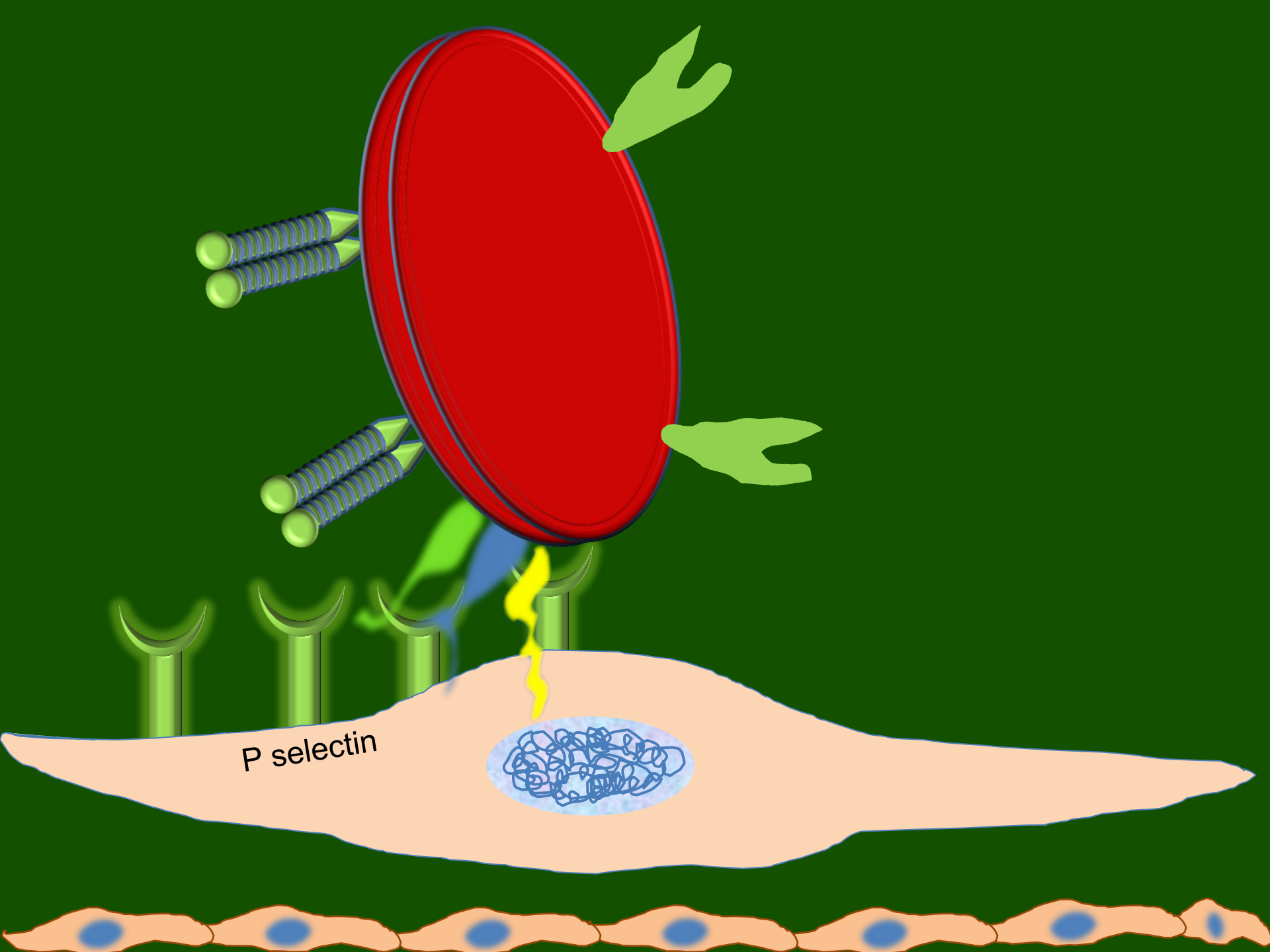
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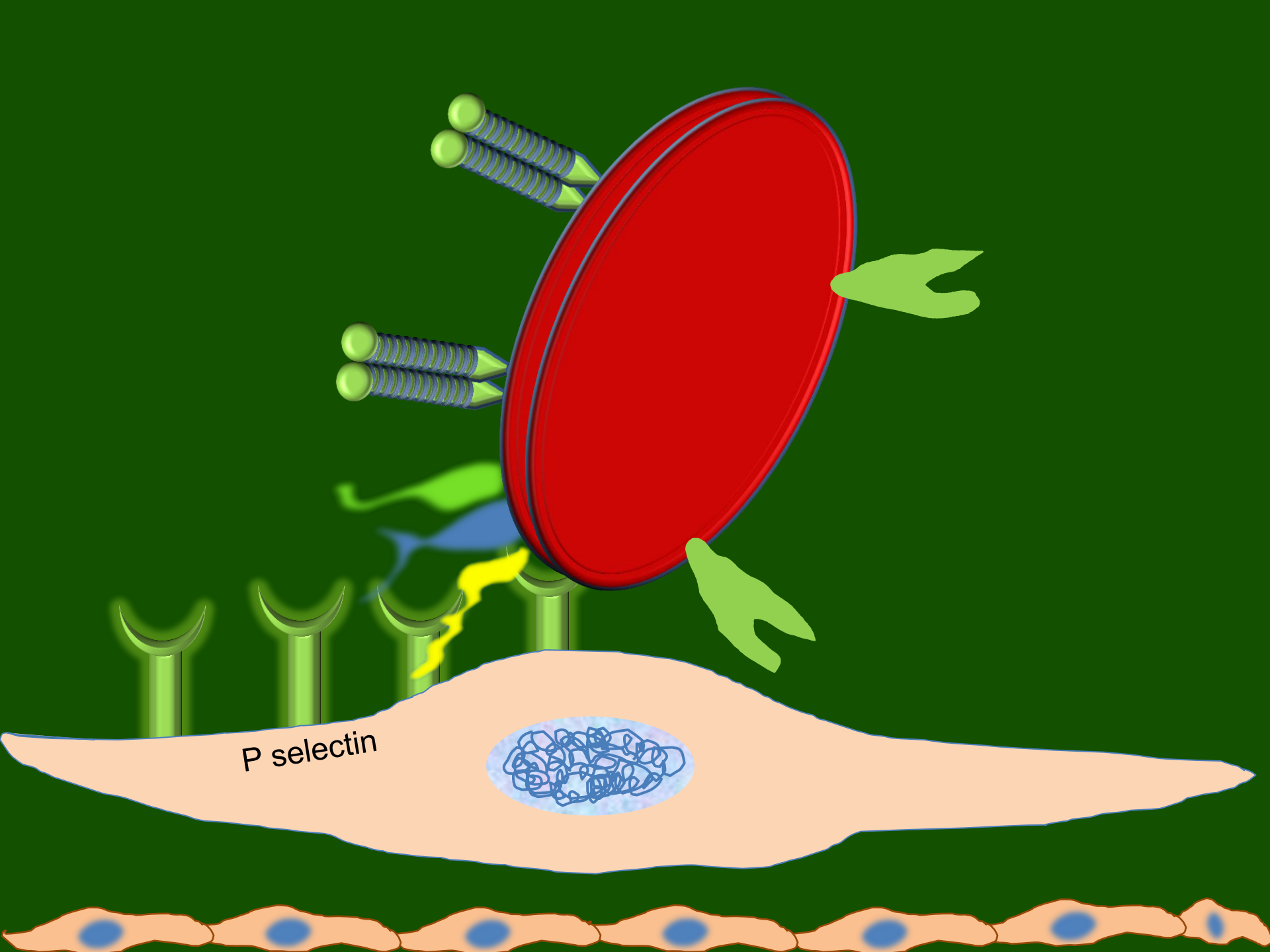
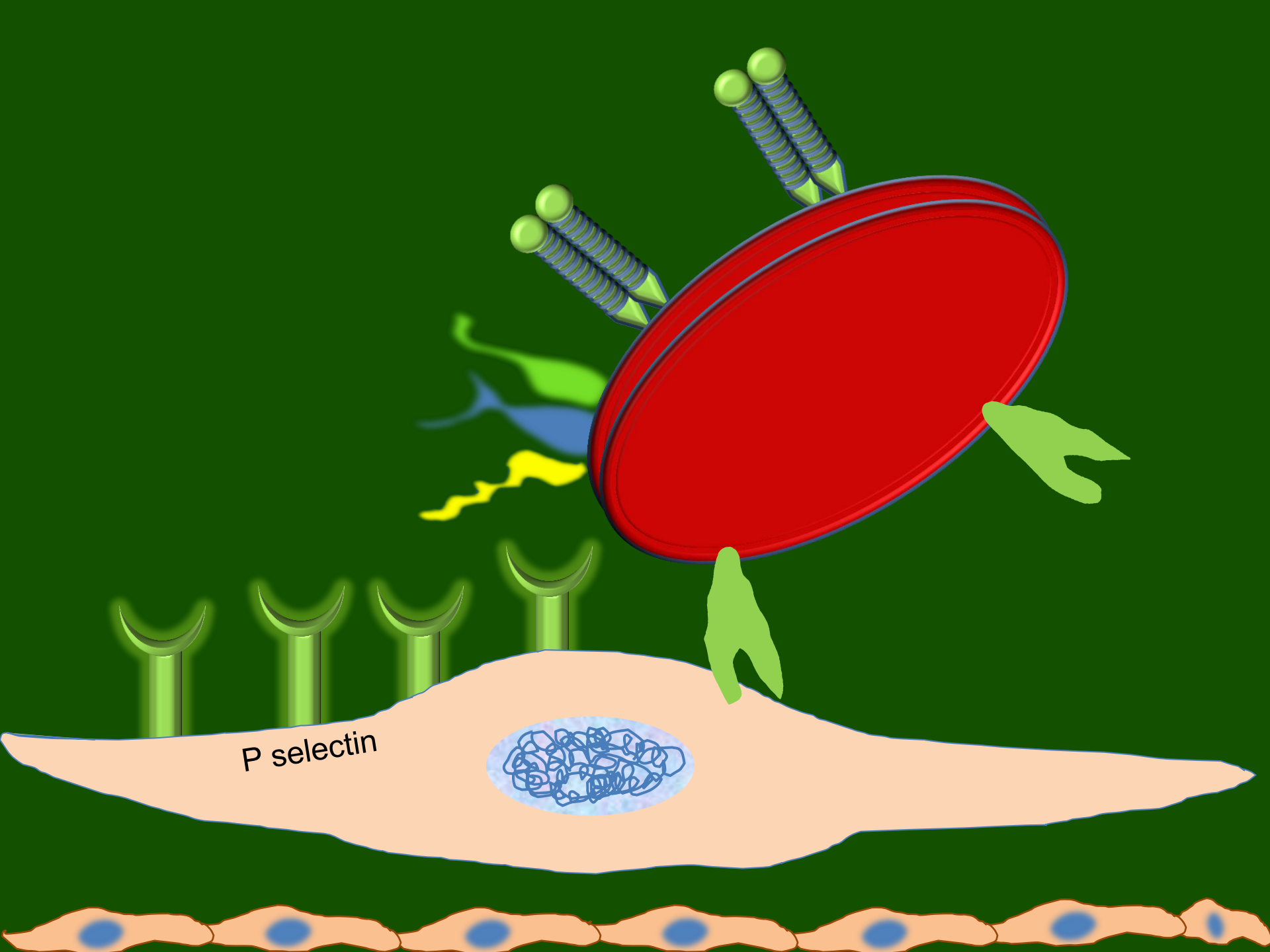

P selectin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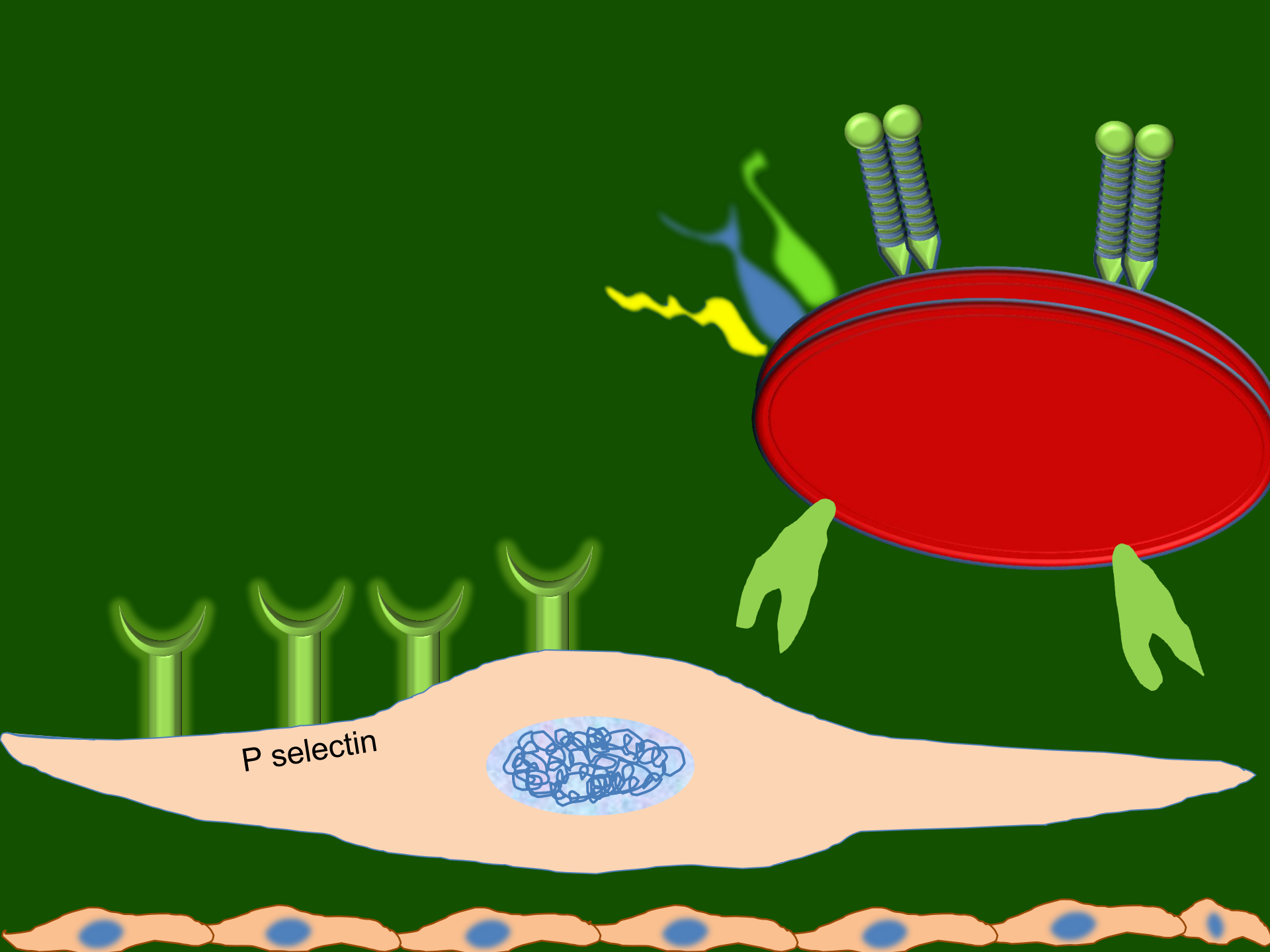
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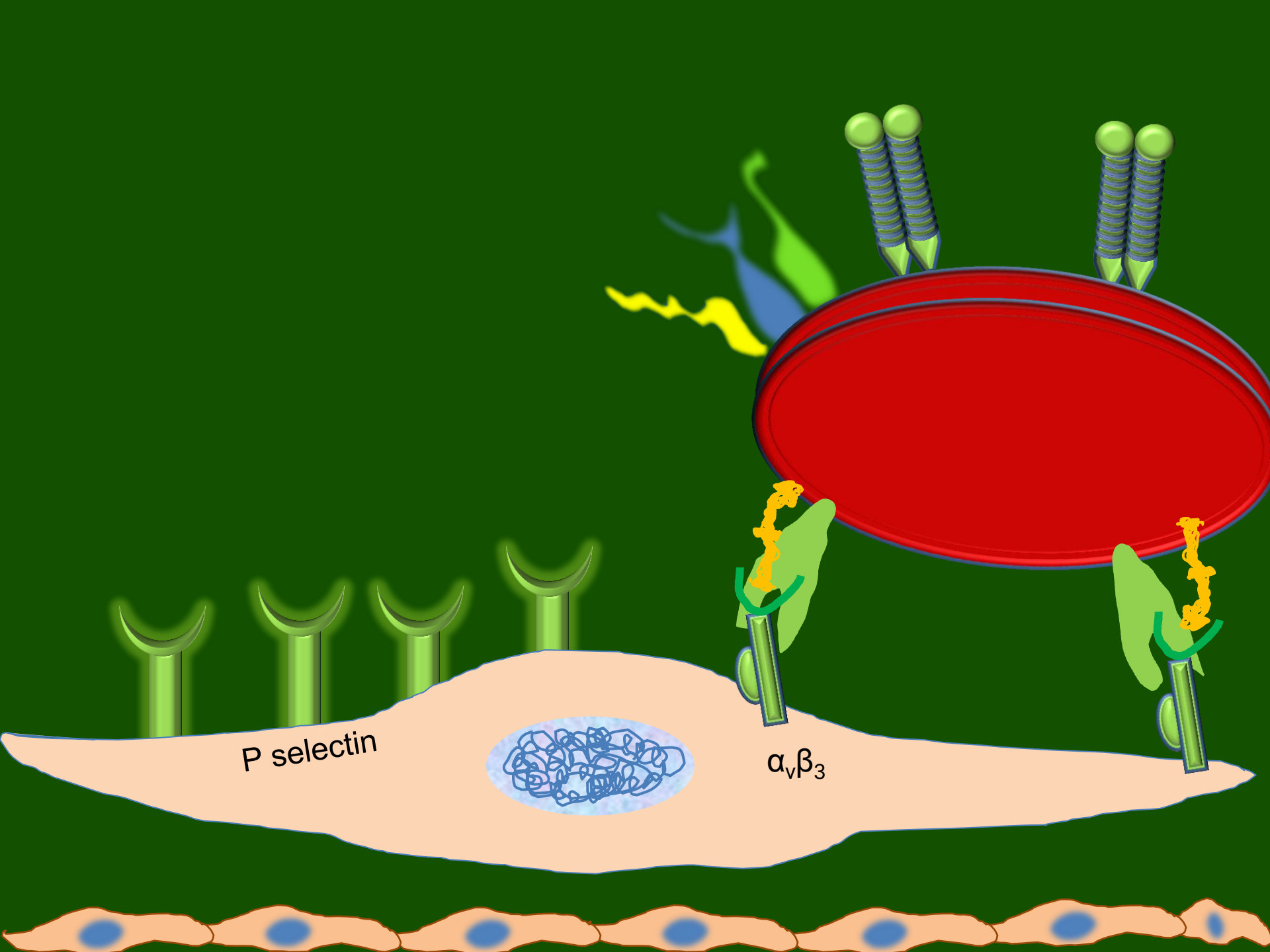
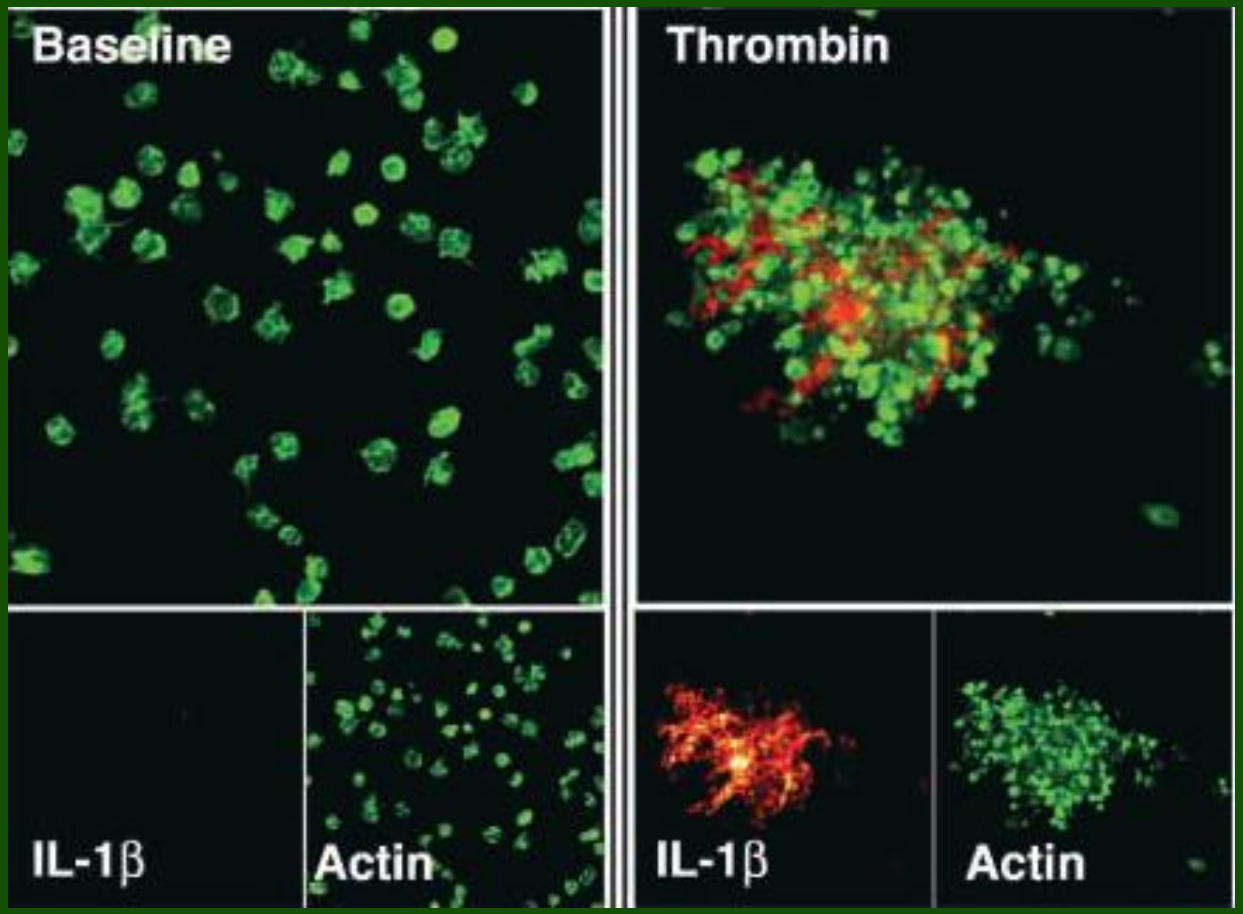

P selectin
$\alpha_v\beta_3$

PLT contact with endothelium activates ECs

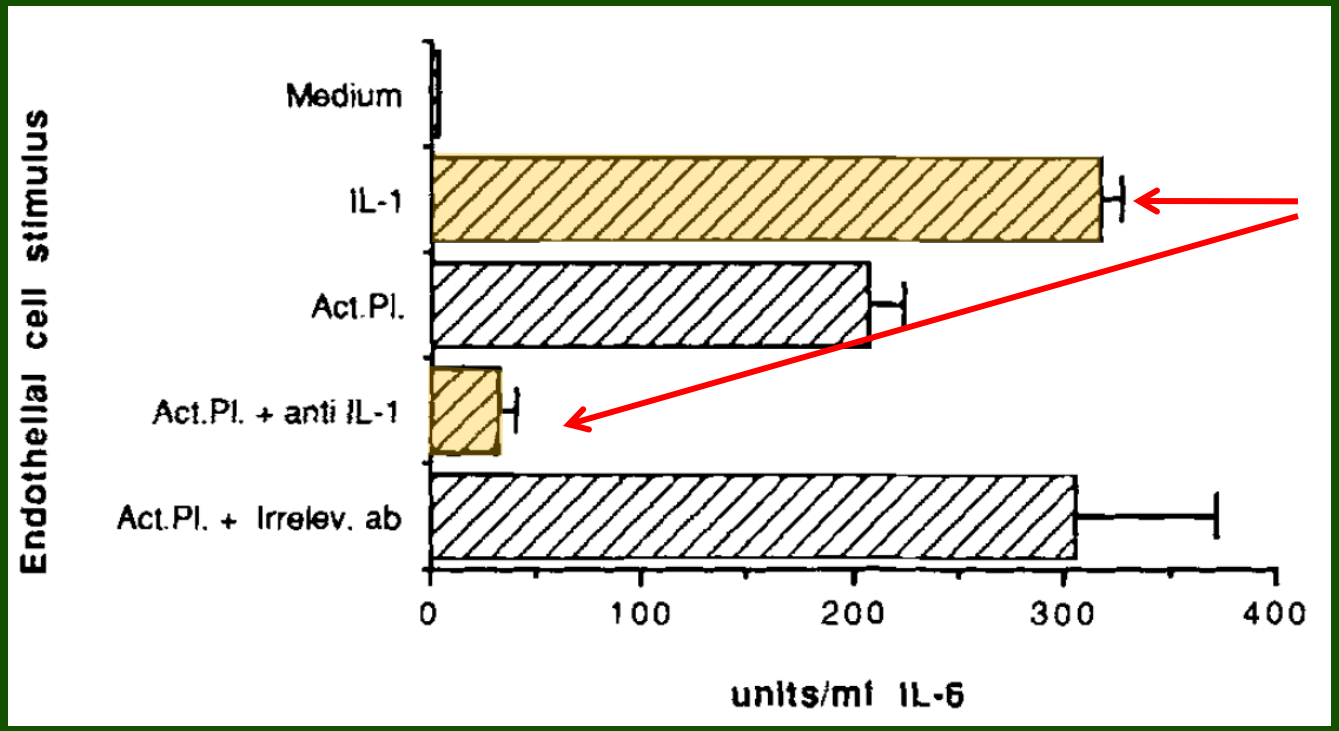
How do Platelets activate Endothelial Cells ?

Activated platelets mediate inflammatory signaling by IL-1 synthesis



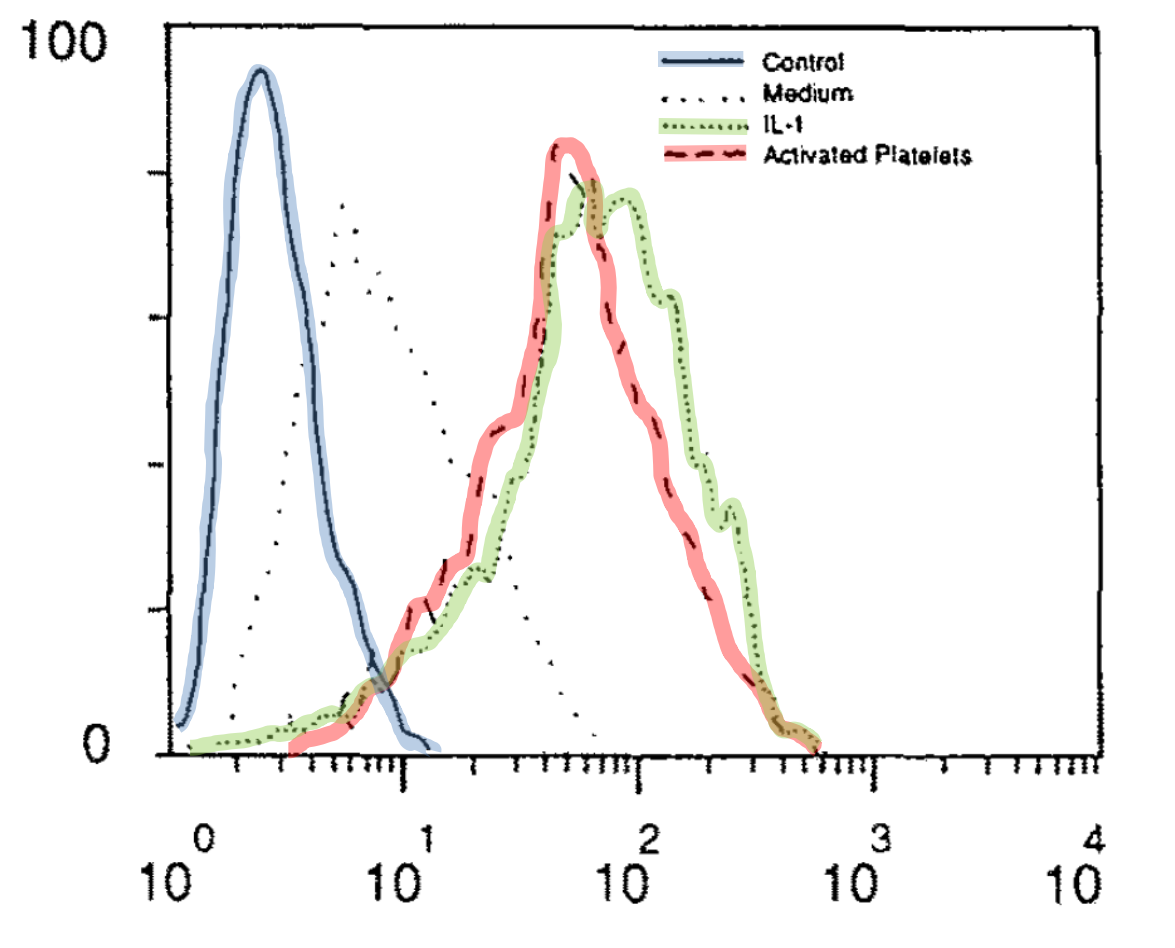
Lindemann S, *J Cell Biol,* 2001

Platelet derived IL-1 stimulates secretion of IL-6 by cultured endothelial cells



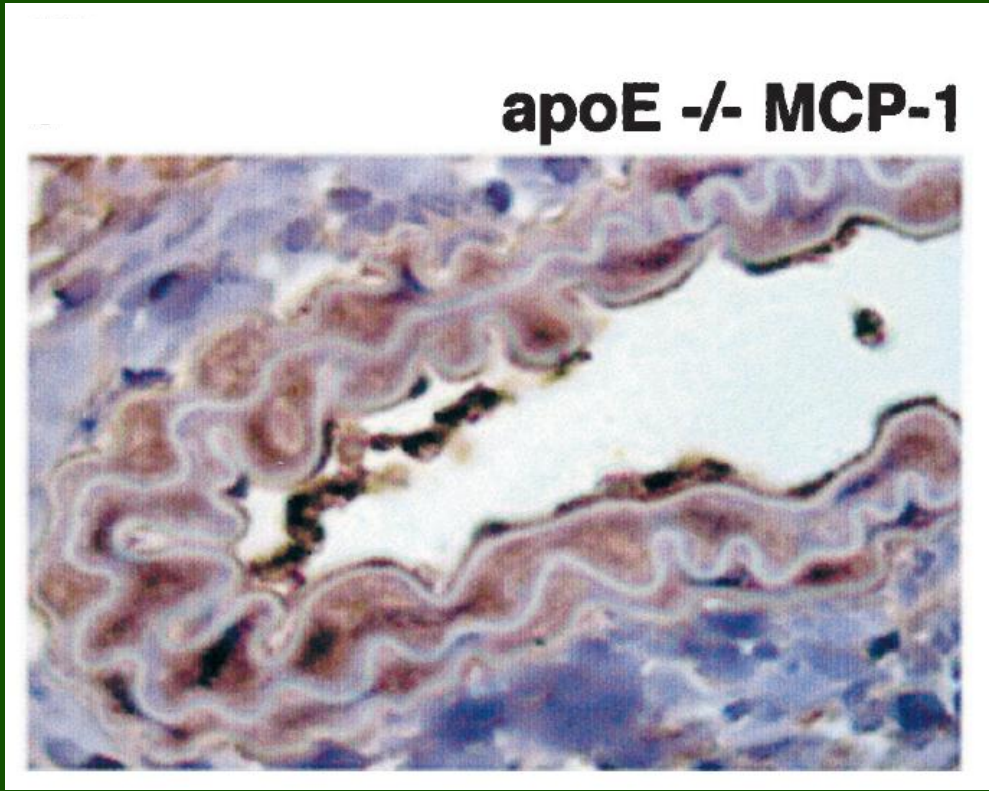
Hawrylowicz C, J Exp Med, 1991

IL-1 mediated ICAM-1 expression by endothelial cells

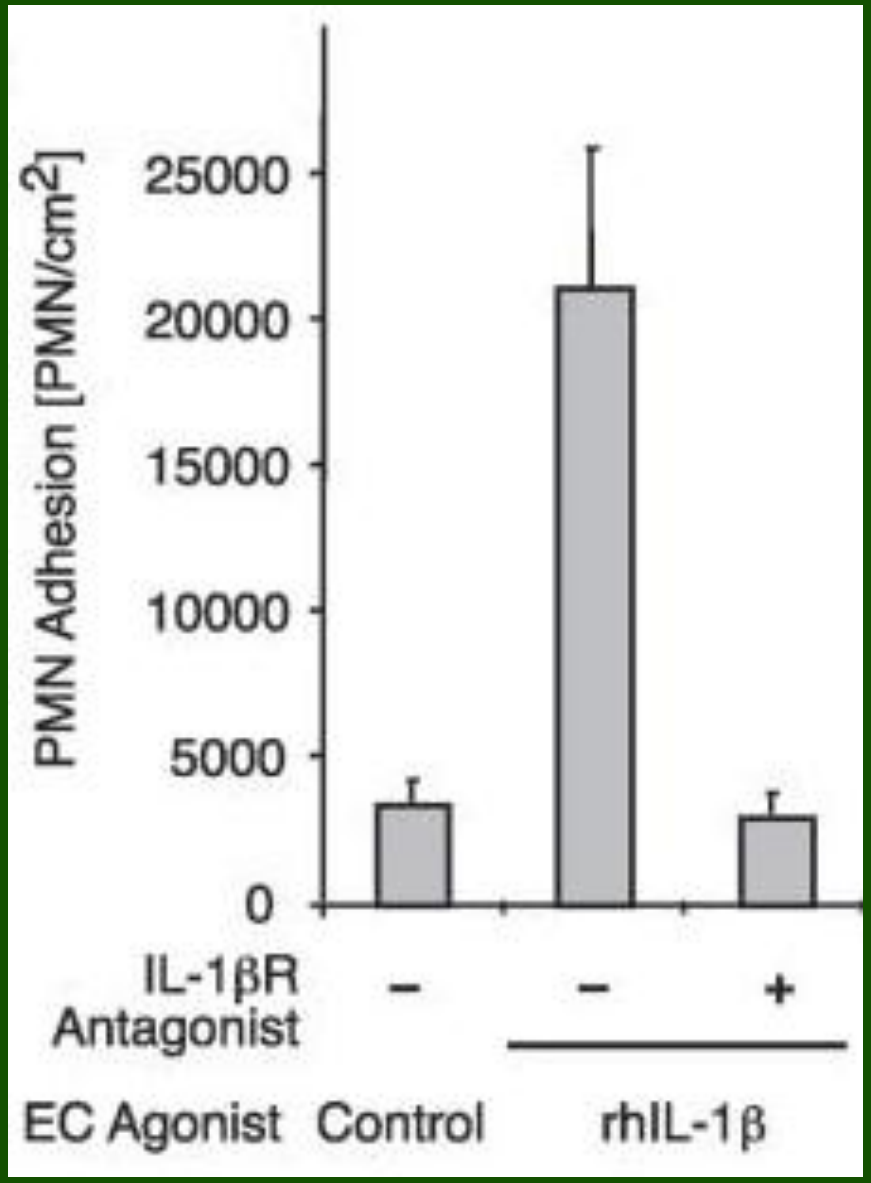


Hawrylowicz C, J Exp Med, 1991

IL-1 mediated MCP-1 expression by endothelial cells followed by …

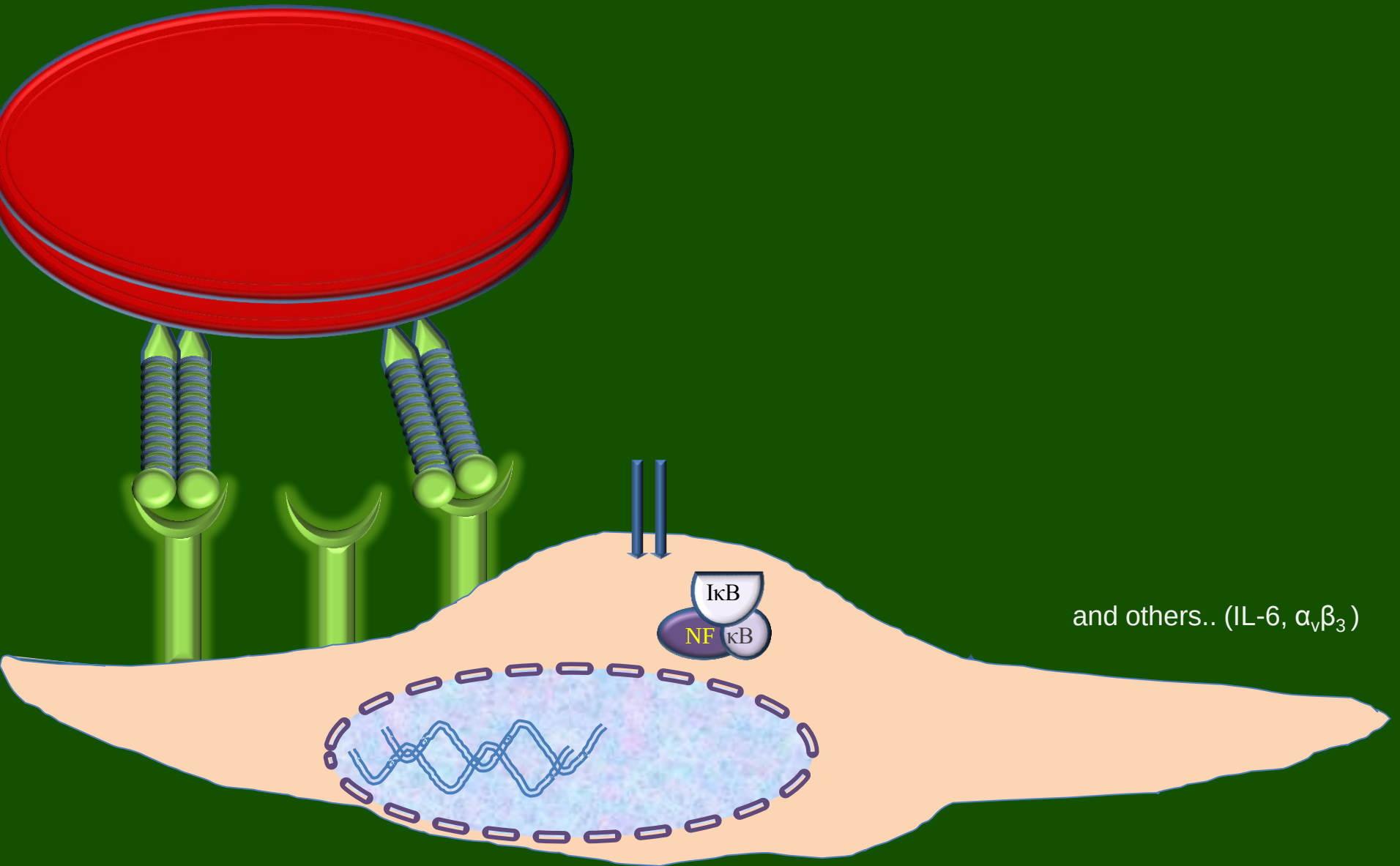


IL-1β induced Neutrophil adhesion to endothelial cells



Lindemann S, *J Cell Biol,* 2001

NF κB dependent chemokine secretion by ECs

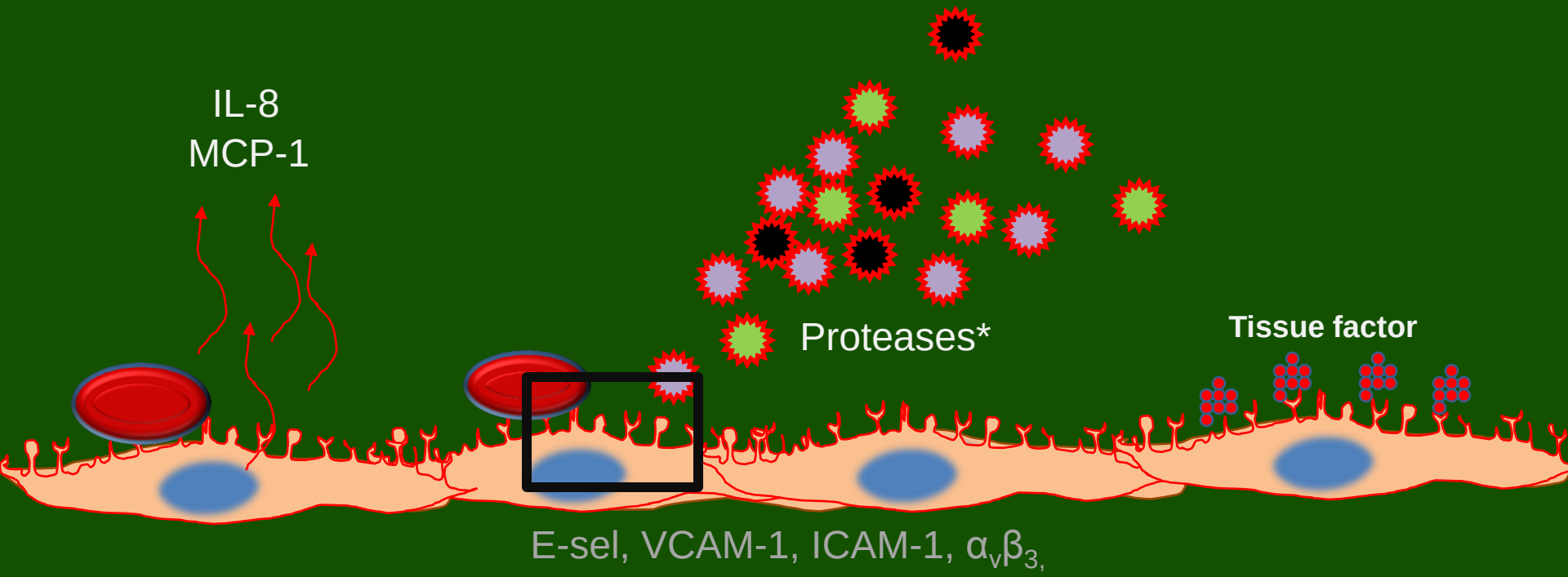


and others.. (IL-6, $\alpha_v\beta_3$)

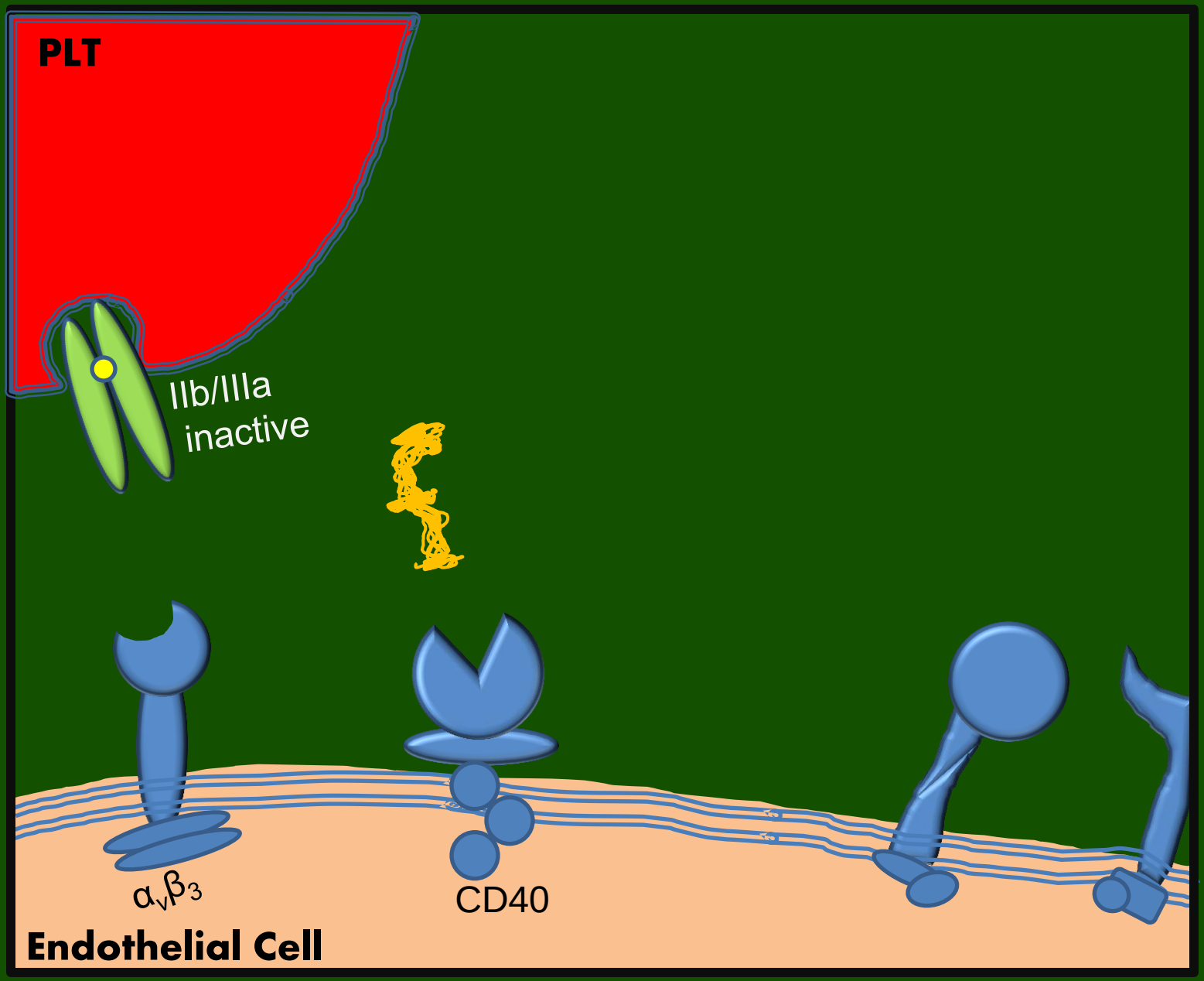
Endothelial activation after PLT – EC interaction (CD40 – CD40L)

CD40L induces the expression of adhesion molecules, cytokines and chemo-attractans, proteases and tissue factor by ligating CD40 on endothelial cells and monocytes.

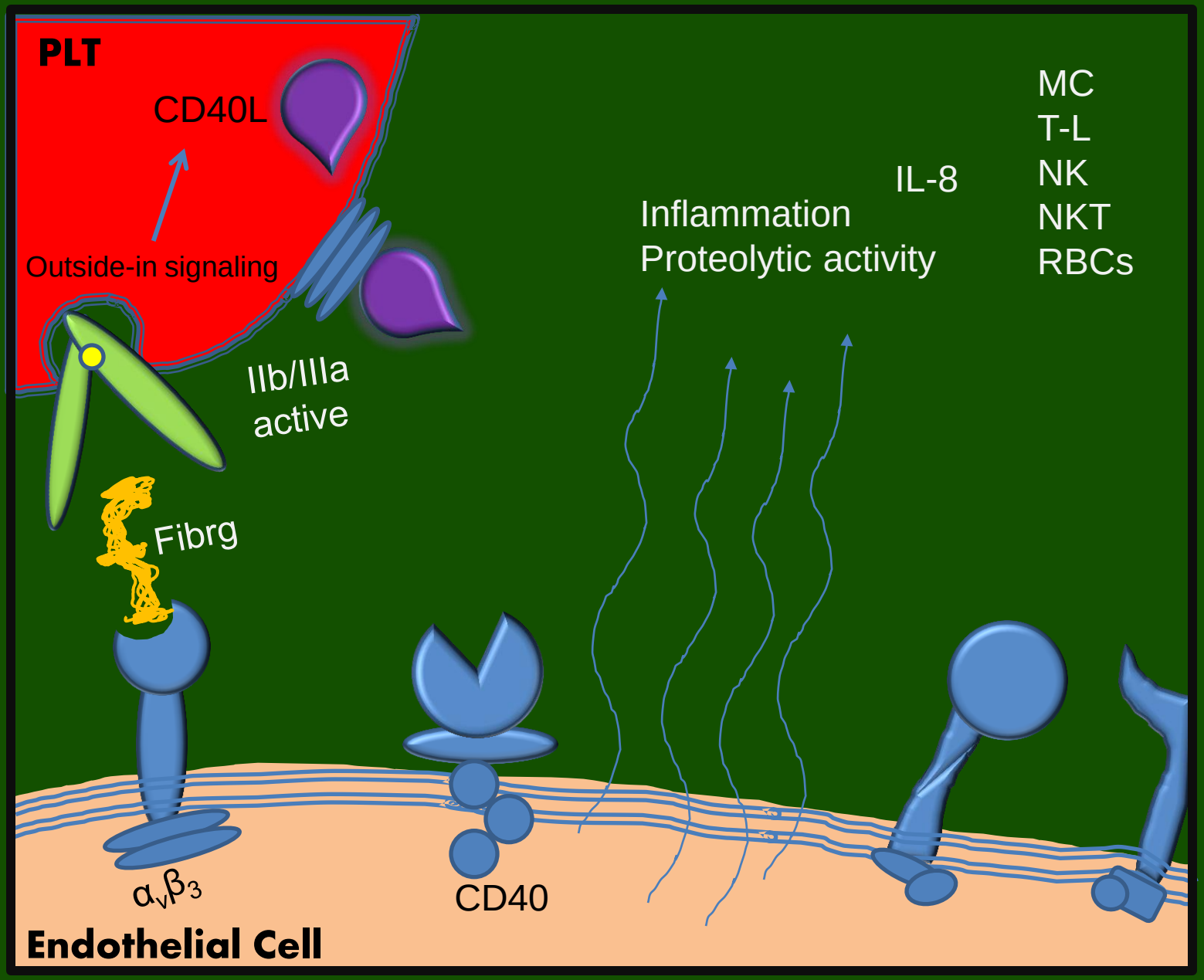
*uPA, tPA, MMP-1, MMP-2, MMP-9 and ROS-
EC proteolytic activity and local inflammation



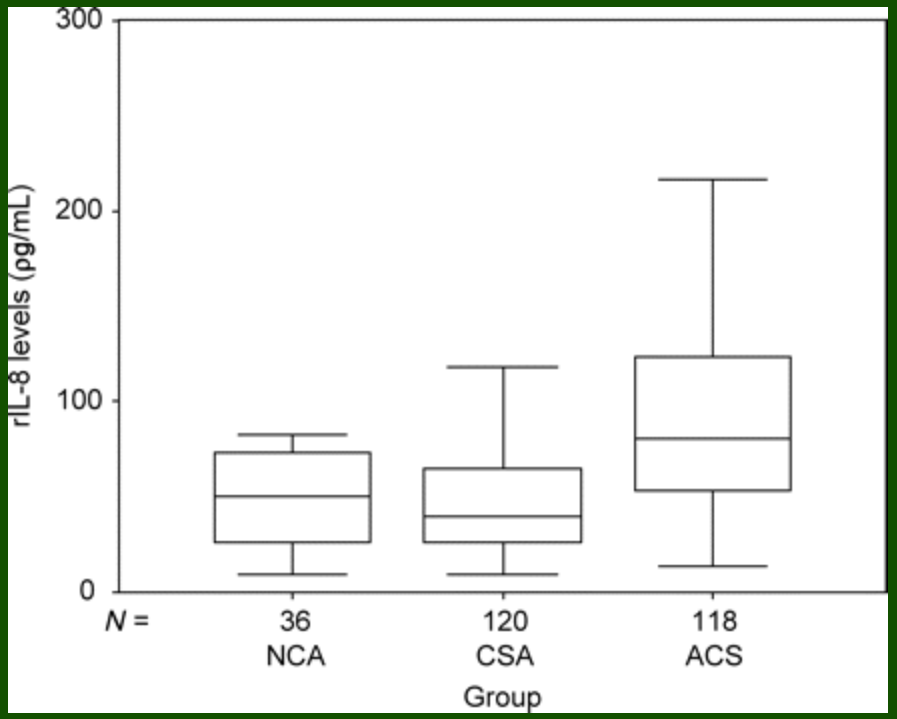
How does this work..

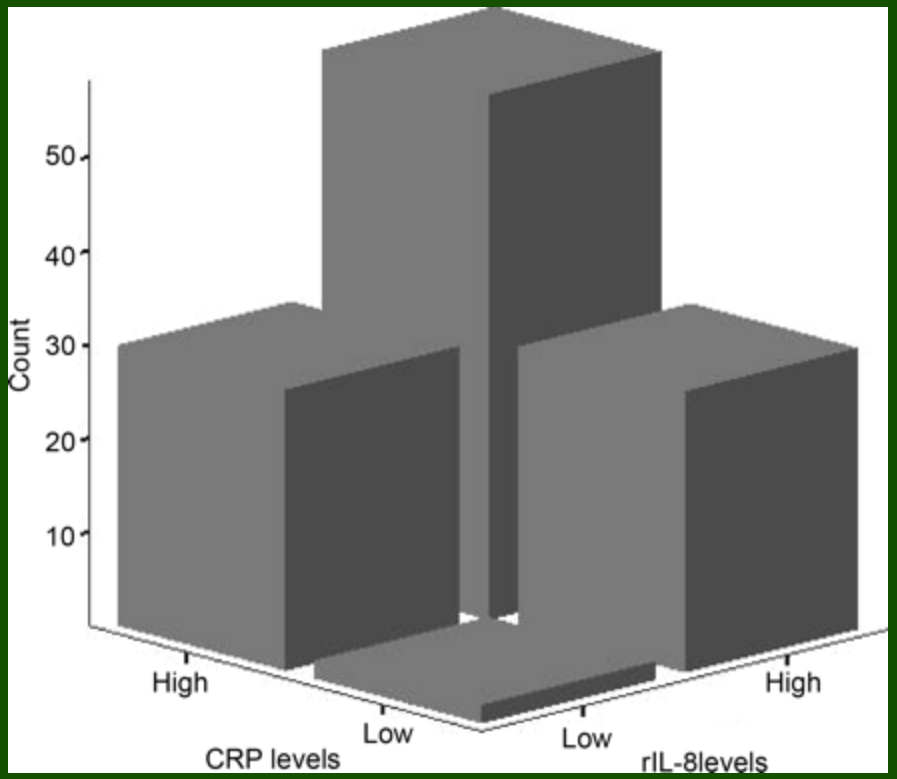


Cd40L released within seconds after GPIIb/IIIa ligation (sCD40 released within minutes to hours)



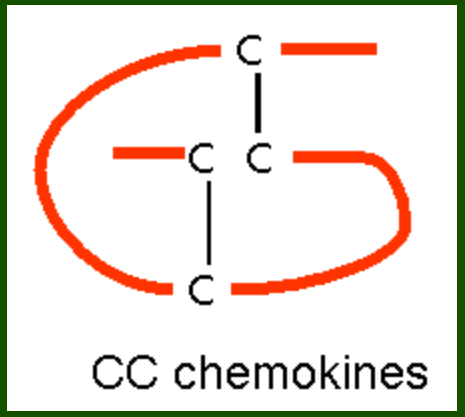
Interleukin-8 is increased in the membrane of circulating erythrocytes in patients with ACS





Tziakas D, Stakos D, Eur Heart J, 2008

Chemokines involved in PLT – EC - Leukocyte interactions



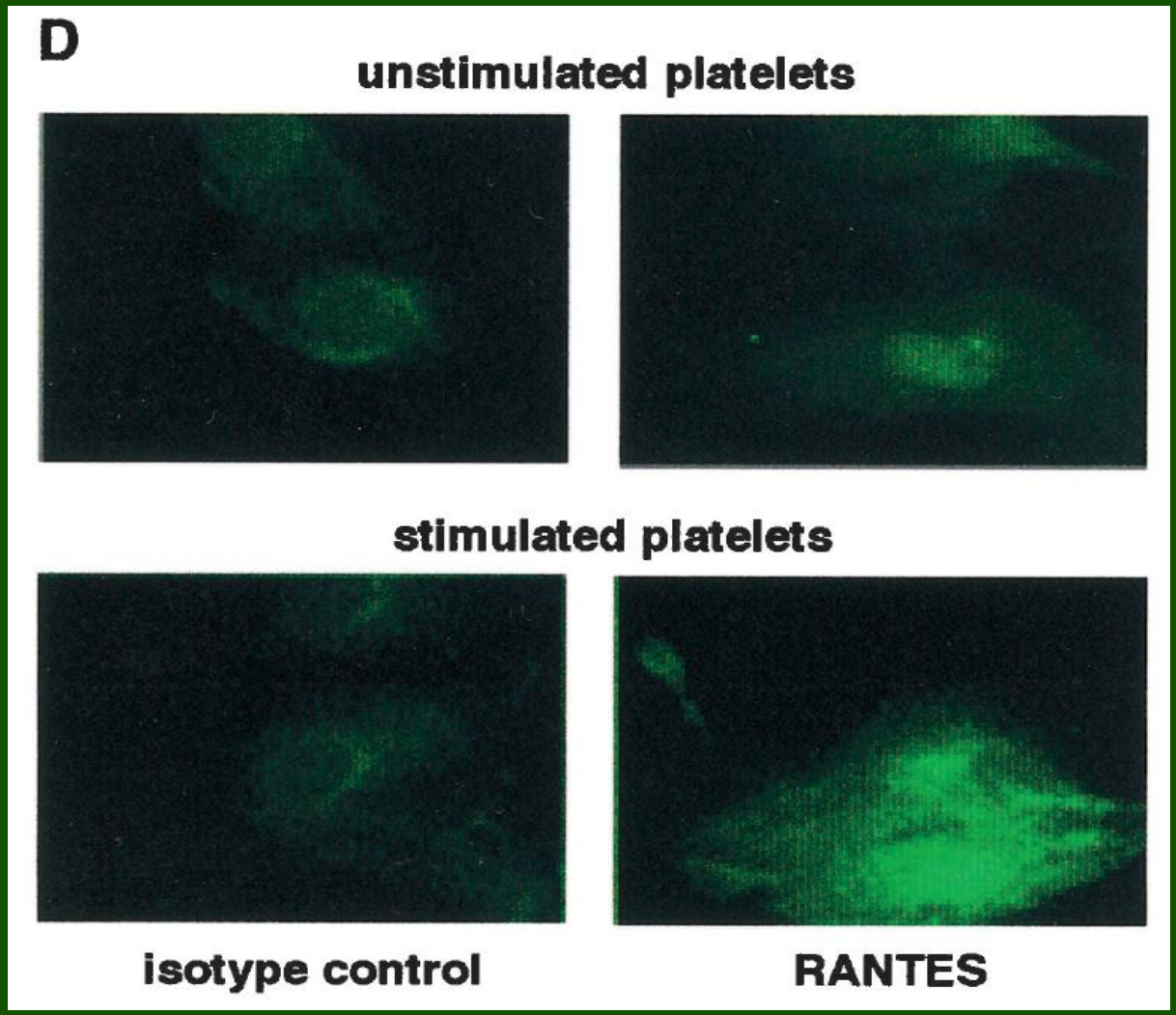
Chemokine (C-C motif) ligand 5 (also **CCL5**) also known as **RANTES***

recruits leukocytes into inflammatory sites

(*Regulated upon Activation, Normal T-cell Expressed, and Secreted)

C: Cystin

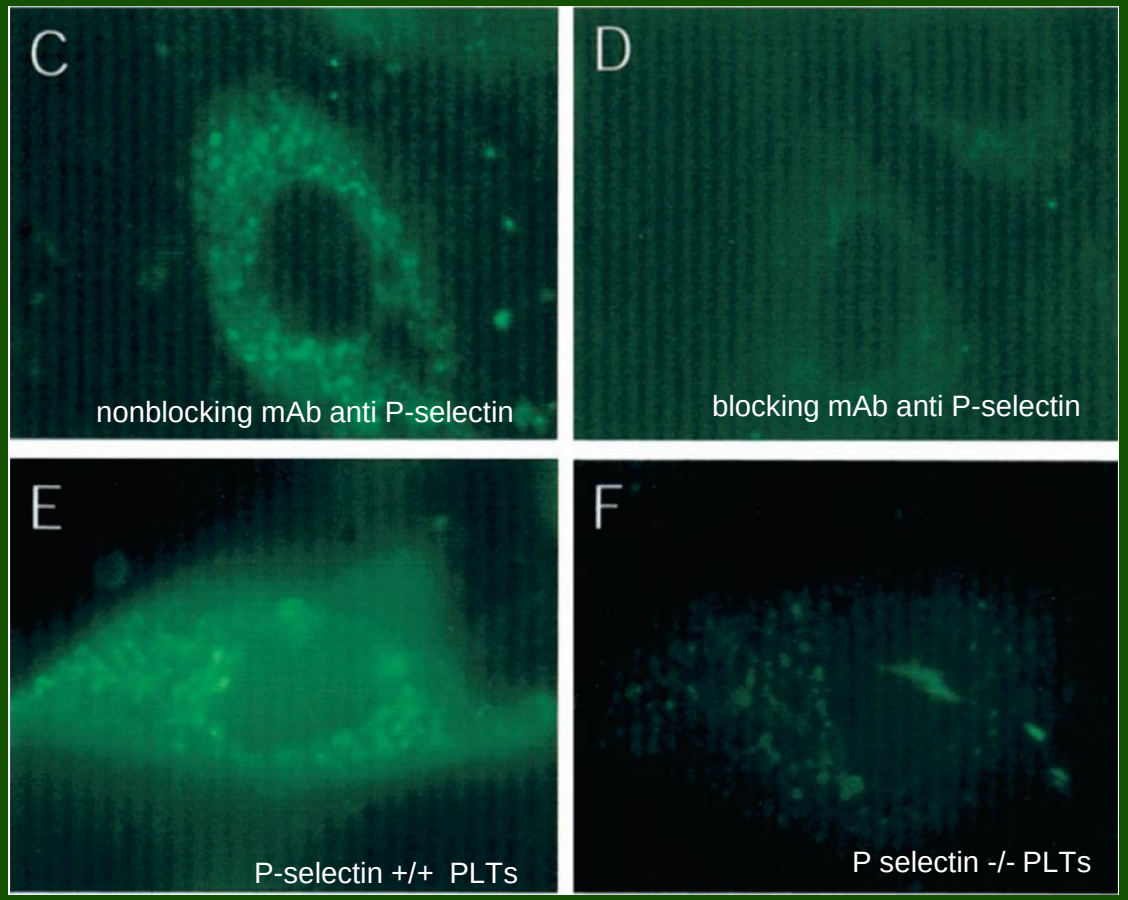
stimulated PLTs on ECs - RANTES secretion and deposition on ECs – Leukocyte recruitment



Von Hundelshausen P, Circulation, 2001

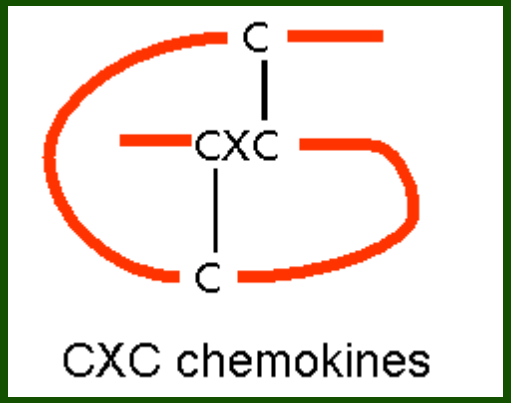
RANTES deposition on ECs requires previous PLT adhesion via P-selectin

RANTES deposition



Schober A, Circulation 2002

Chemokines involved in PLT – EC - Leukocyte interactions



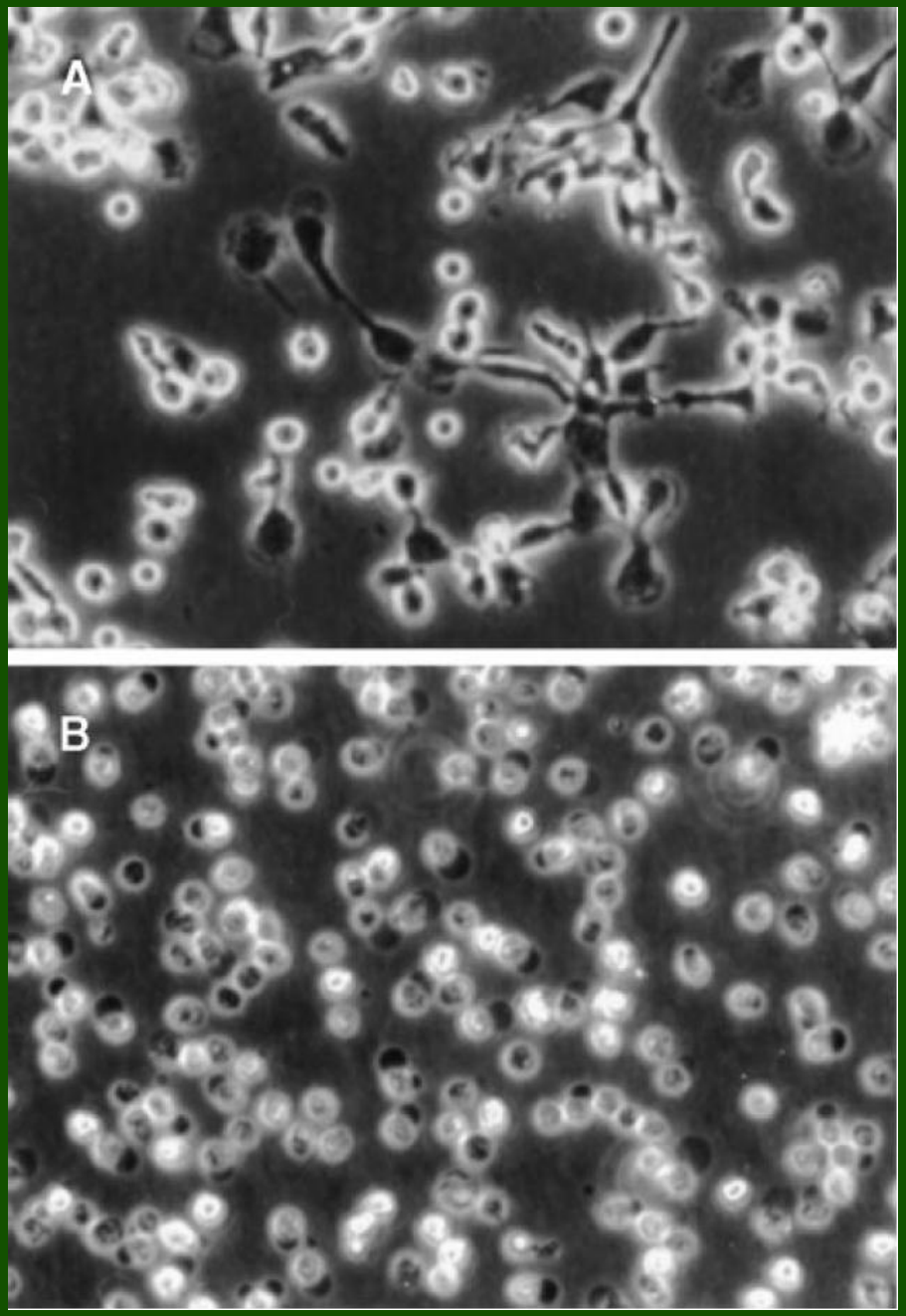
CXC chemokines

Chemokine (CXC motif) ligand 4 (also CXCL4) it is also known as **PF4***

chemotactic and differentiating factor for neutrophils and monocytes

(*Platelet Factor 4)

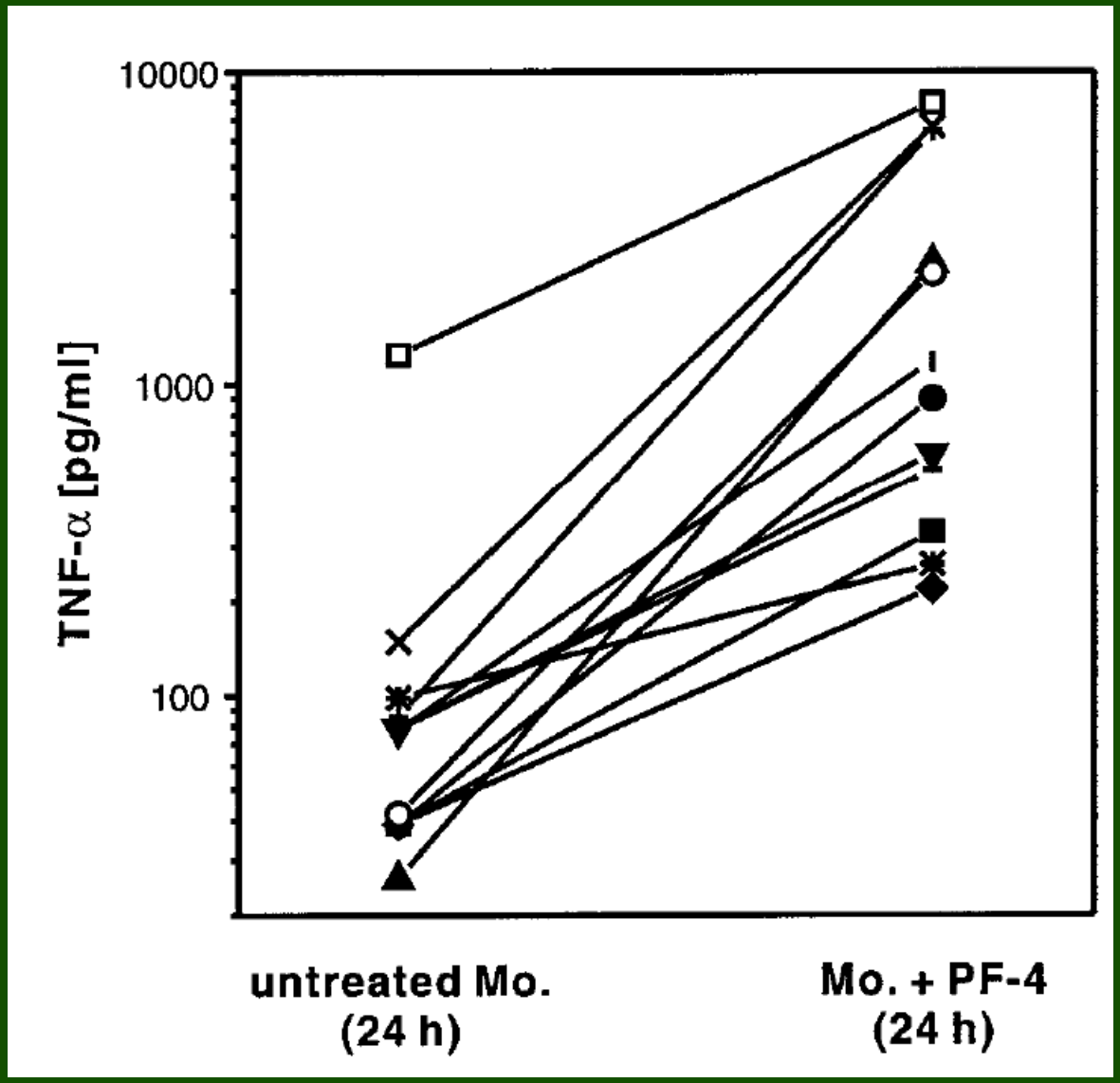
PF4 induces morphological alterations to monocytes



Treated with PF4

Untreated Monocytes

PF4 induces TNF-α release from monocytes

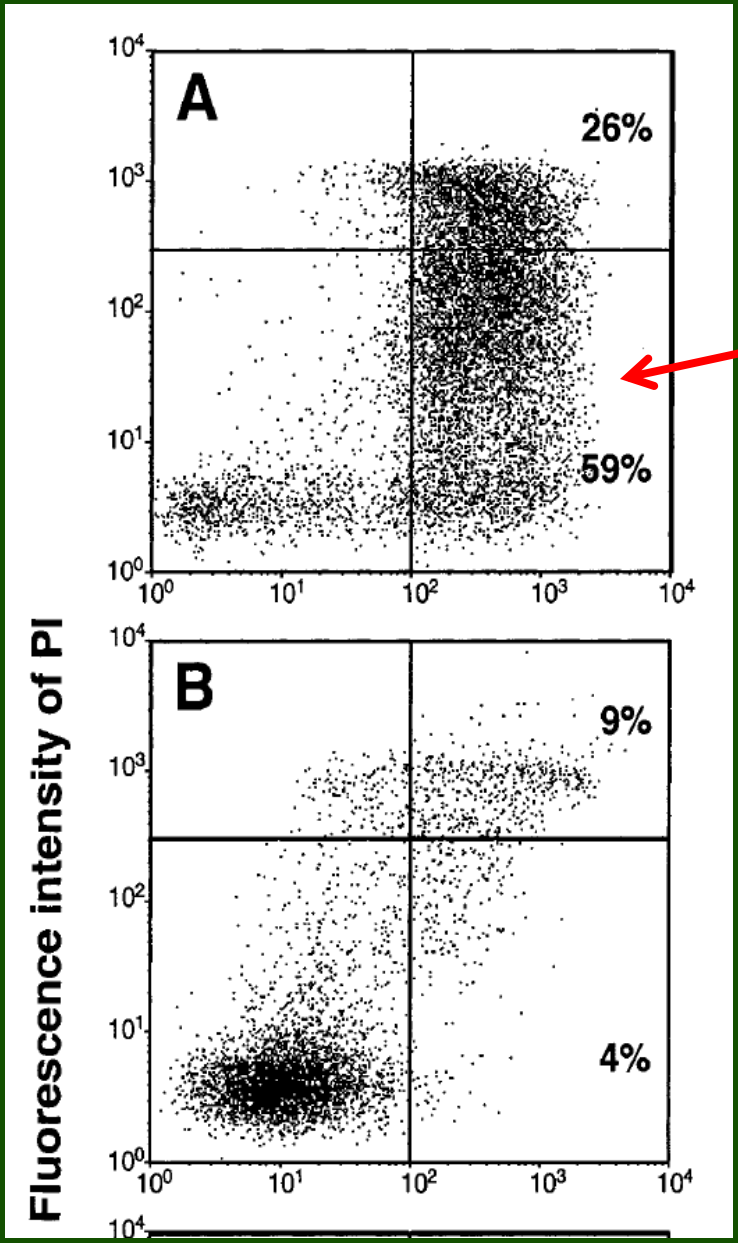


Scheuerer B, Blood, 2000

PF4 promotes differentiation of monocytes into macrophages and inhibit their apoptosis

No PF4

PF4



Necrotic cells

Apoptotic ells

Unstimulated monocytes rapidly undergo apoptosis

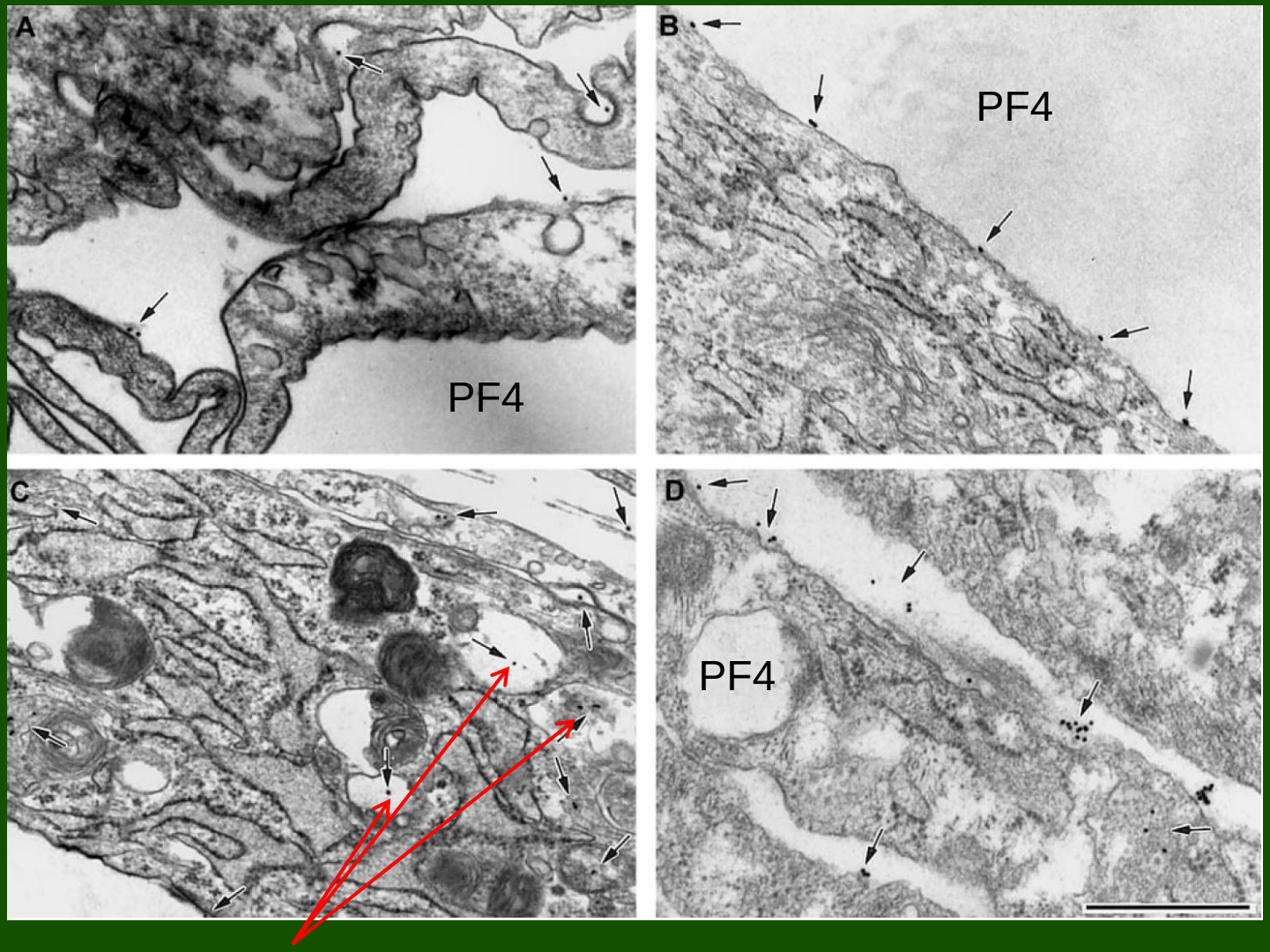
Necrotic cells

Apoptotic ells

Scheuerer B, Blood, 2000

PF4 interferes with LDL endocytosis
A key point of how PLTs promote atherogenesis in its initial steps

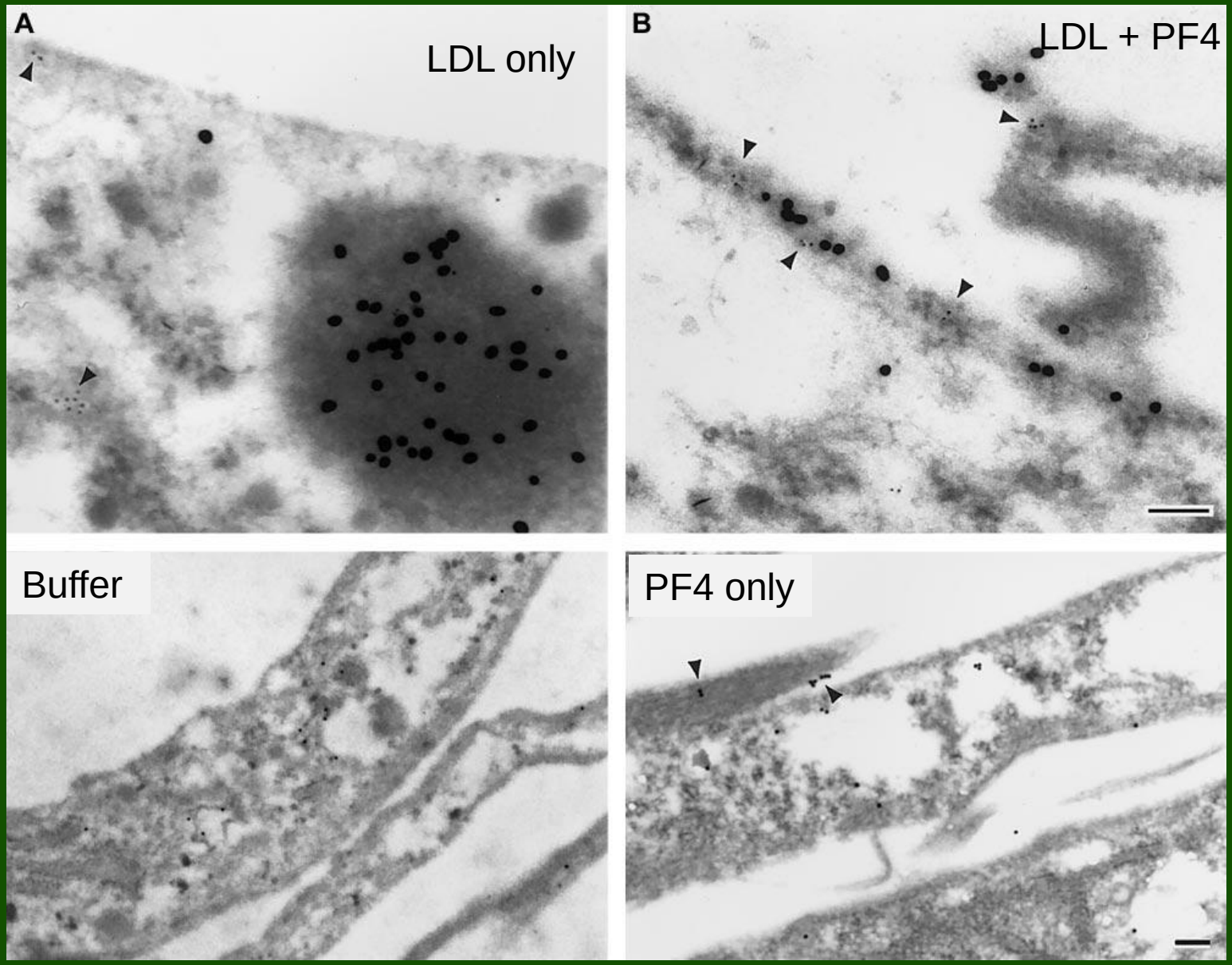
PF4 inhibits LDL-R dependent binding and internalization of LDL cholesterol by vascular cells



LDL in coated pits

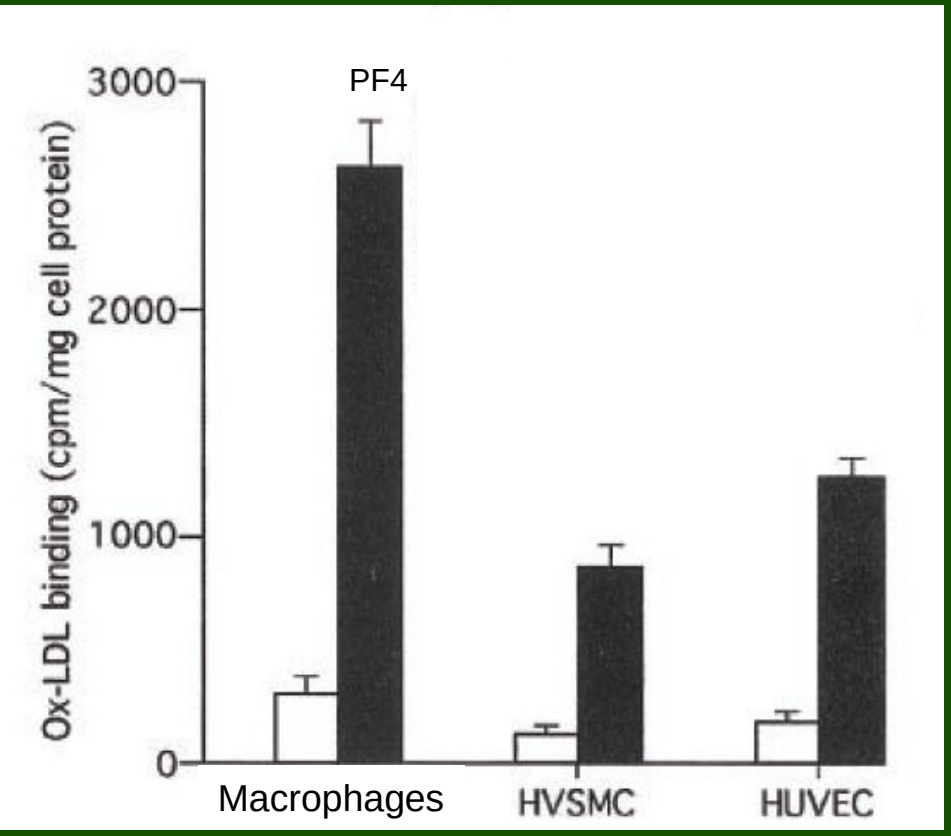
Sachais BC, Blood, 2002

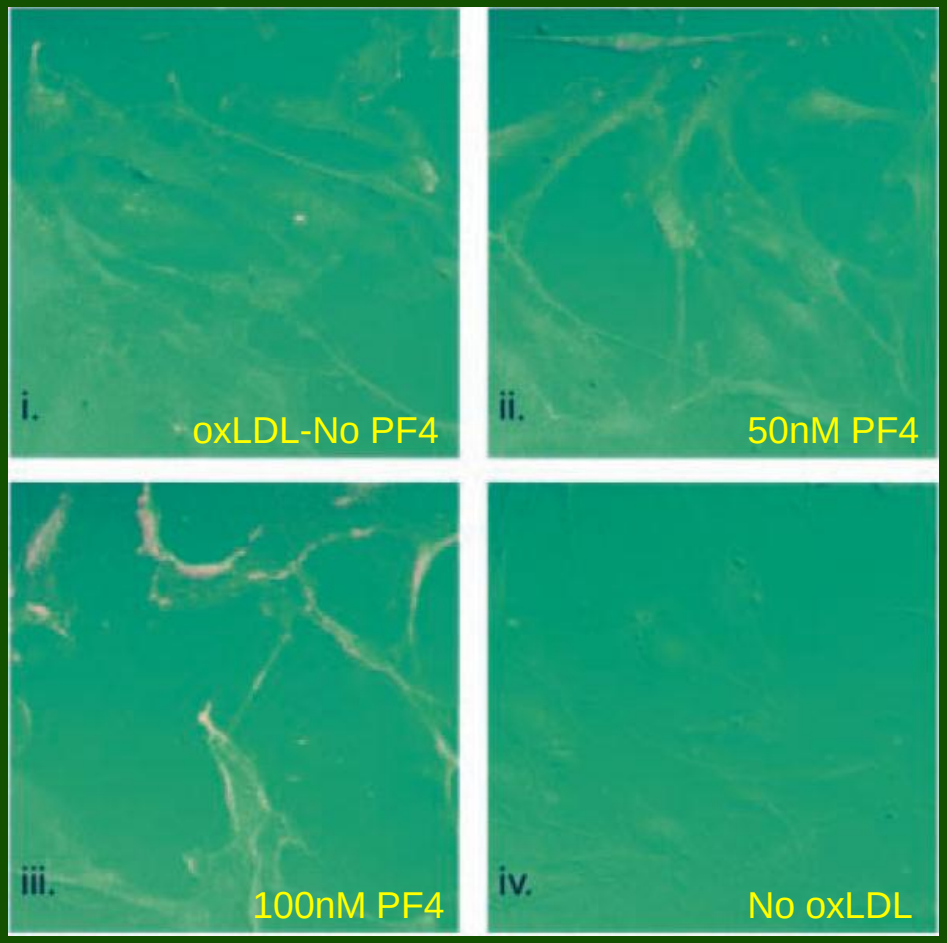
LDL – LDLR clusters in cell surface in the presence of PF4



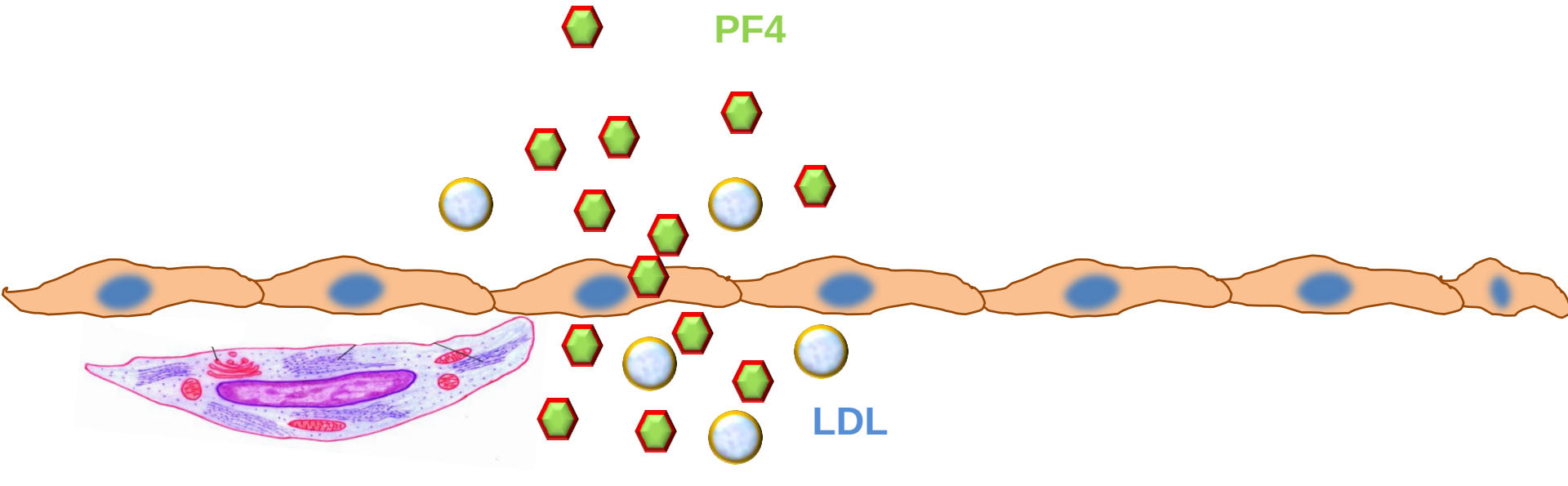
Sachais BC, Blood, 2002

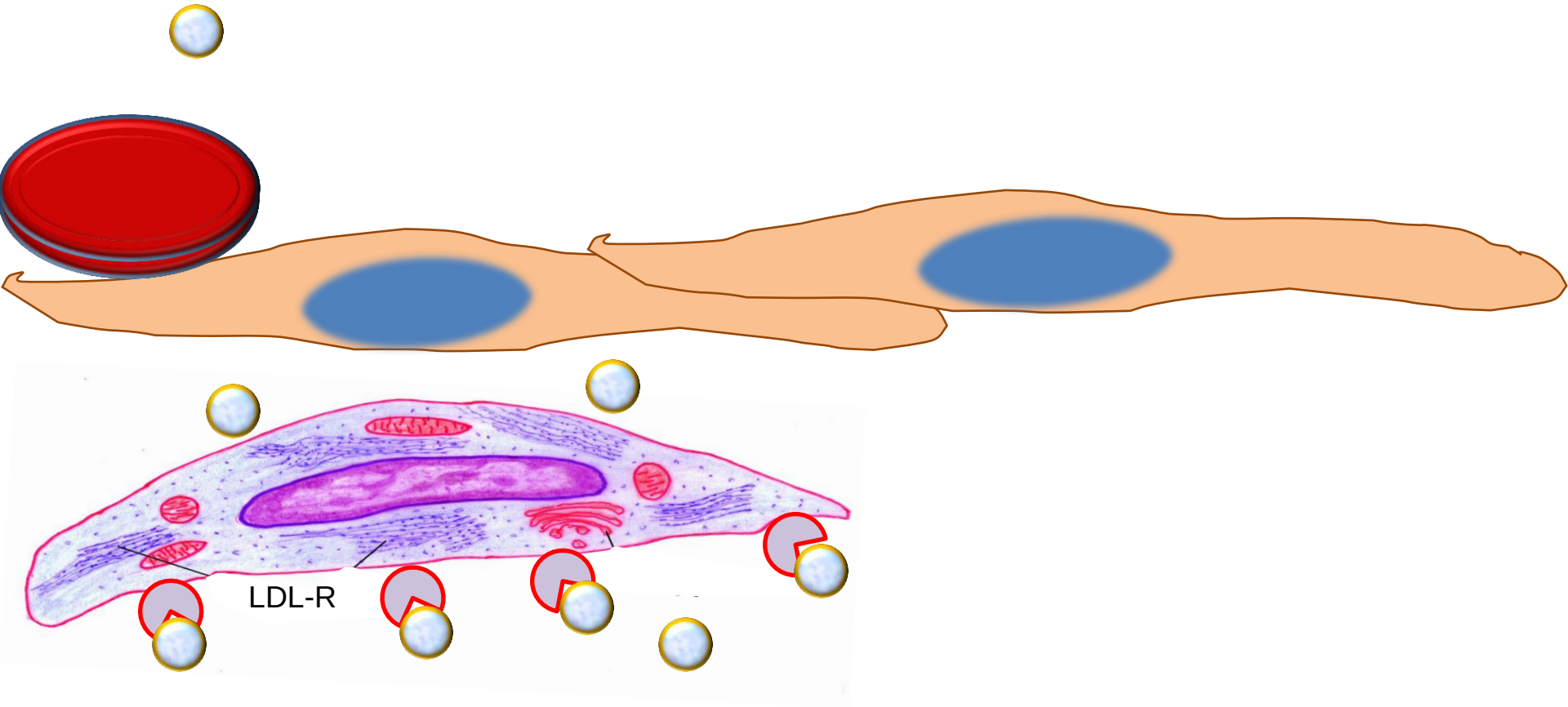
PF4 stimulates binding of ox-LDL to ECs and macrophages

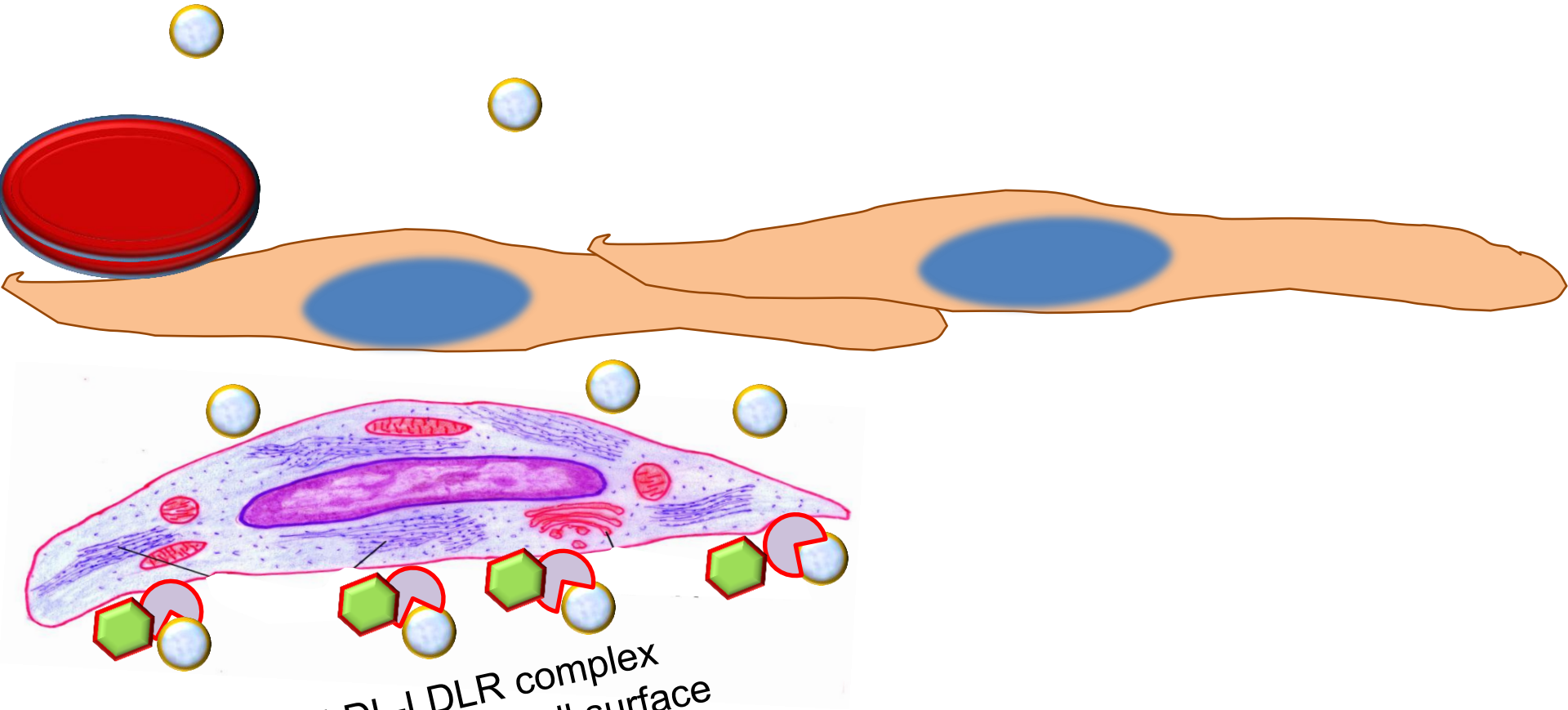




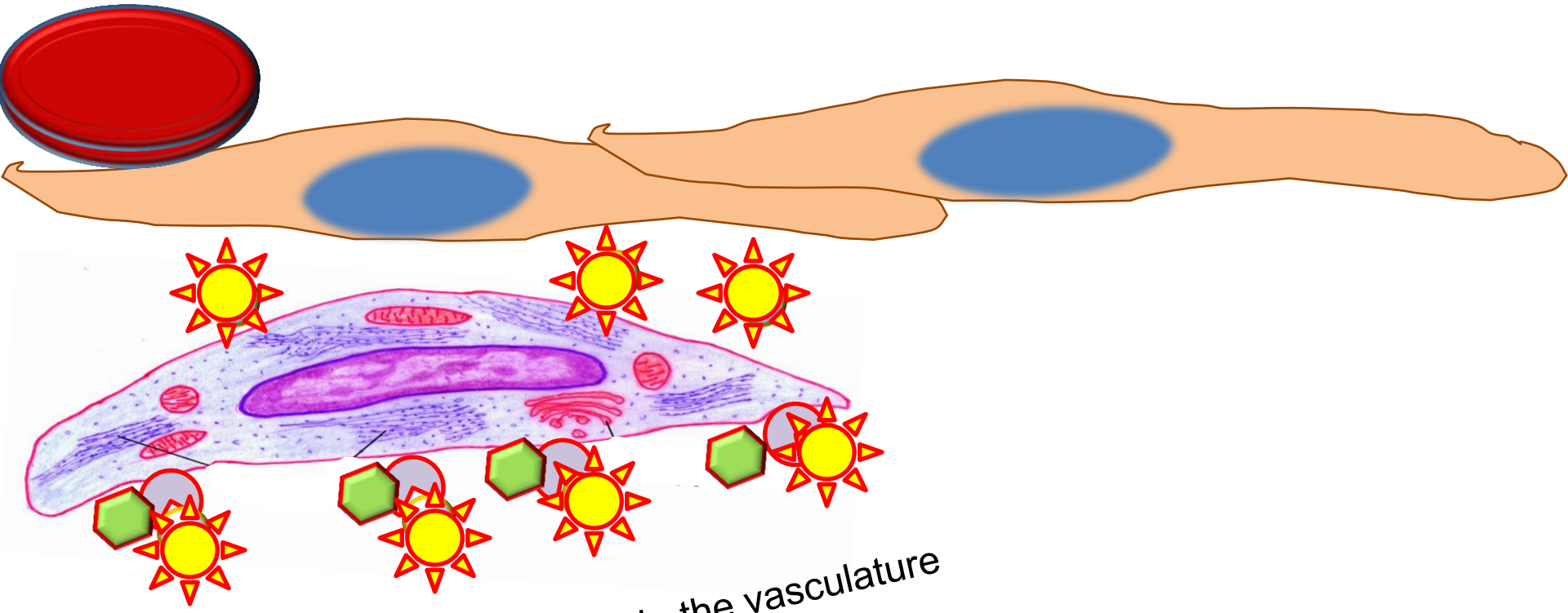
Nassar T, J Biol Chem, 2003


PF4
LDL


LDL-R

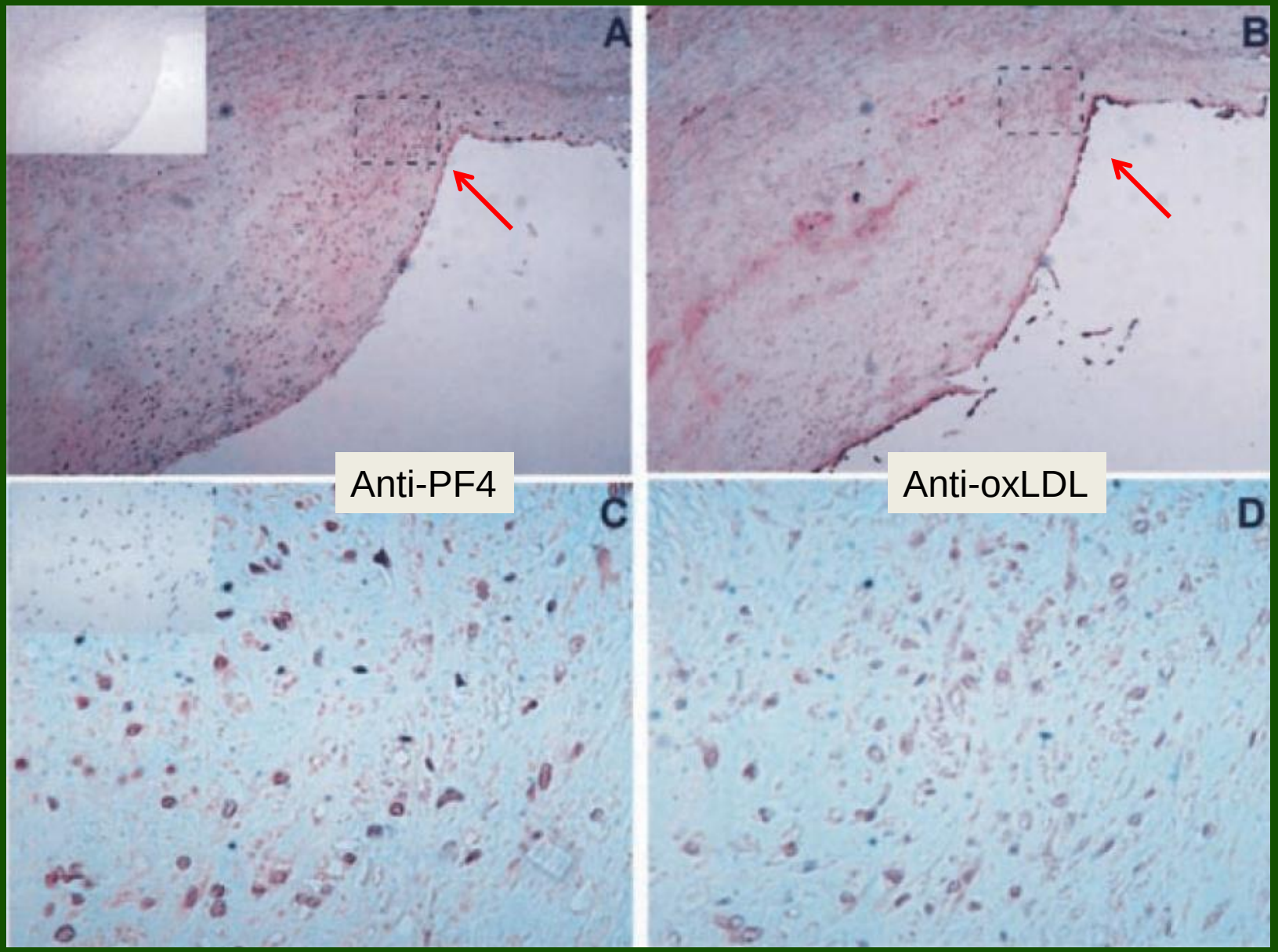


LDL-LDLR complex
Retention on cell surface



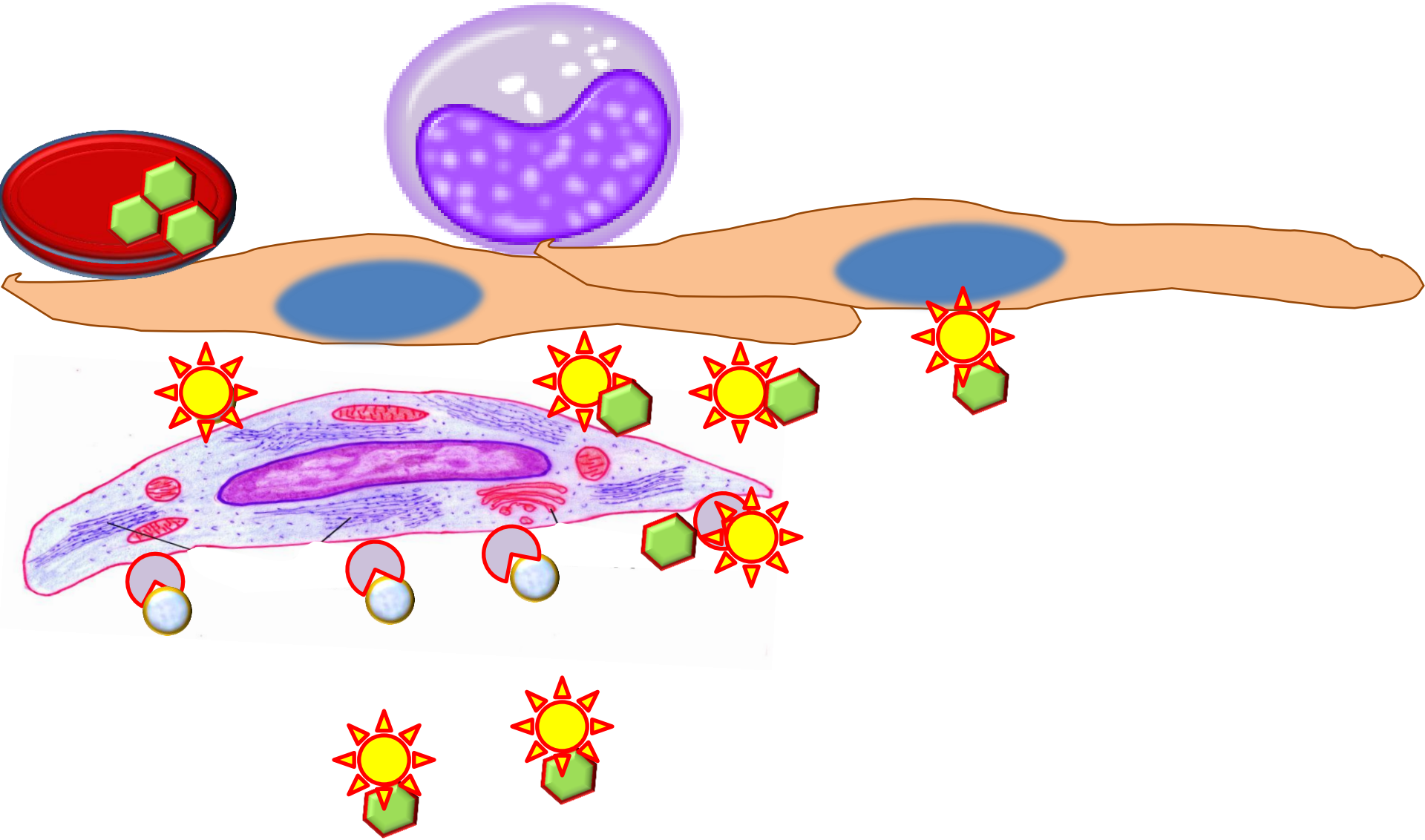
Increased residence time in the vasculature
→ oxidative modification of LDL

Co-localization of PF4 and ox-LDL in human atherosclerotic vessels

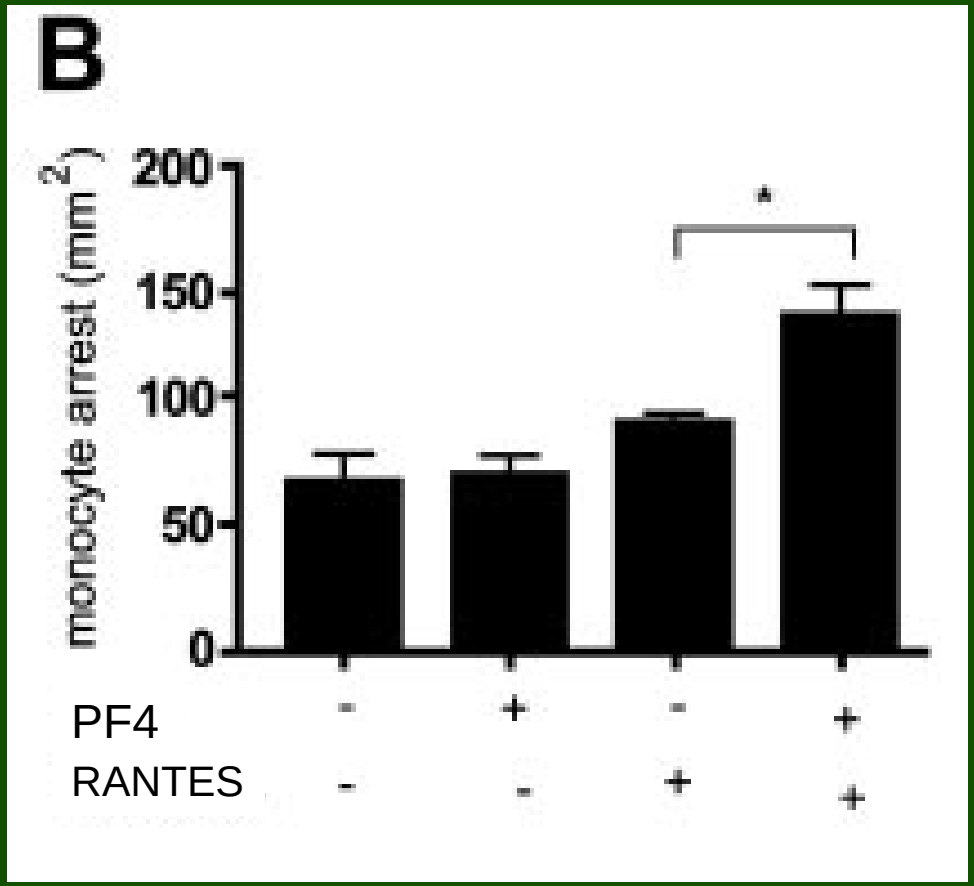


Nassar T, J Biol Chem, 2003

PF4-mediated monocyte recruitment ...

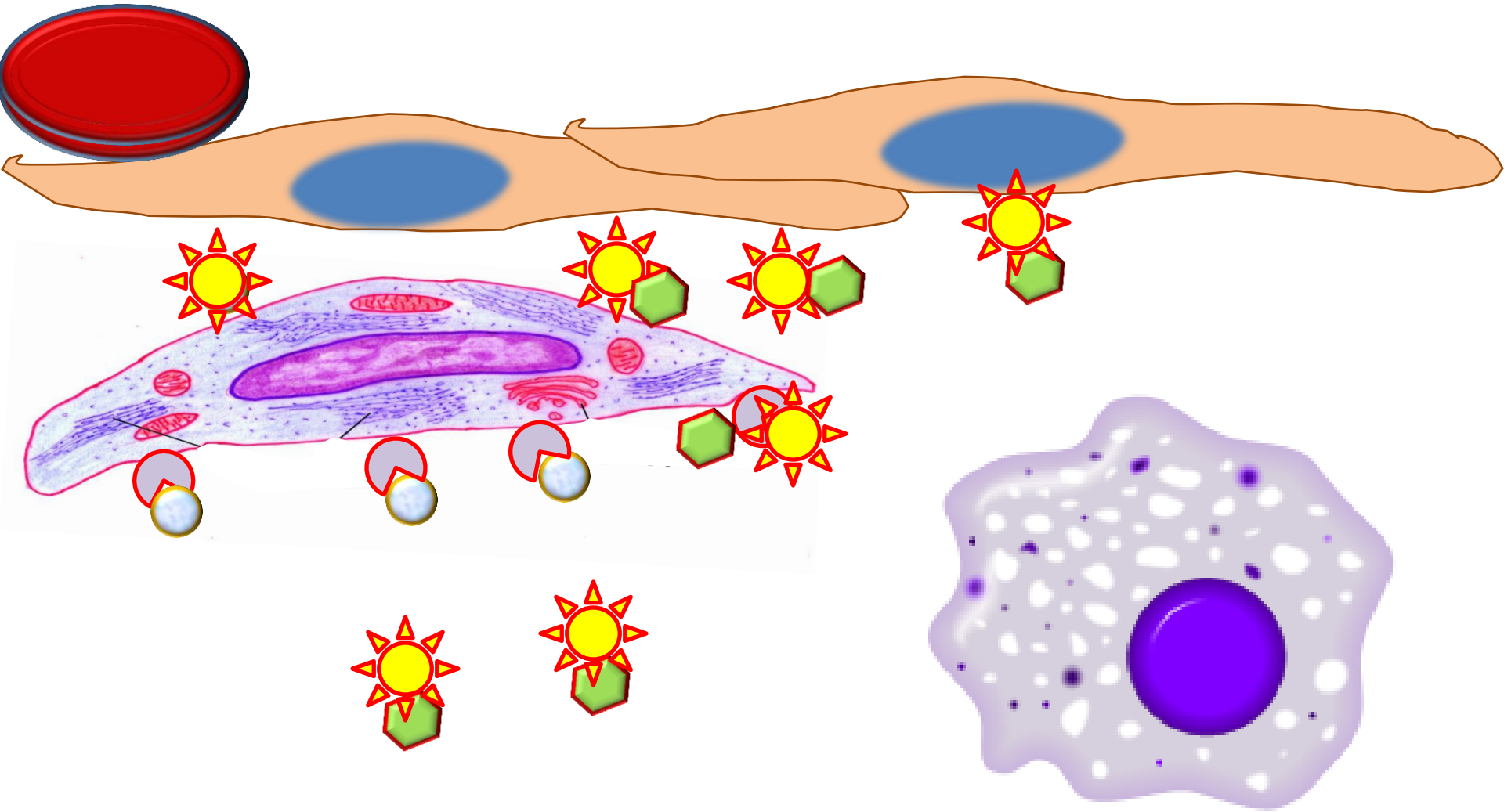


PF4 enhanced the arrest of RANTES-stimulated monocytes on activated ECs under flow

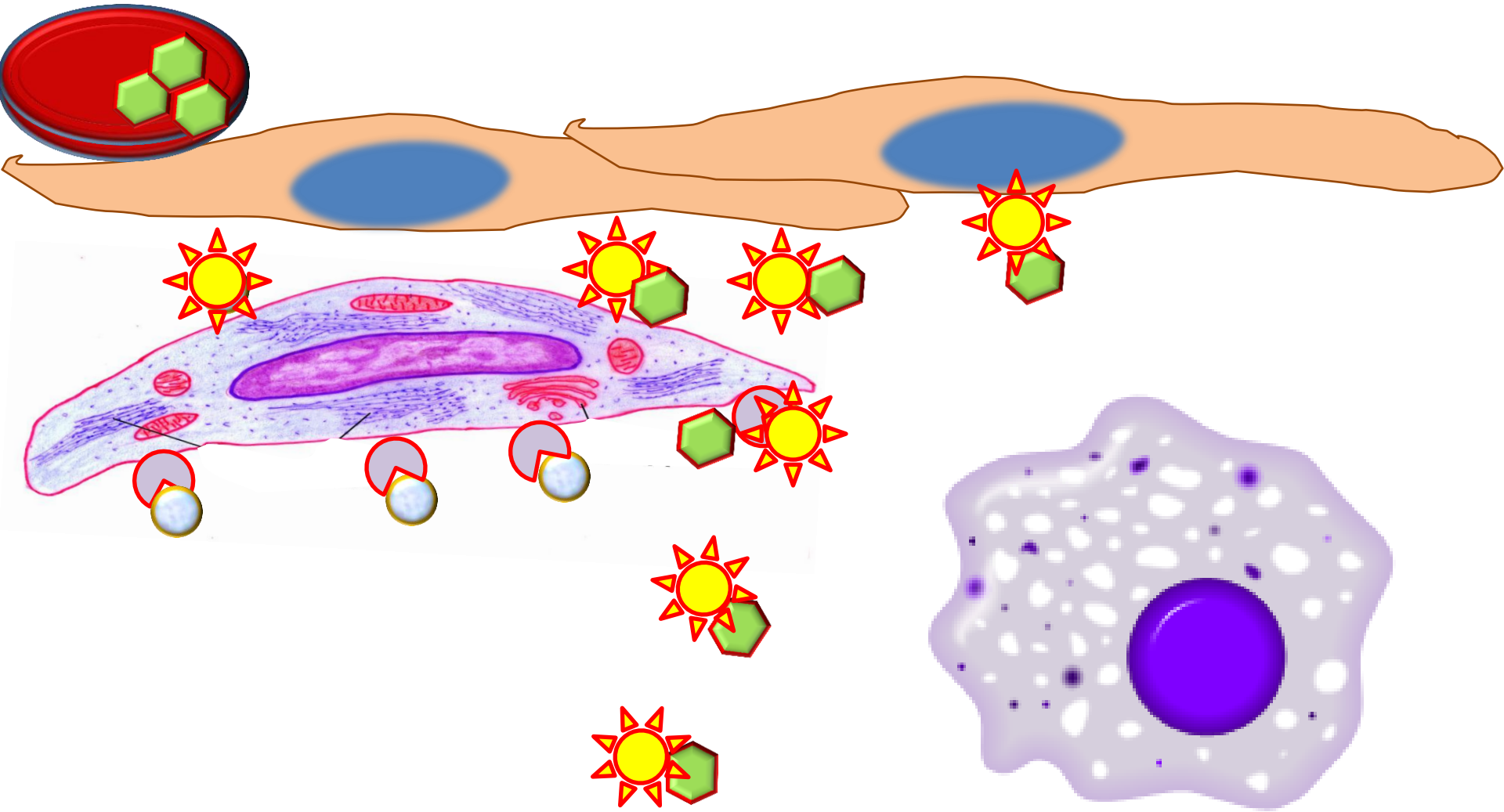


Von Hundelshausen P, Blood, 2005

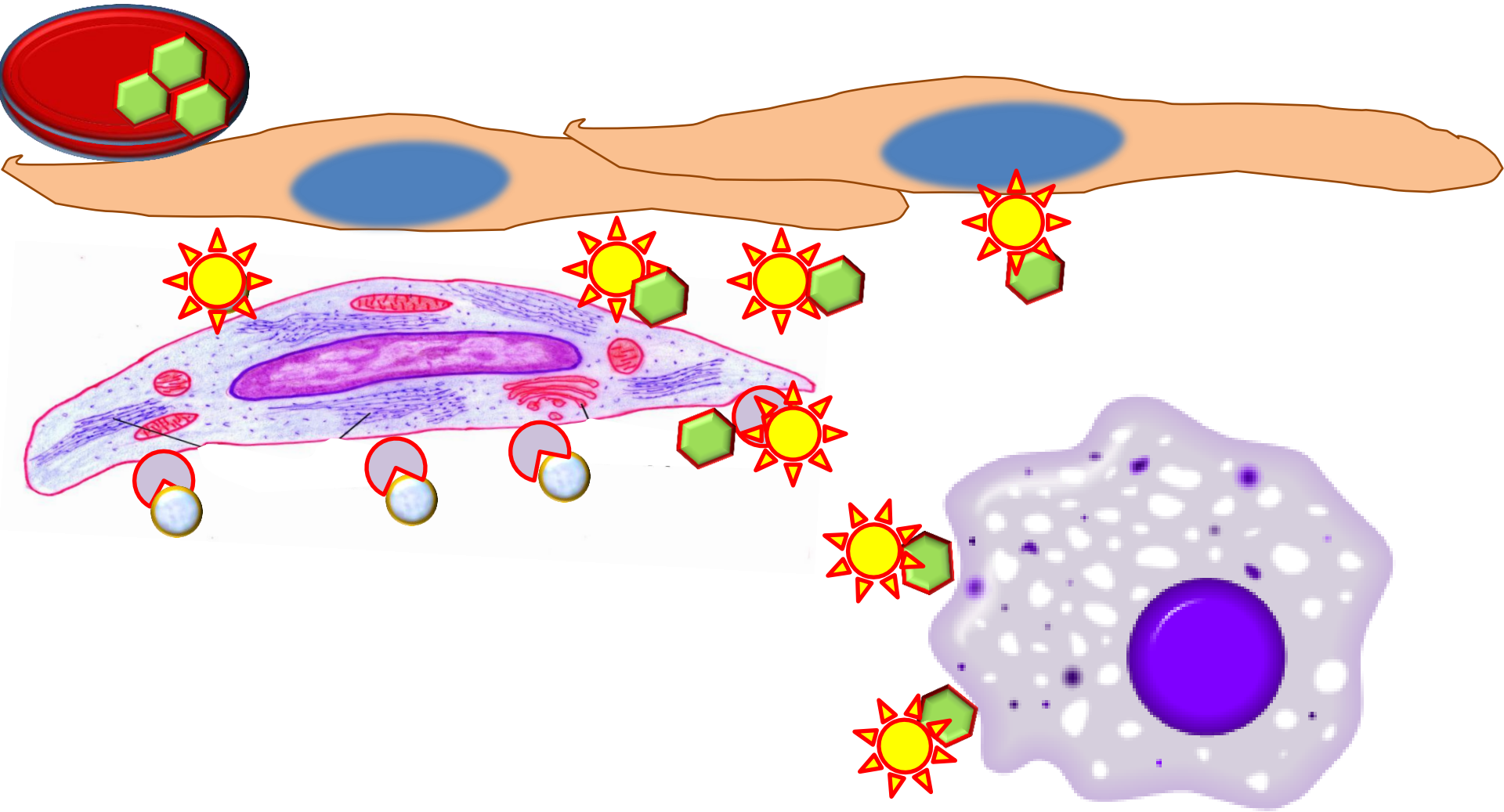
...and differentiation into macrophages



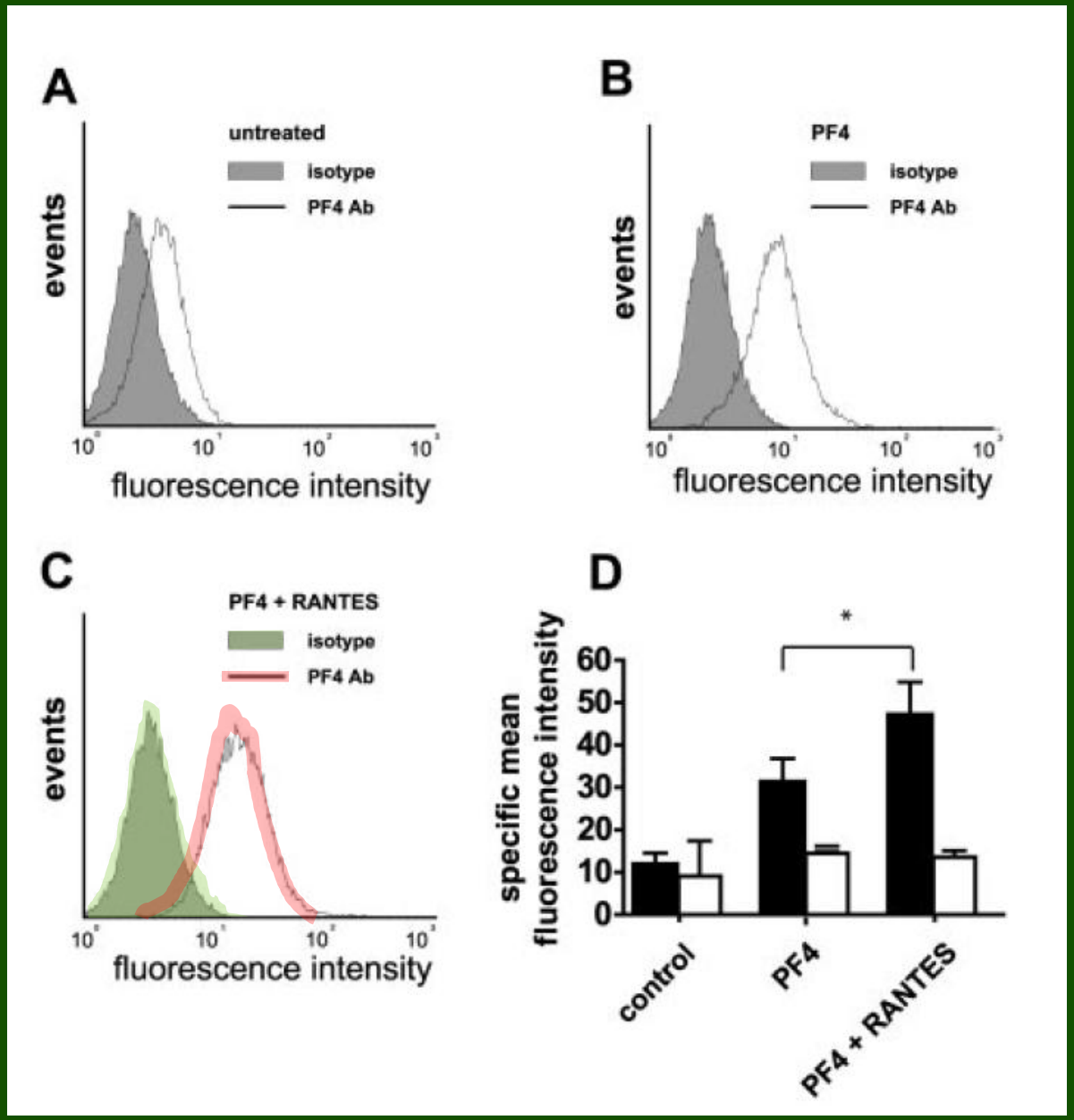
PF4 enhances binding of oxLDL from macrophages



PF4 enhances binding of oxLDL from macrophages

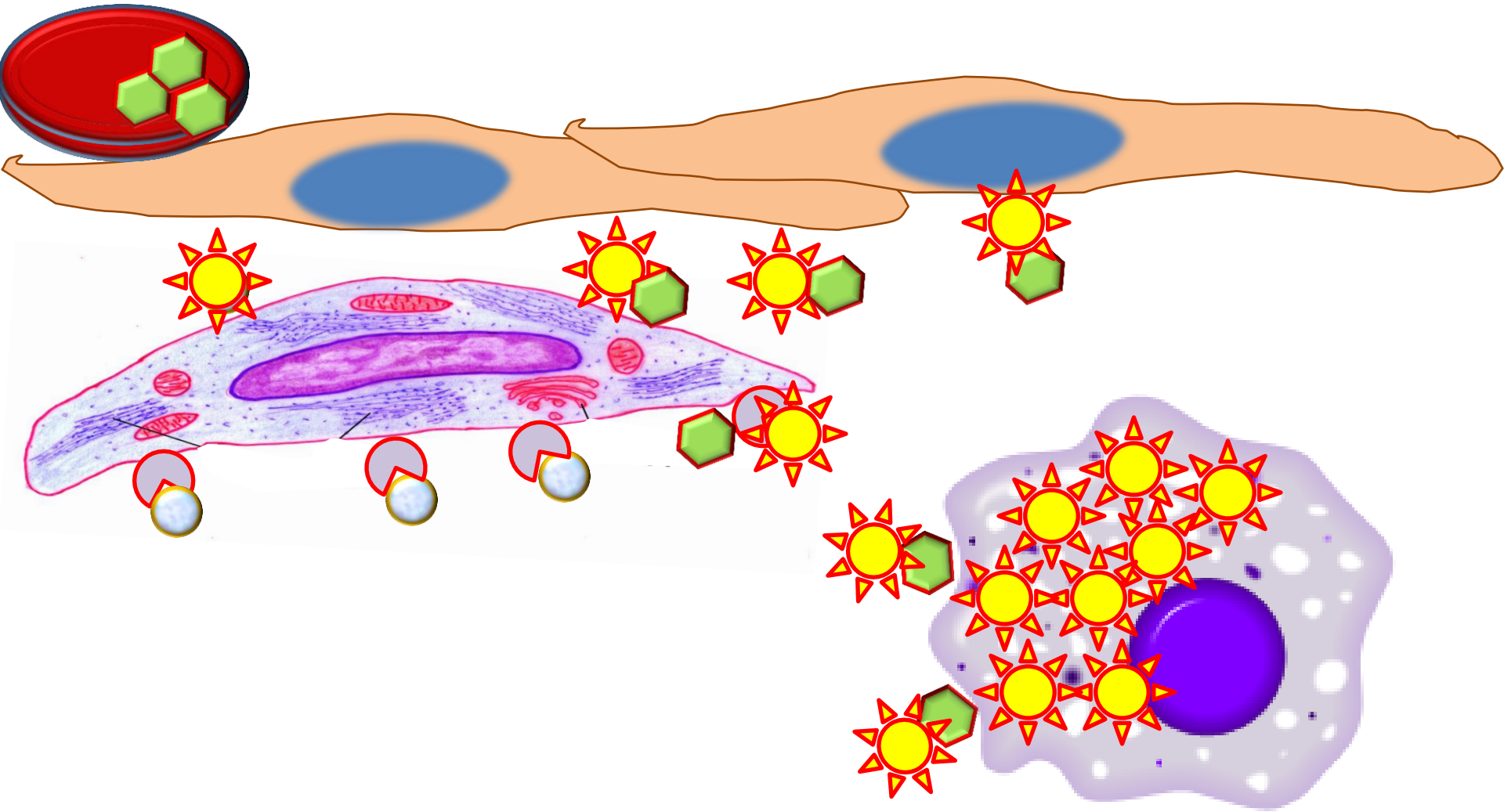


Binding of PF4 to the monocyte surface was increased in the presence of RANTES

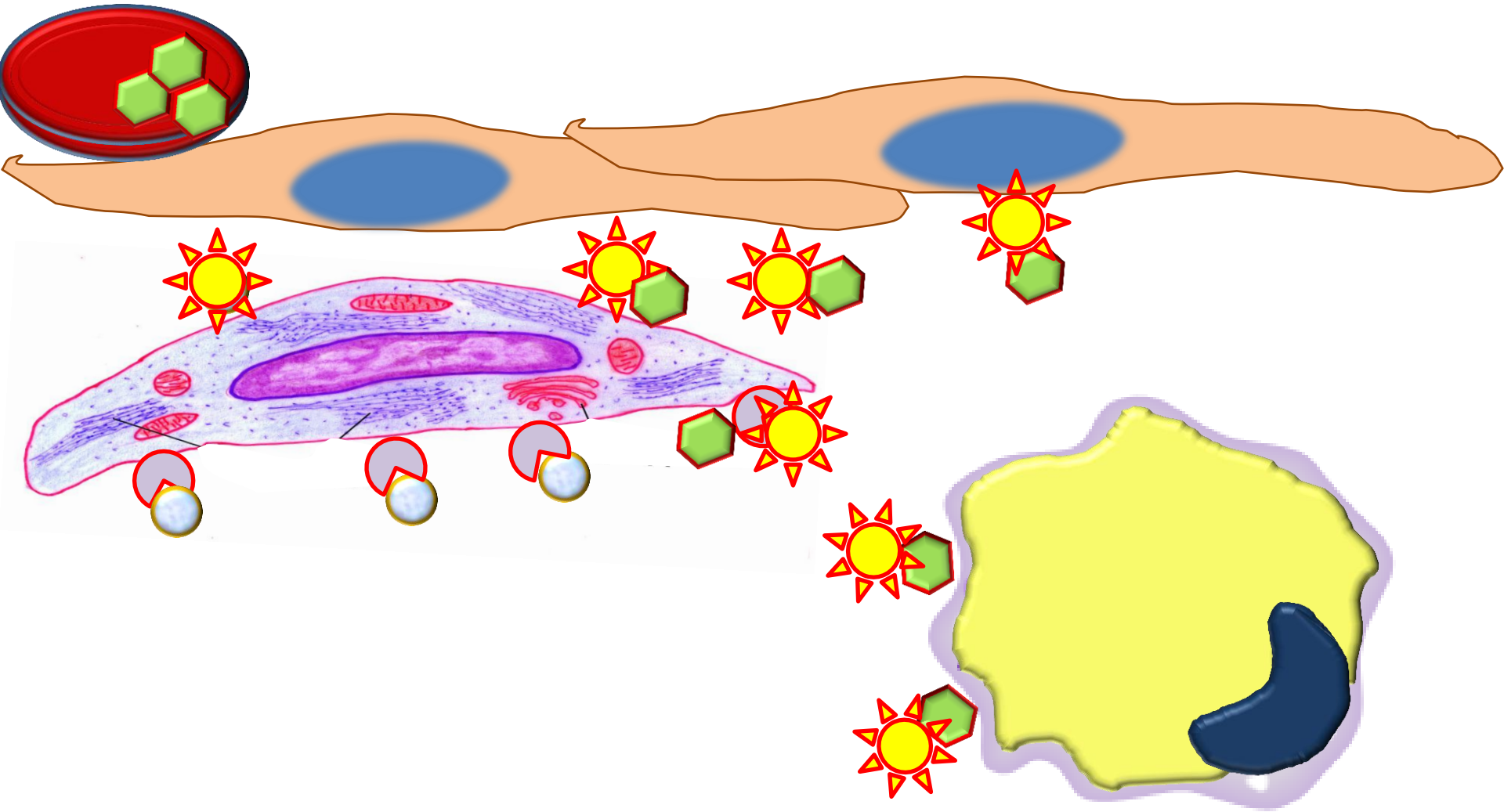


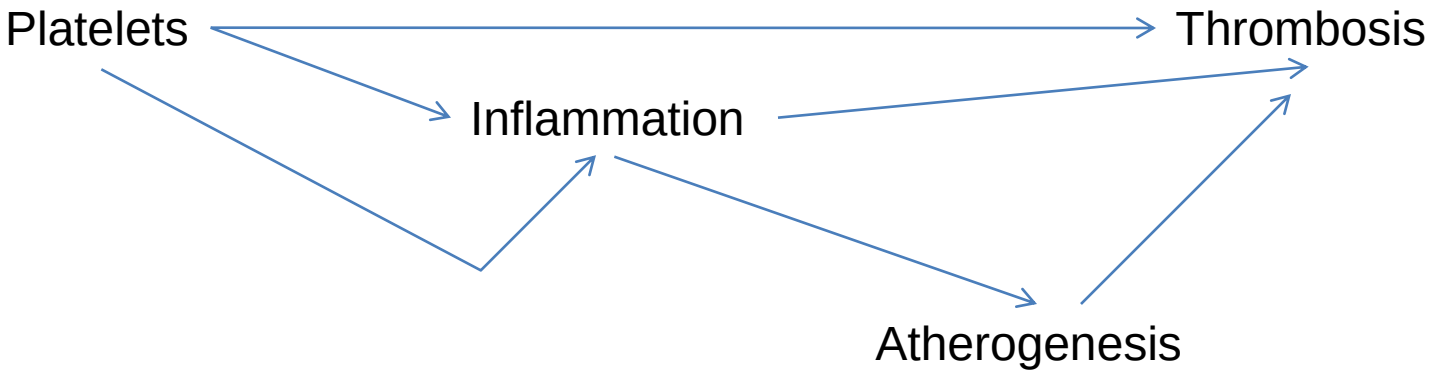
Von Hundelshausen P, Blood, 2005

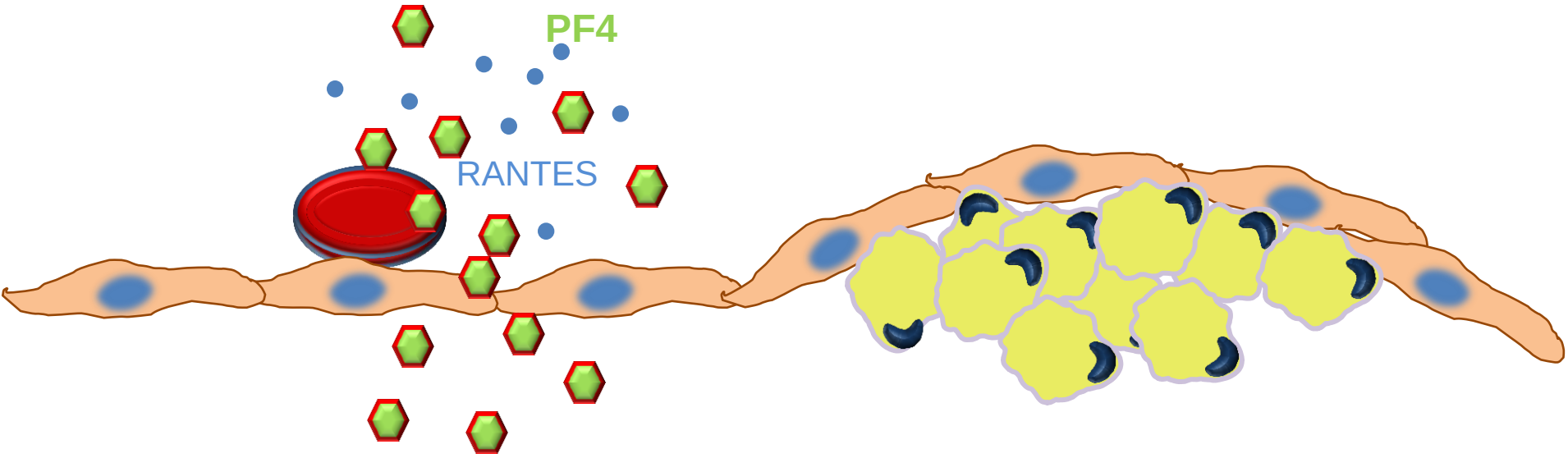
PF4 enhances binding and endocytosis of oxLDL from macrophages



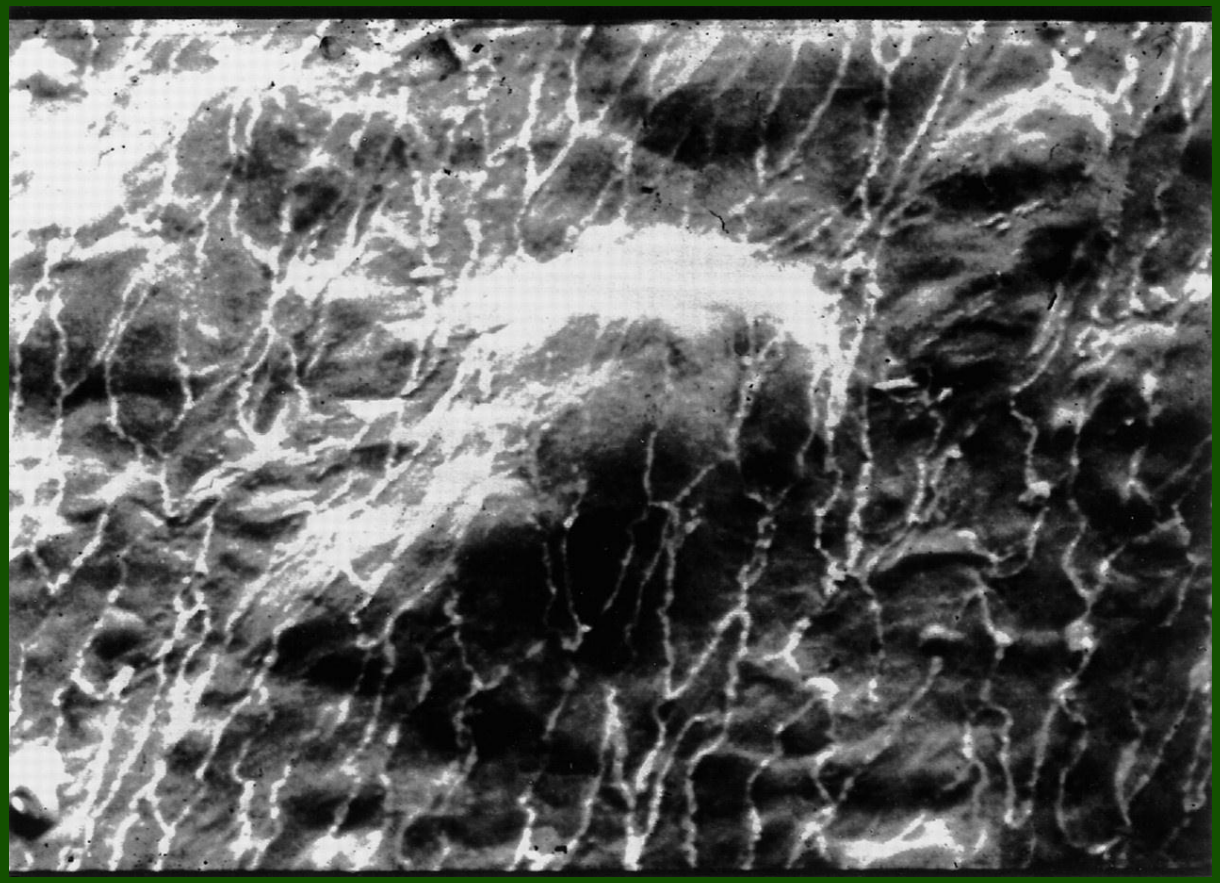
Subsequent esterification of ox-LDL in macrophages accelerating the formation of foam cells.




Platelets
Thrombosis
Inflammation
Atherogenesis

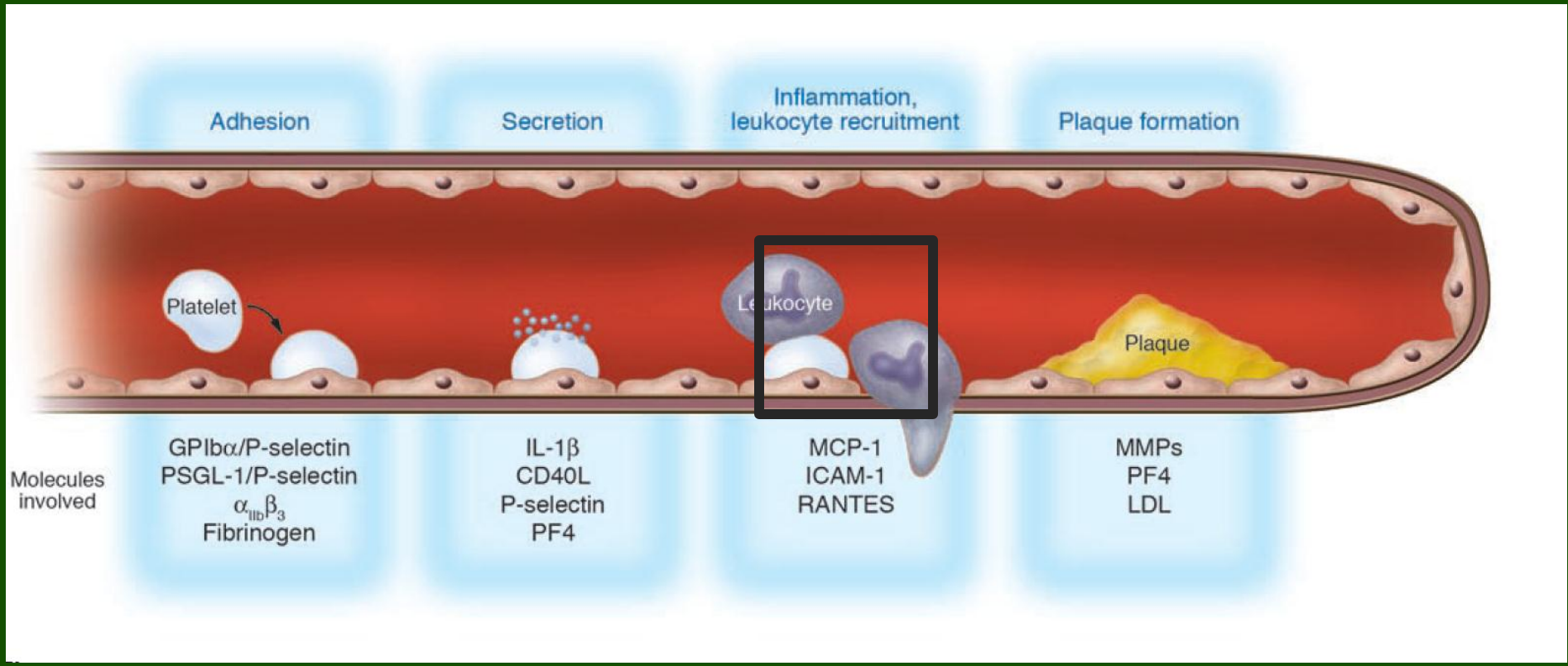

PF4
RANTES

Very early development of atherosclerosis in a nonhuman primate beneath an intact endothelium.



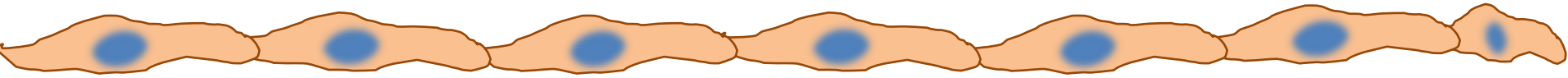
What we have seen until now..

Platelet interaction with endothelium and at site recruitment of WBCs may initiate atherosclerosis



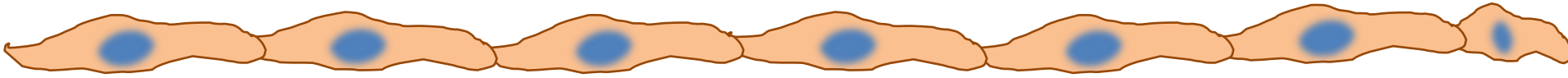
Platelet-leukocyte interactions

Platelet – leukocyte interactions at site

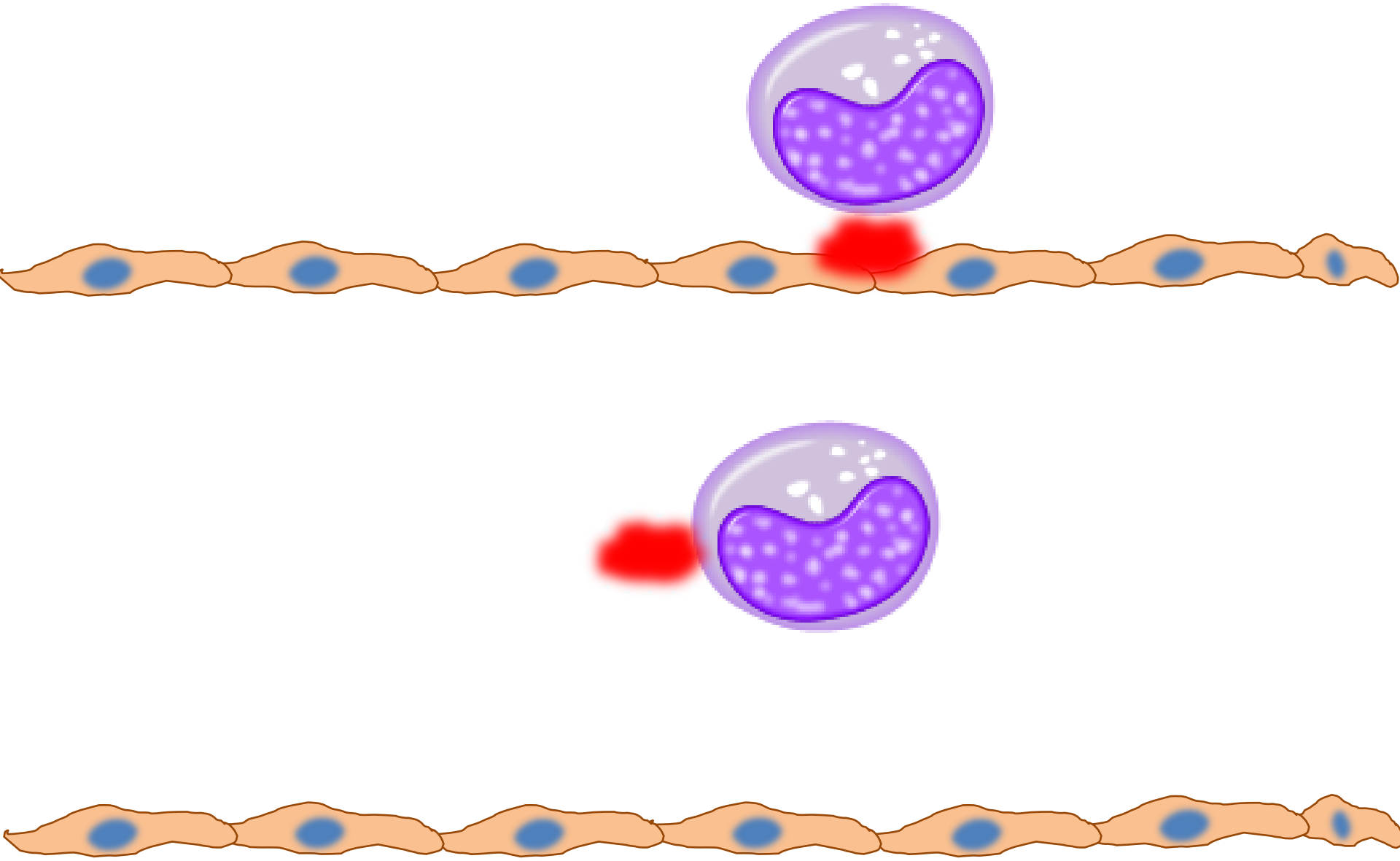


Platelet – leukocyte interactions in circulation

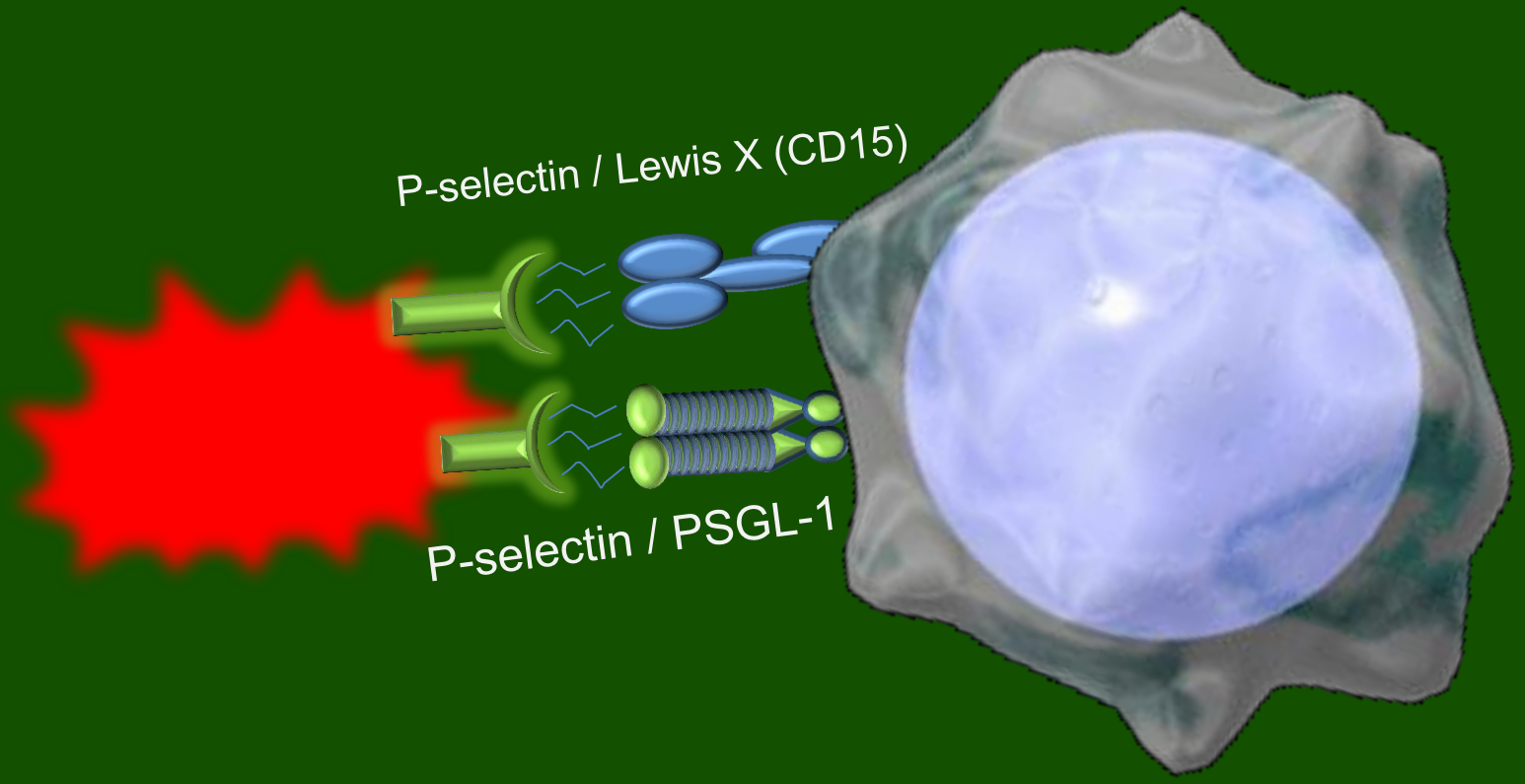
(turbulent flow, cytokines, inflammatory conditions, AMI, after rolling in activated Ecs)



Platelet – Leukocyte Aggregates

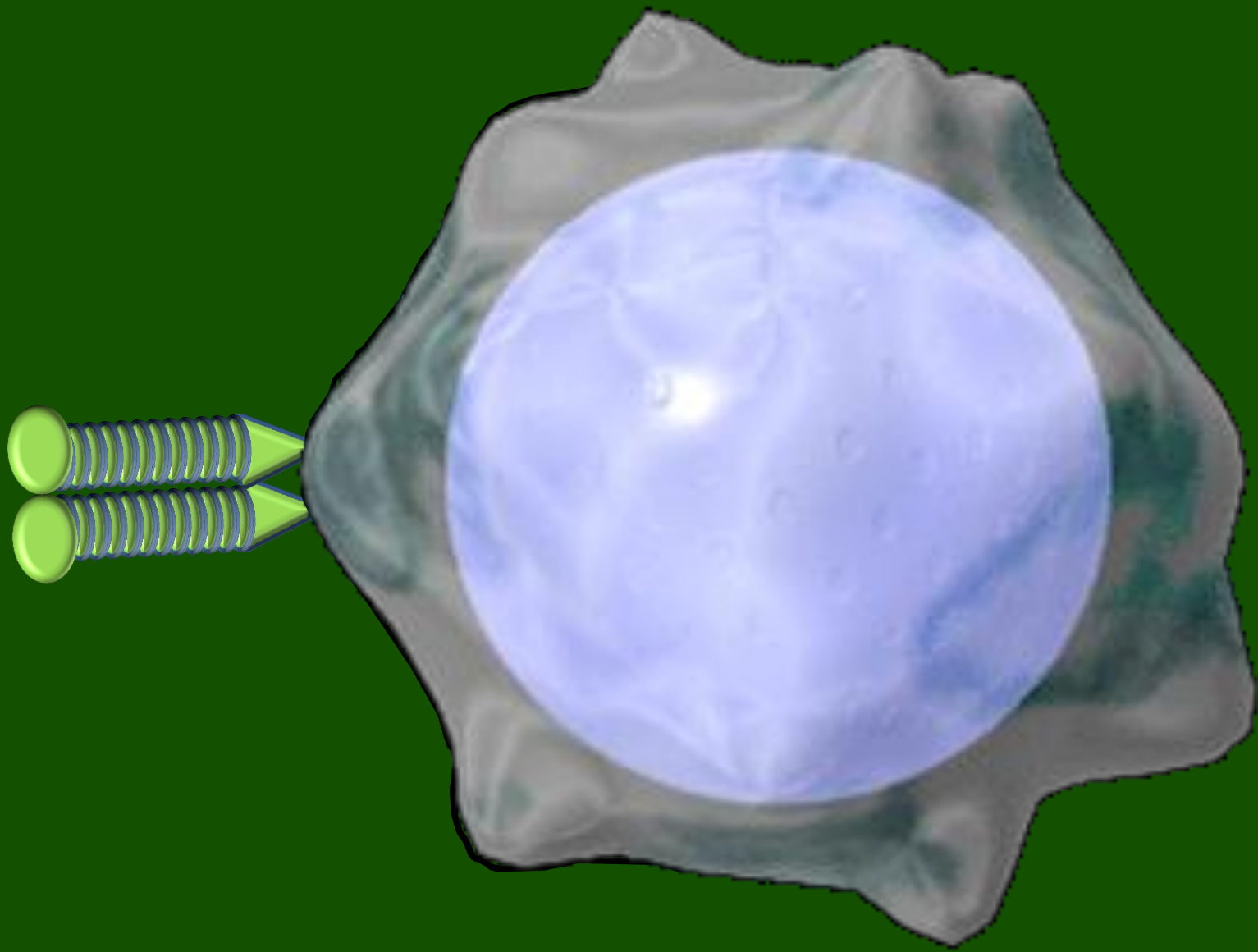


PLT – Leukocyte initial tethering

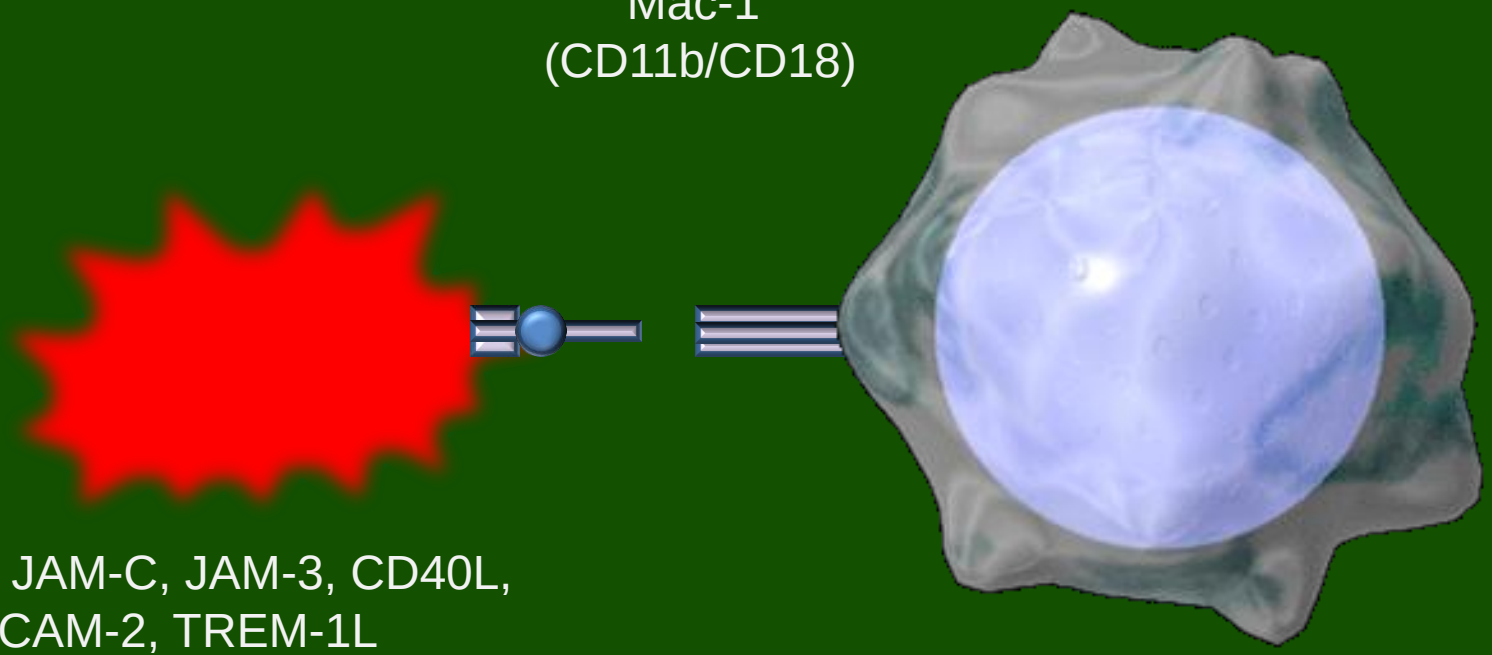


May A, ATVB, 2008

PSGL-1 ligation prepare rolling through actin cytoskeleton

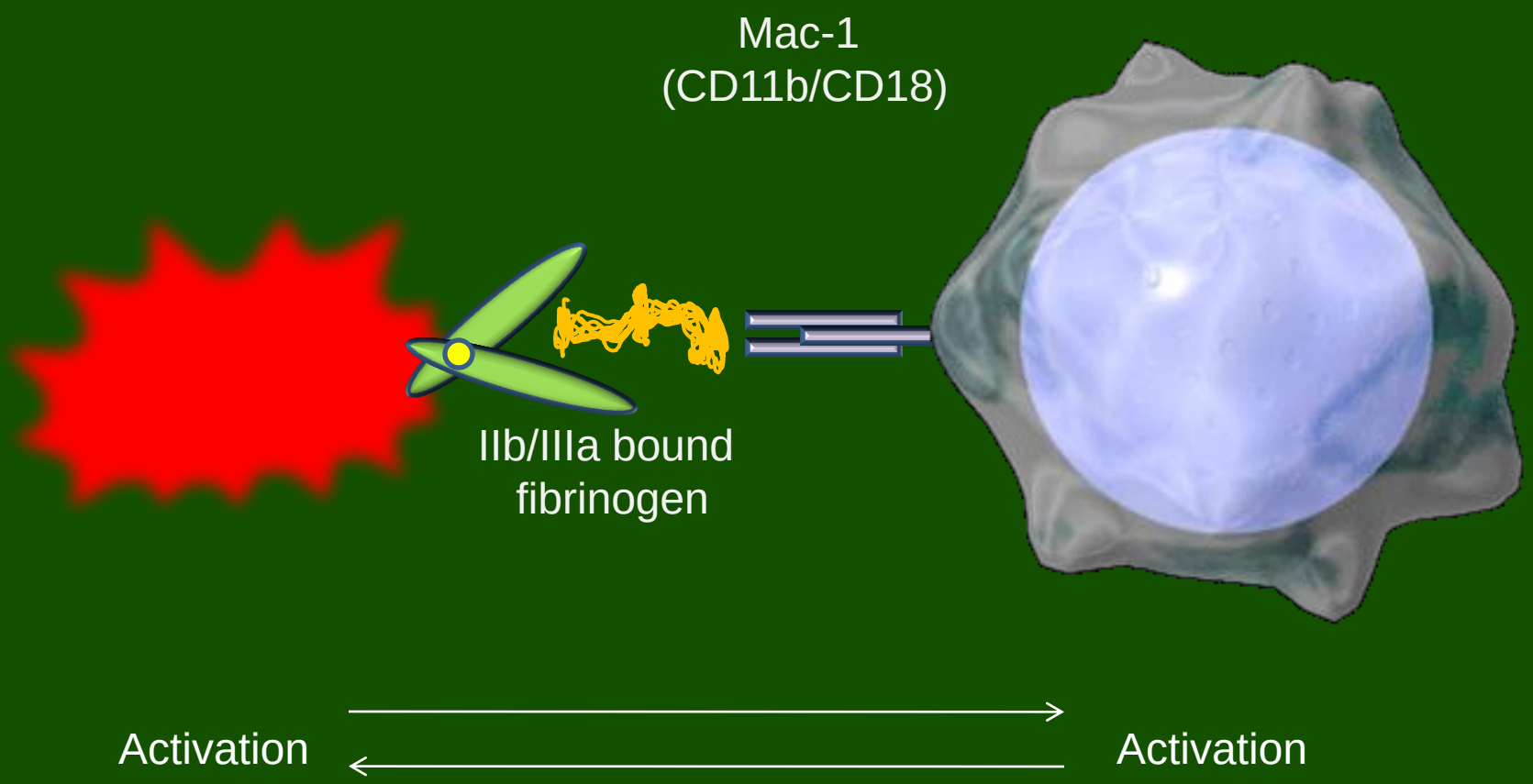


PLT – Leukocyte firm adhesion

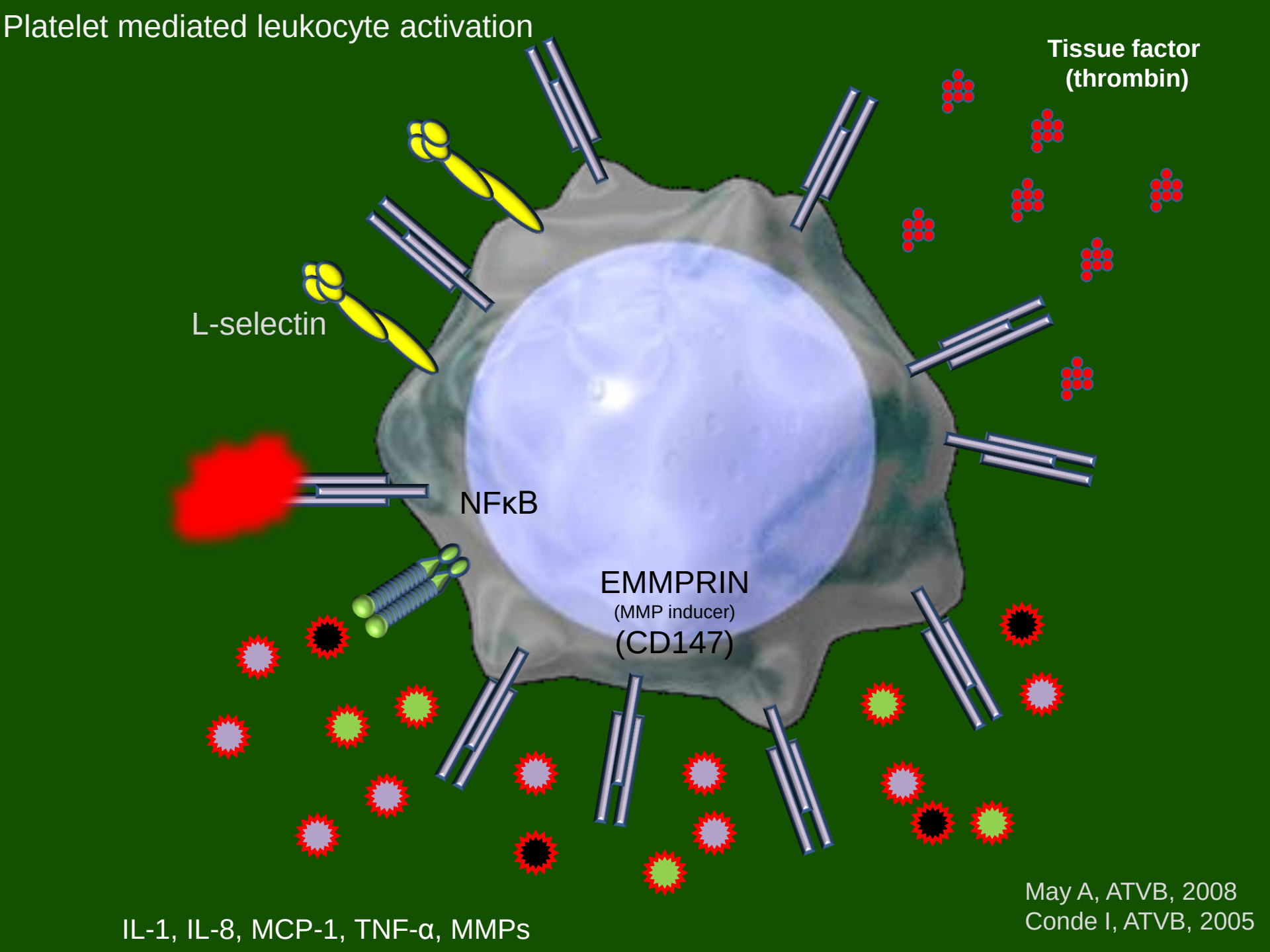


May A, ATVB, 2008

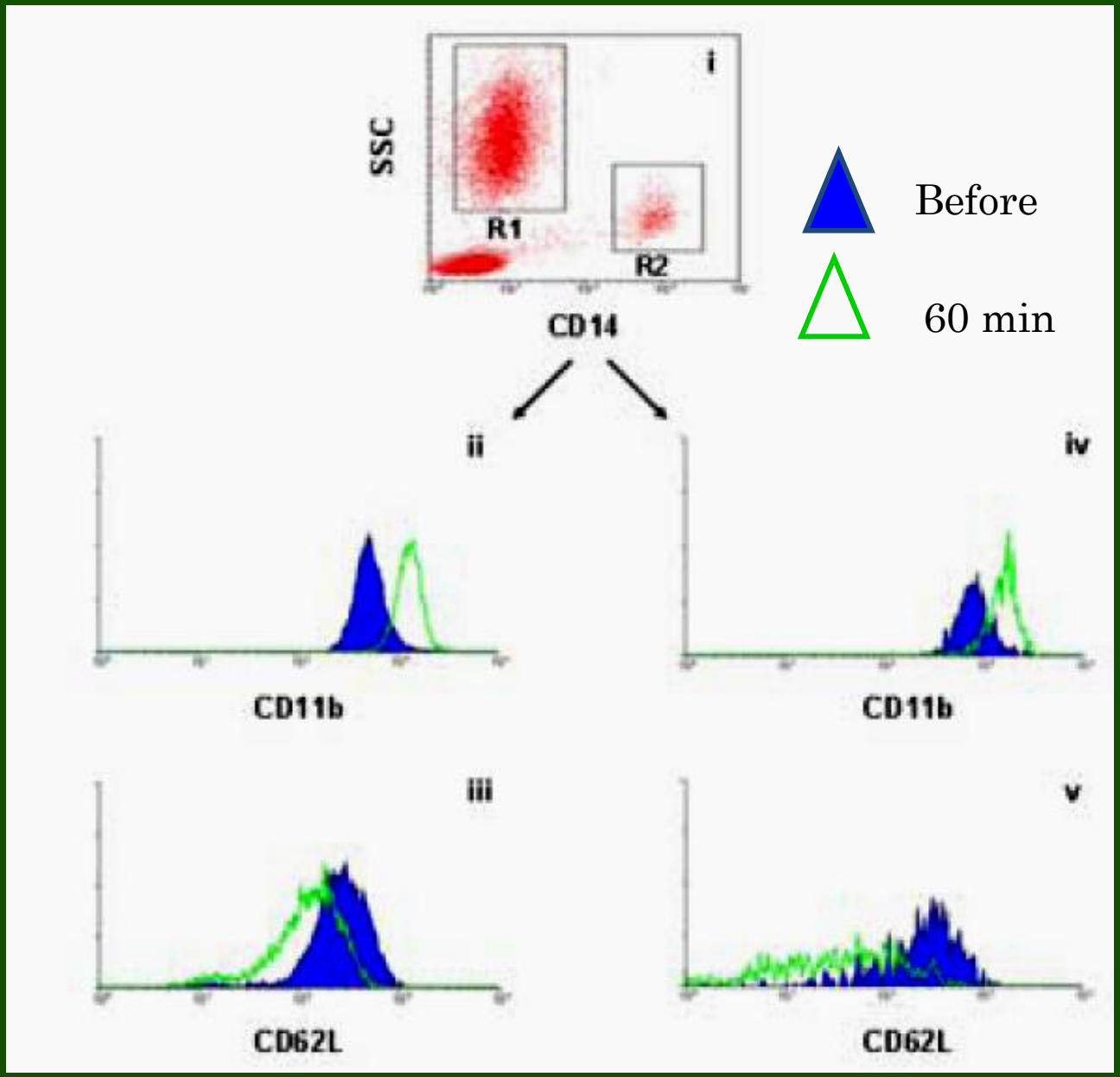
PLT – Leukocyte firm adhesion



May A, ATVB, 2008

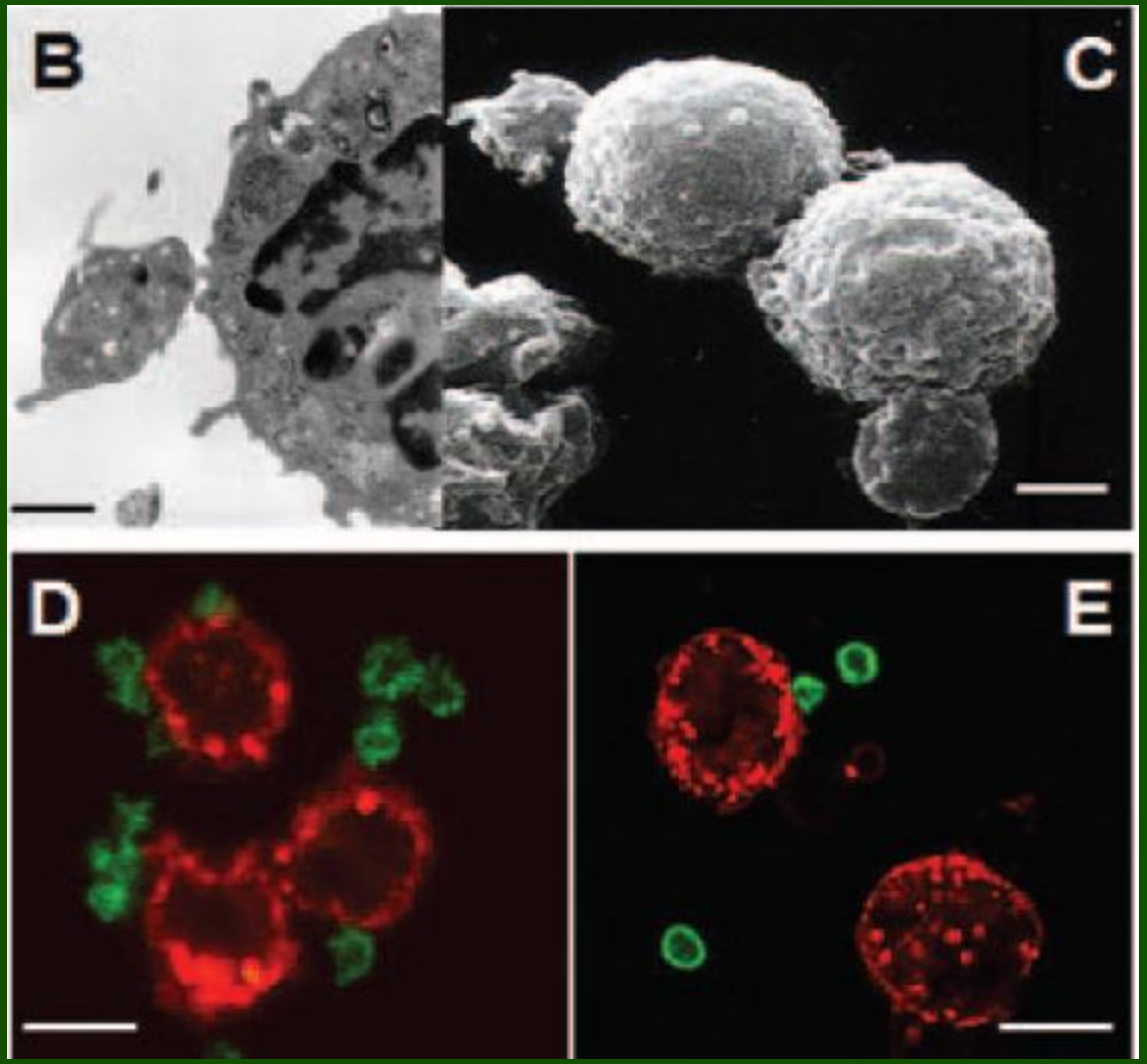

Platelet mediated leukocyte activation
Tissue factor
(thrombin)
L-selectin
NFκB
EMMPRIN
(MMP inducer)
(CD147)
IL-1, IL-8, MCP-1, TNF-α, MMPs
May A, ATVB, 2008
Conde I, ATVB, 2005

CD11b/CD18 up-regulation and CD62L shedding after endothelial injury in humans



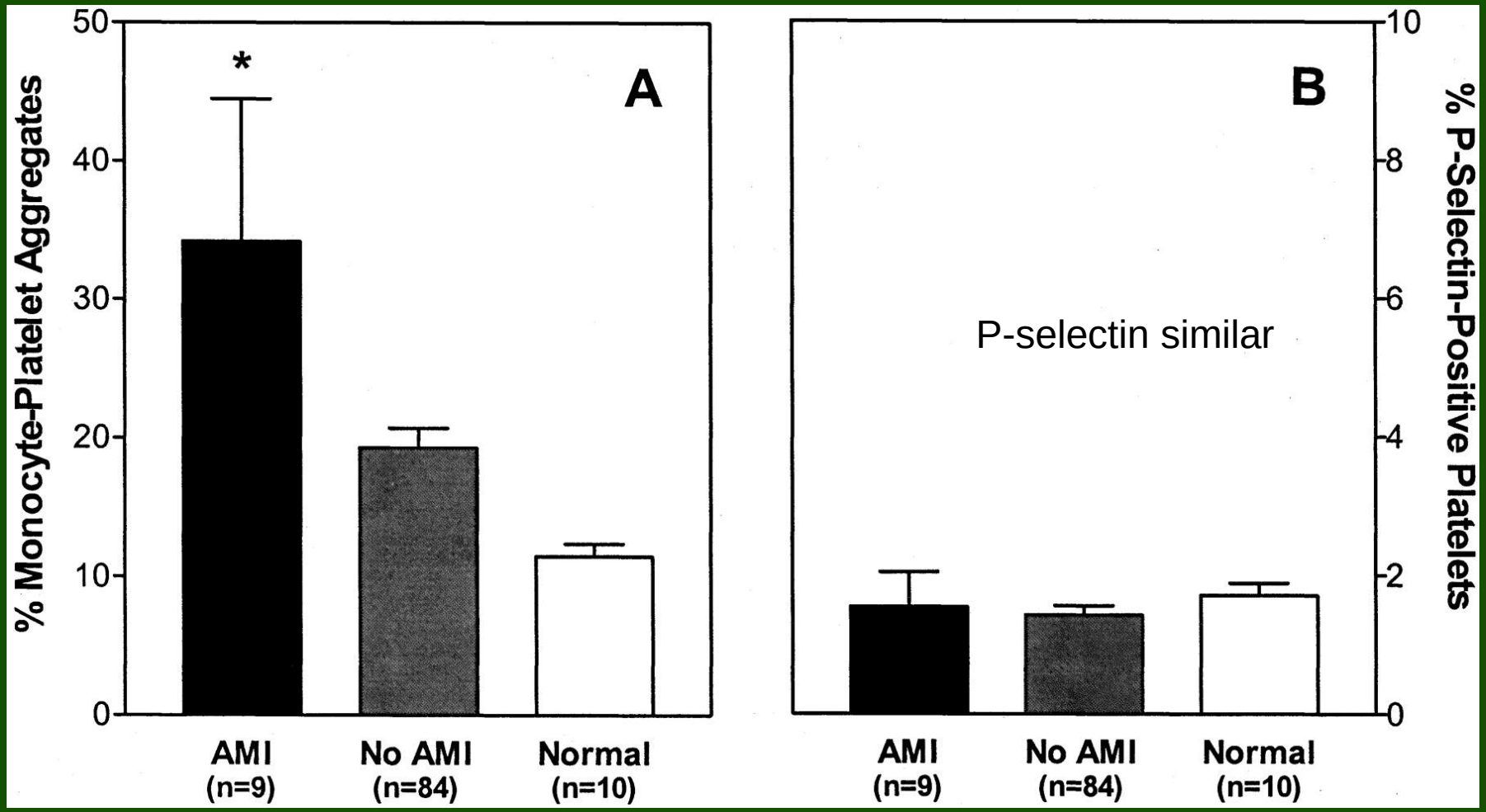
Stakos DA, Coron Artery Dis, 2007

PLT – Leukocyte aggregates (beautiful images! – any clinical importance?)



Bournazos S, ATVB, 2008

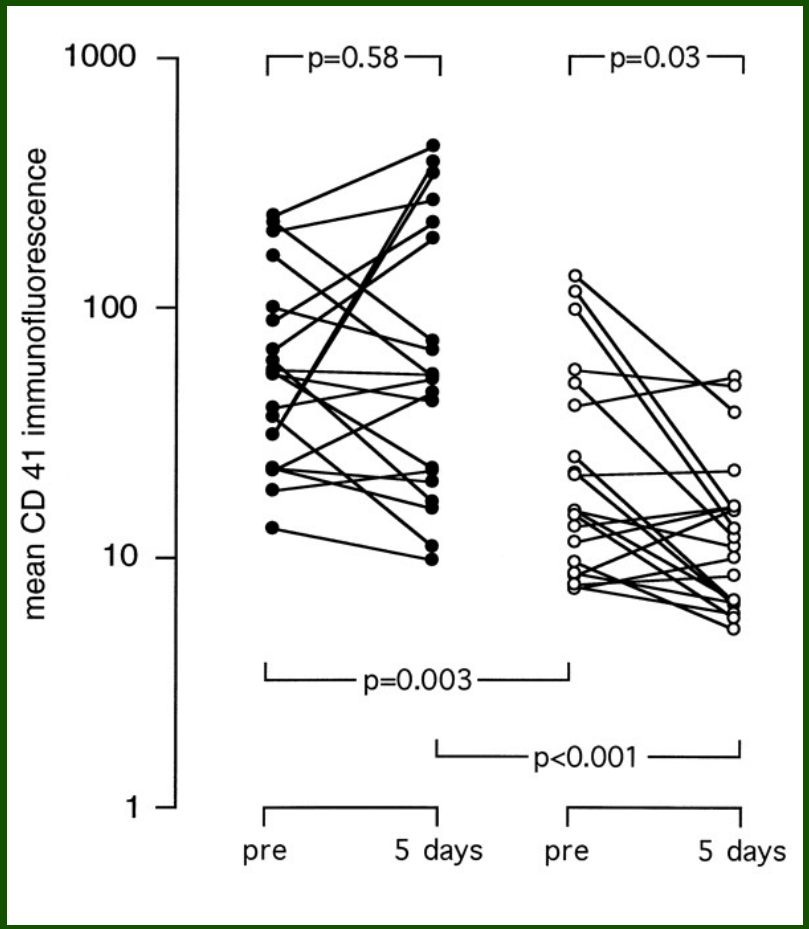
Circulating Monocyte-Platelet Aggregates Are a More Sensitive Marker of In Vivo Platelet Activation Than Platelet Surface P-Selectin. Diagnostic tool?



Michelson AD, Circulation, 2001

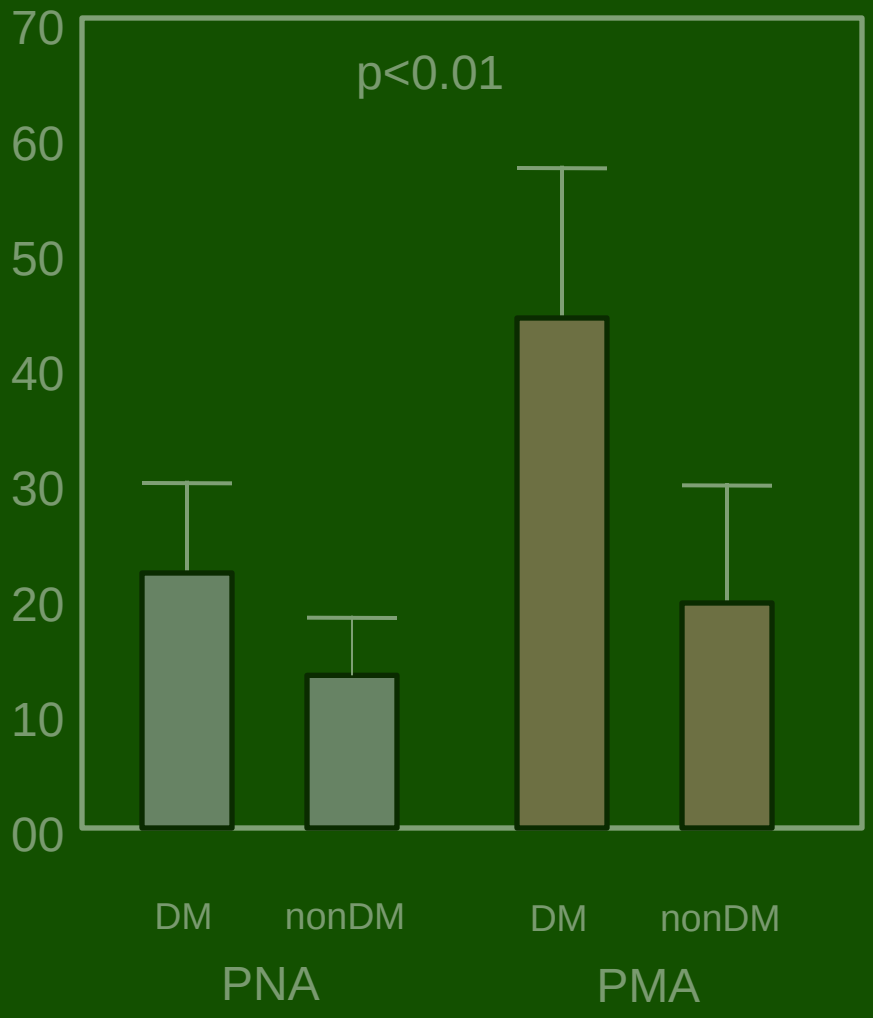
Higher prevalence of PLT-Monoc aggregates in AMI are reduced after successful revascularization

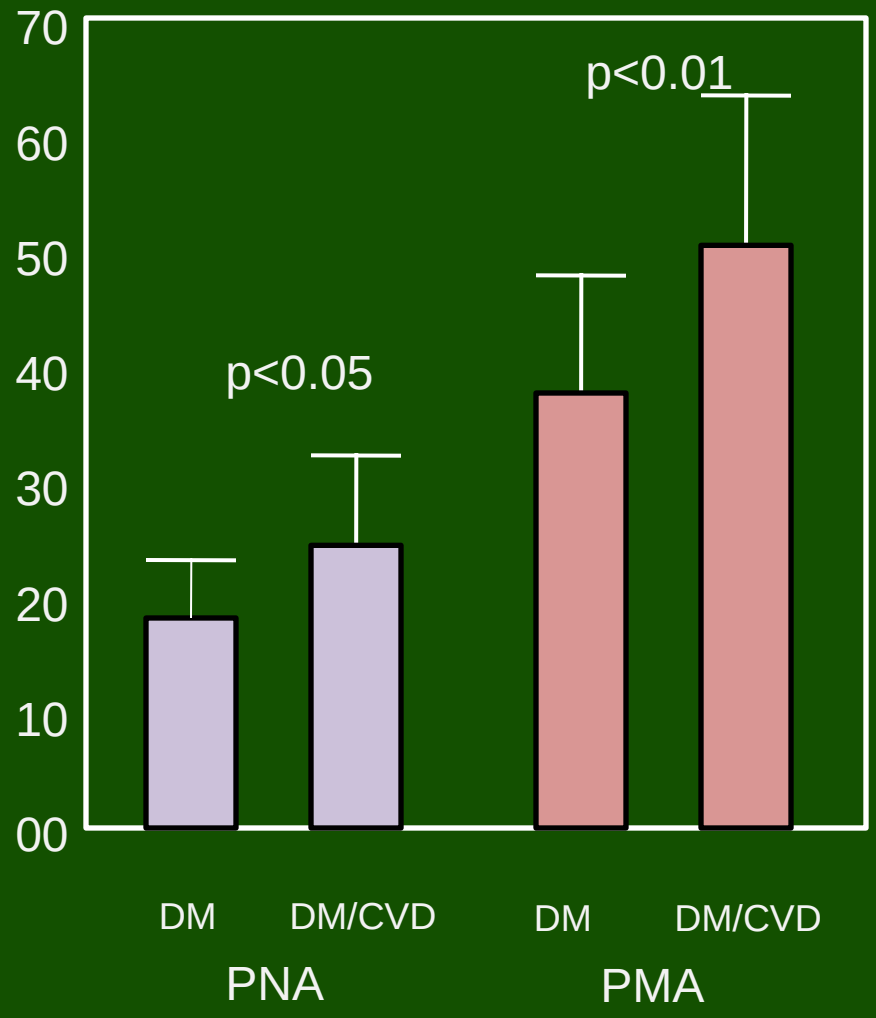
A prognostic marker?



Neumann FJ, Circulation, 1997

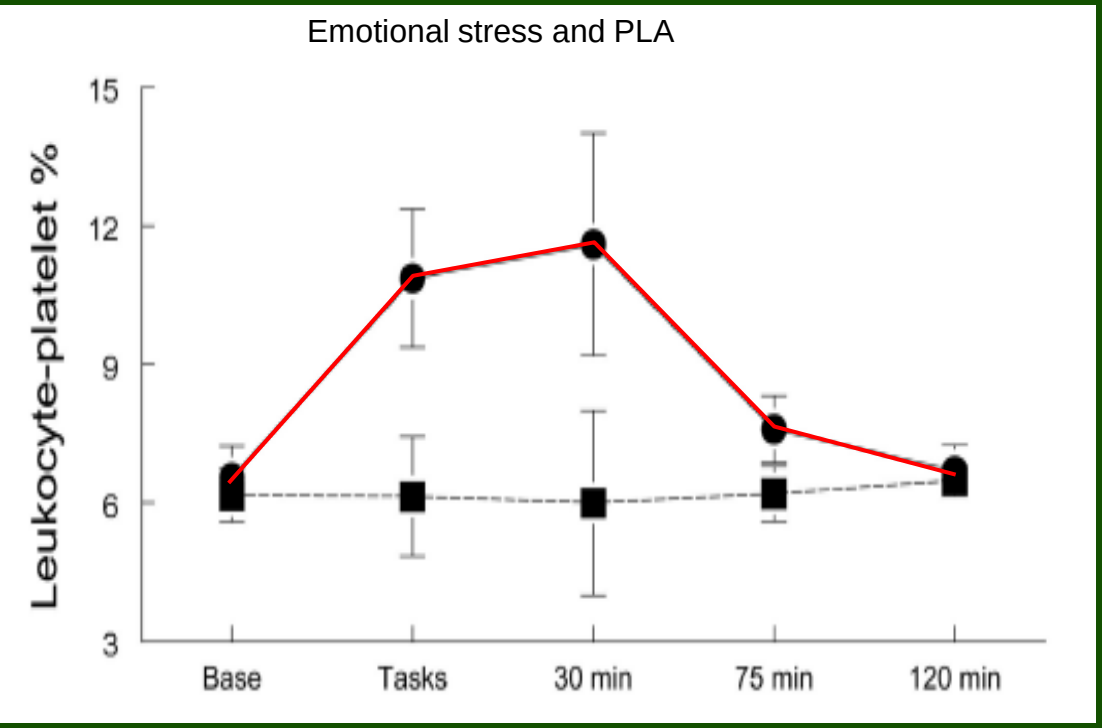
Leuk – PLT Aggregates as markers of CVD in diabetes. Risk stratification?



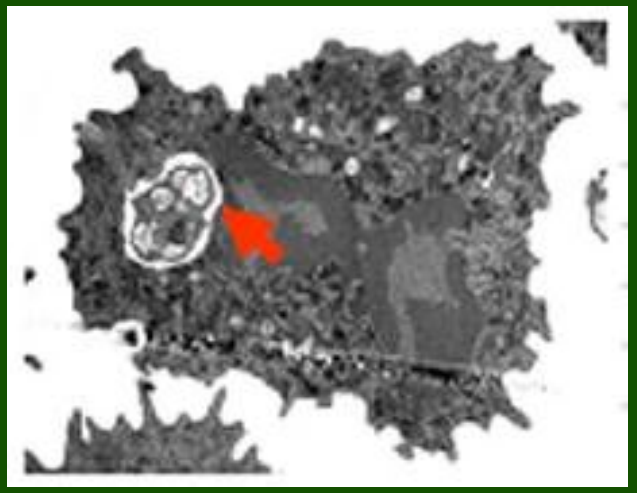


Elalamy I, Thromb Res, 2008

Phagocytic clearance of activated platelets by leukocytes (markers)..



Strike PC, PNAS, 2005

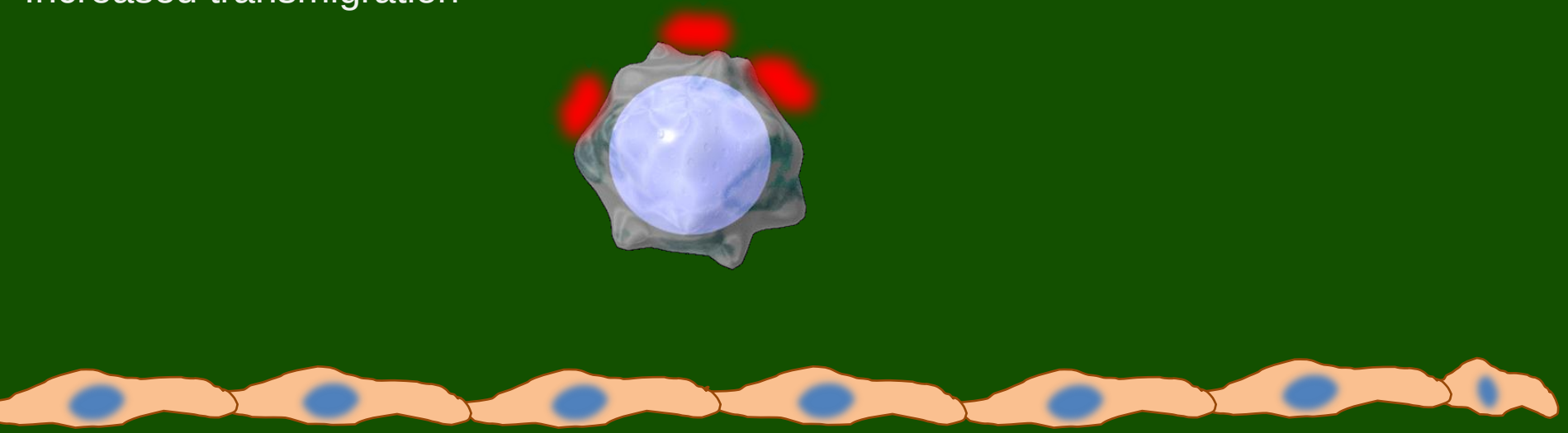


Maugeri N, Blood, 2011

…or active players?

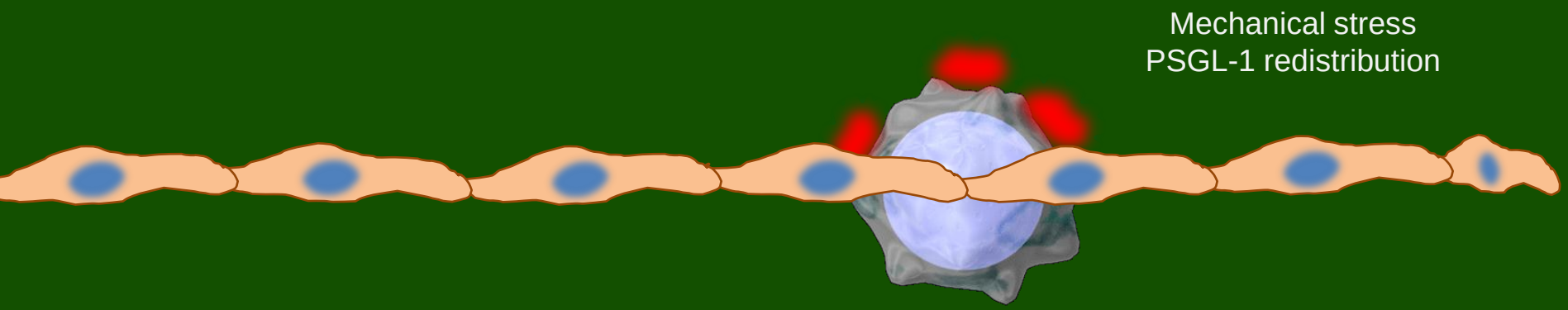
PMA have … compared to PLT-free monocytes

Increased primary (Leuk –EC) and secondary (Leuk-Leuk) tethering
Increased integrin expression and activity
Increased transmigration

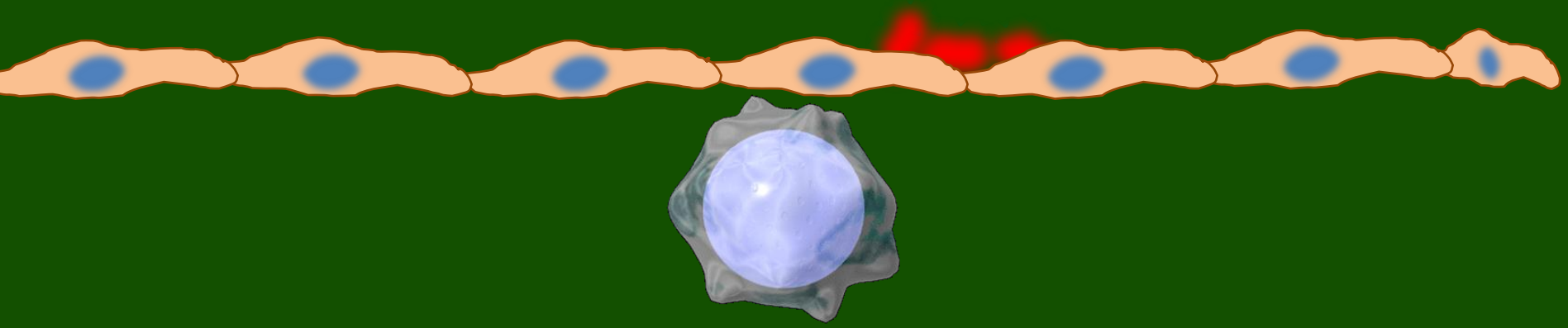


PMA have … compared to PLT-free monocytes

Increased primary (Leuk –EC) and secondary (Leuk-Leuk) tethering
Increased integrin expression and activity
Increased transmigration



Contribute to (PMA mediated) PLT deposition



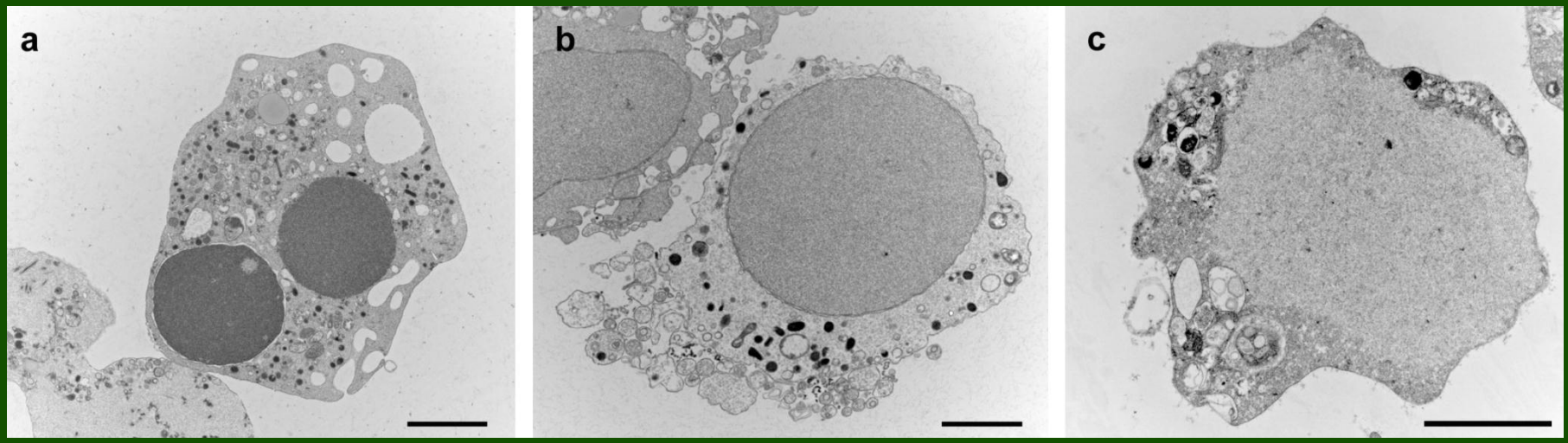
Newer forms of PLT-Leukocyte interactions

The Neutrophil Extracellular Traps (NETs)

NETs are extracellular DNA fibers (taken out of the cell) and neutrophil antimicrobial proteins after Neutrophil suicide in tissues or blood stream

Neutrophil Extracellular Traps (NET) interactions with Platelets

Neutrophil treated with anti-Fas antibodies



apoptotic morphology

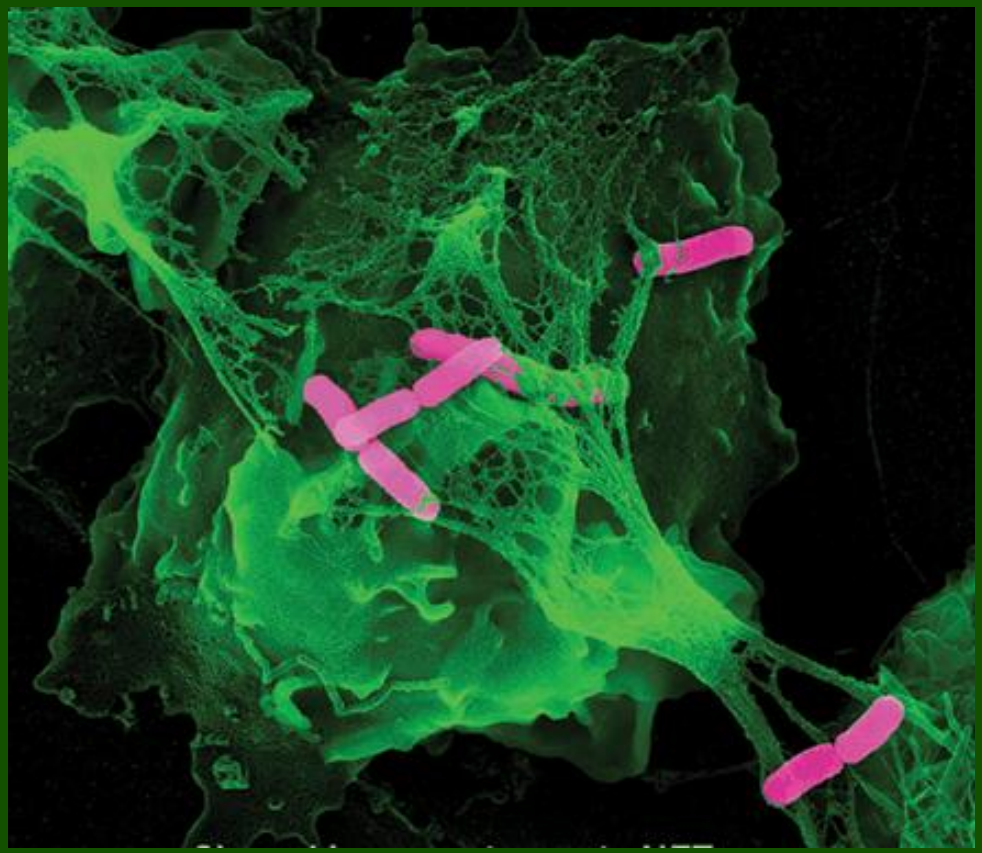
Nuclear alterations

mixing of nuclear, cytoplasmic, and granular components

cell death and NET formation

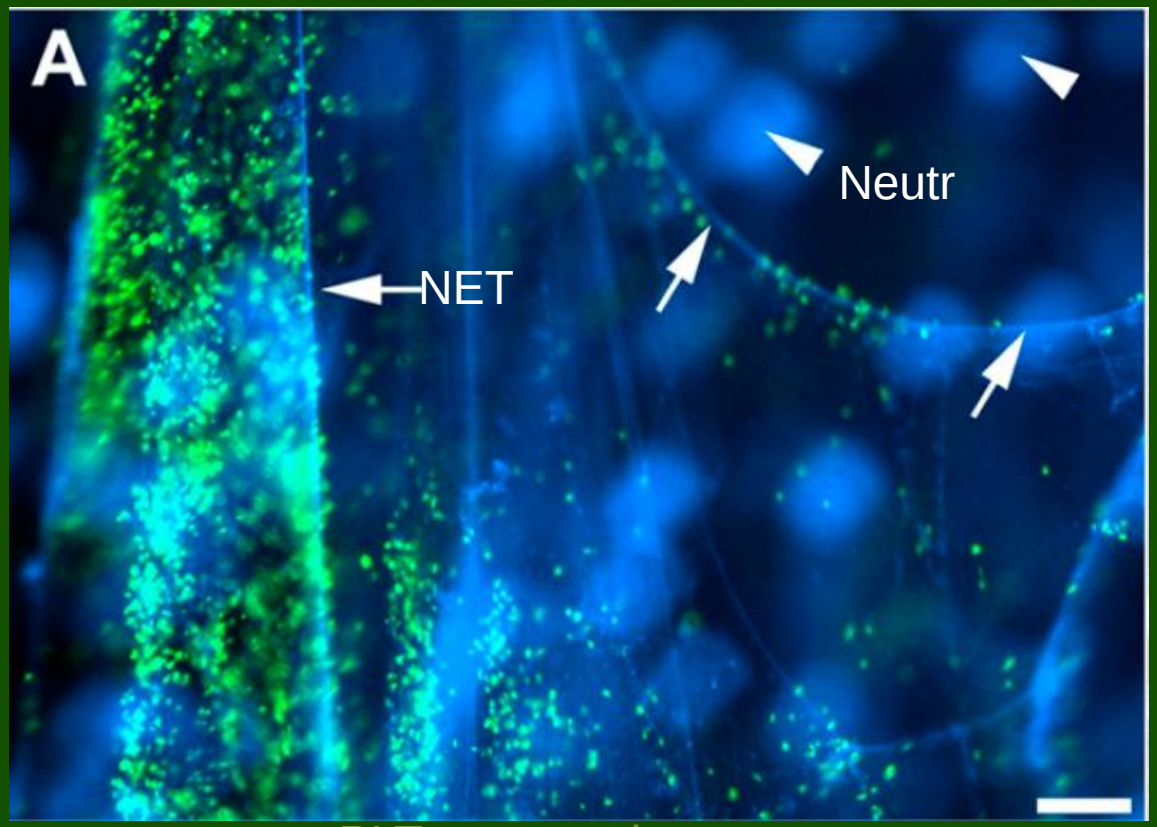
Fuchs TA, J Cell Biol, 2007

Neutrophil Extracellular Traps (NET) interactions

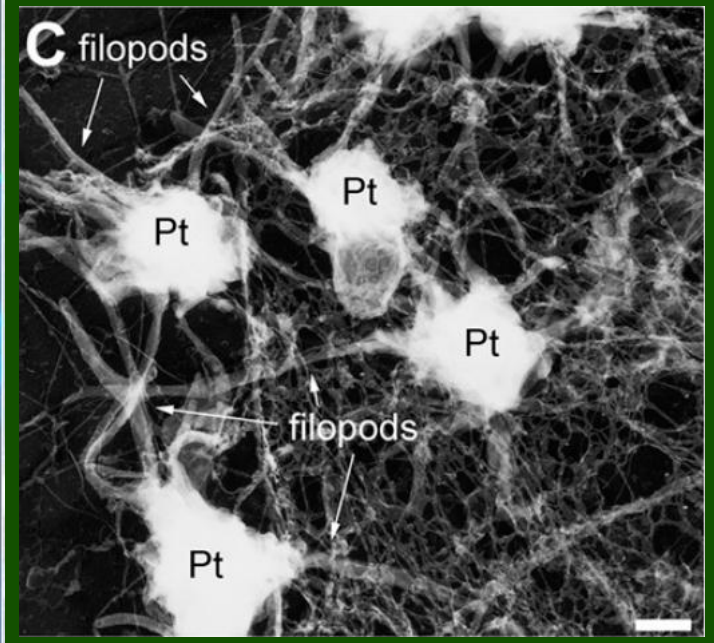


Brinkmann V, Science, 2004

Neutrophil Extracellular Traps (NET) - Platelet immobilization – activation – thrombus?



PLTs captured green



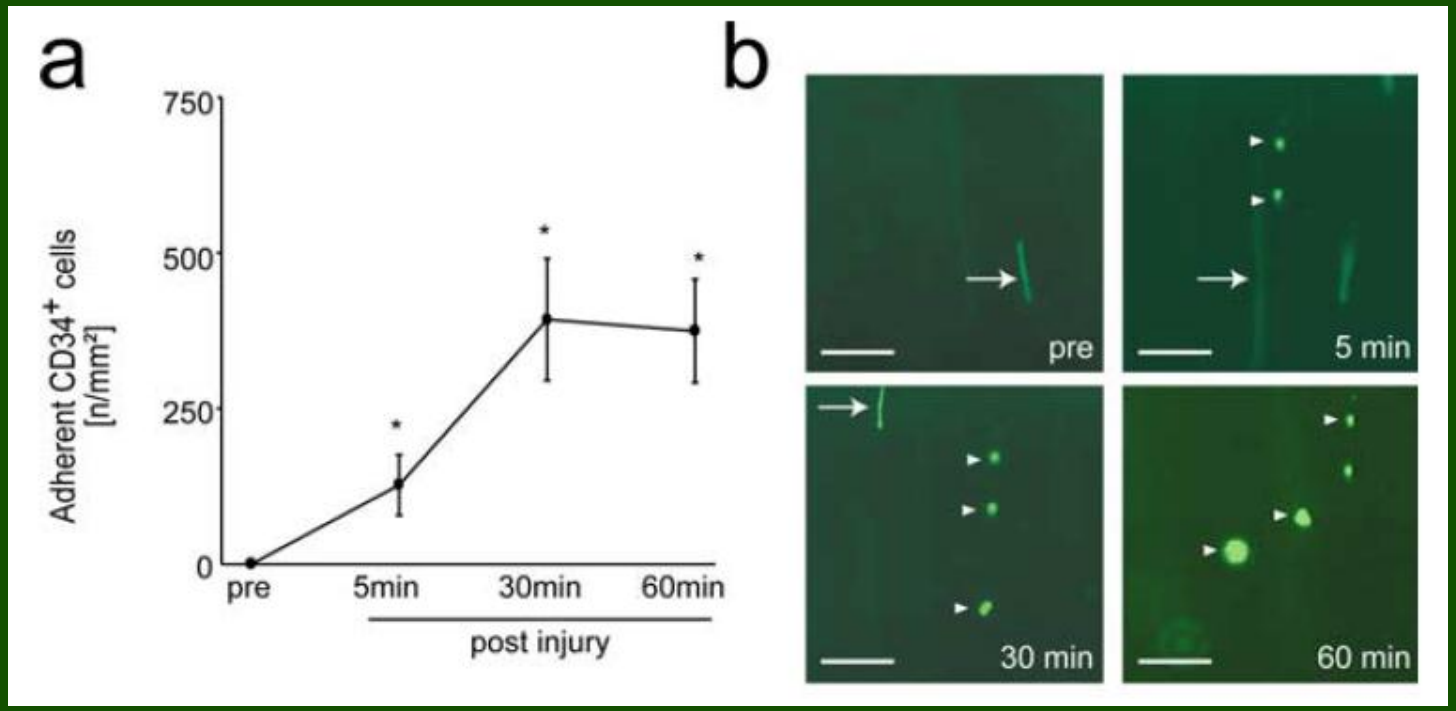
Cause or effect ?

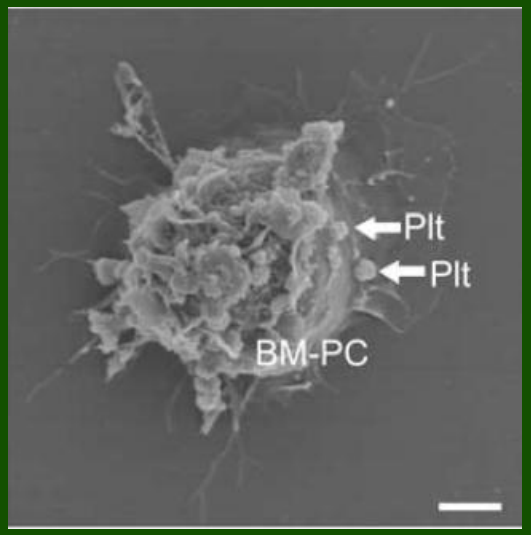
NETs are present in red thrombi together with RBCs

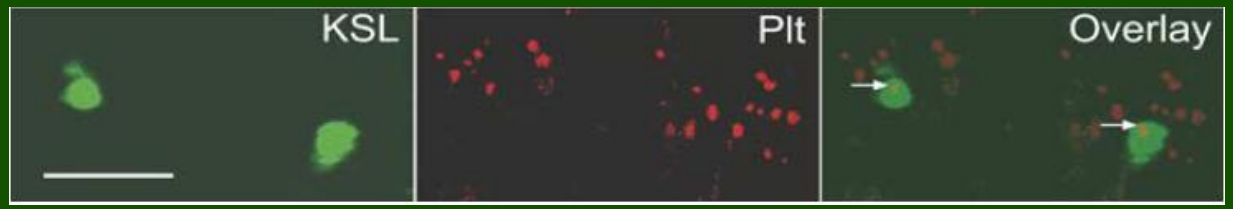
Fuchs TA, PNAS, 2010

Platelet and endothelial progenitor cells

Platelet recruit endothelial progenitor cells at sites of vascular injury

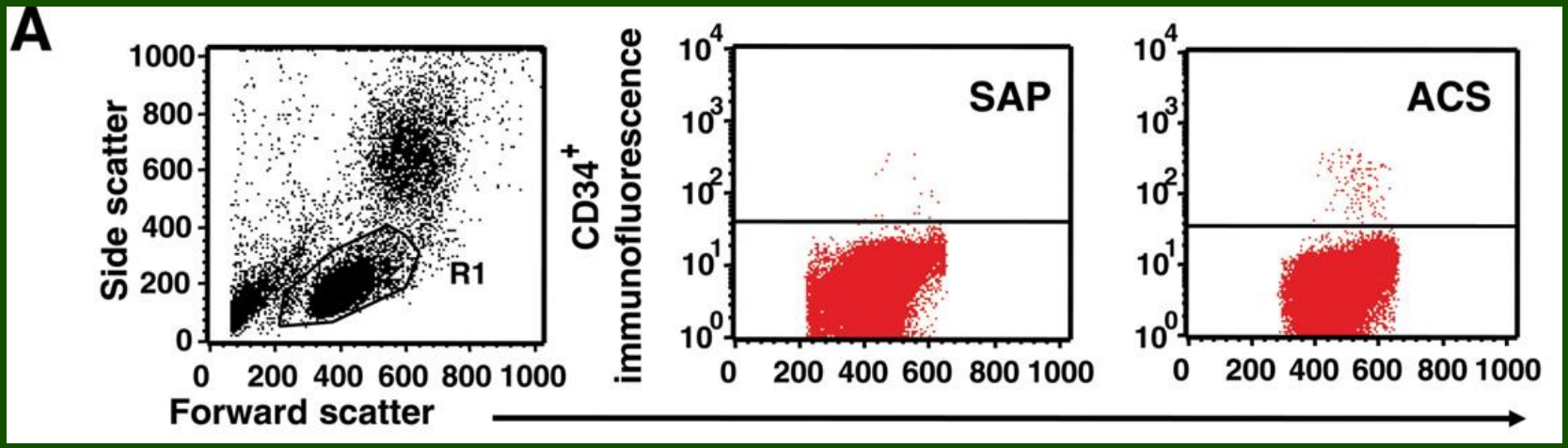


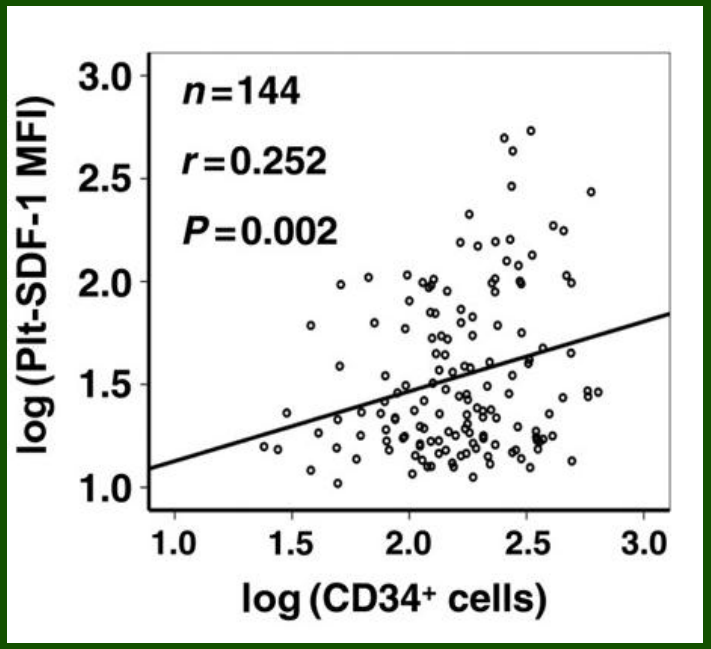




Massberg S, J Exp Med, 2006

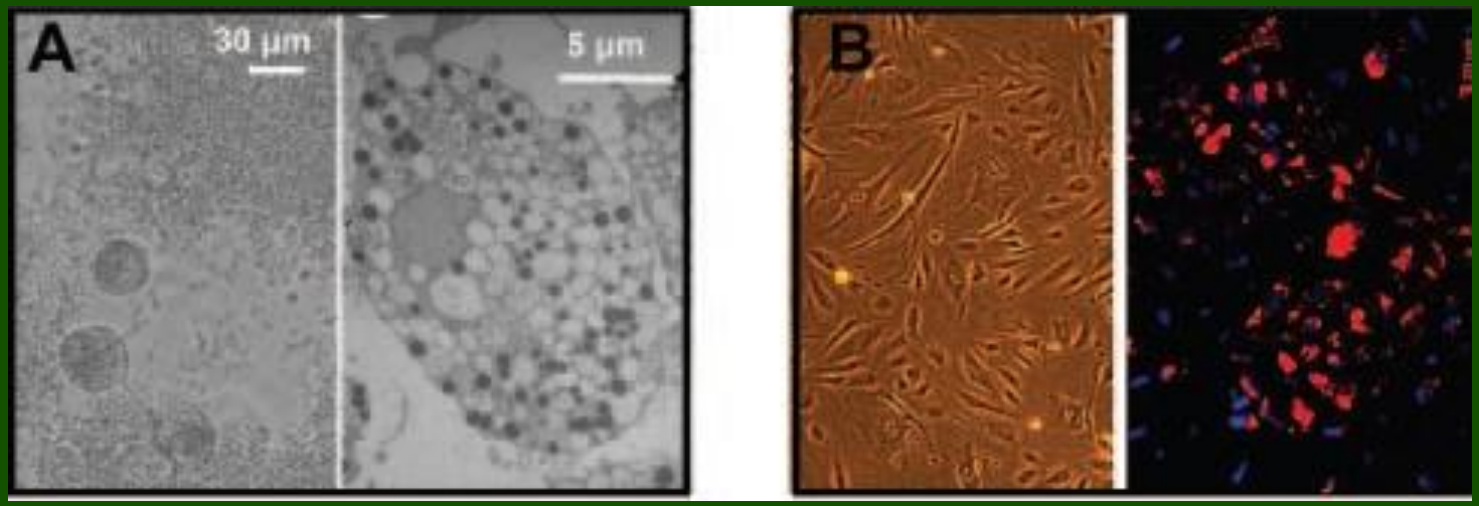
Platelet recruit endothelial progenitor cells at sites of vascular injury via SDF-1





Stellos K, Stakos D. Eur Heart J, 2009

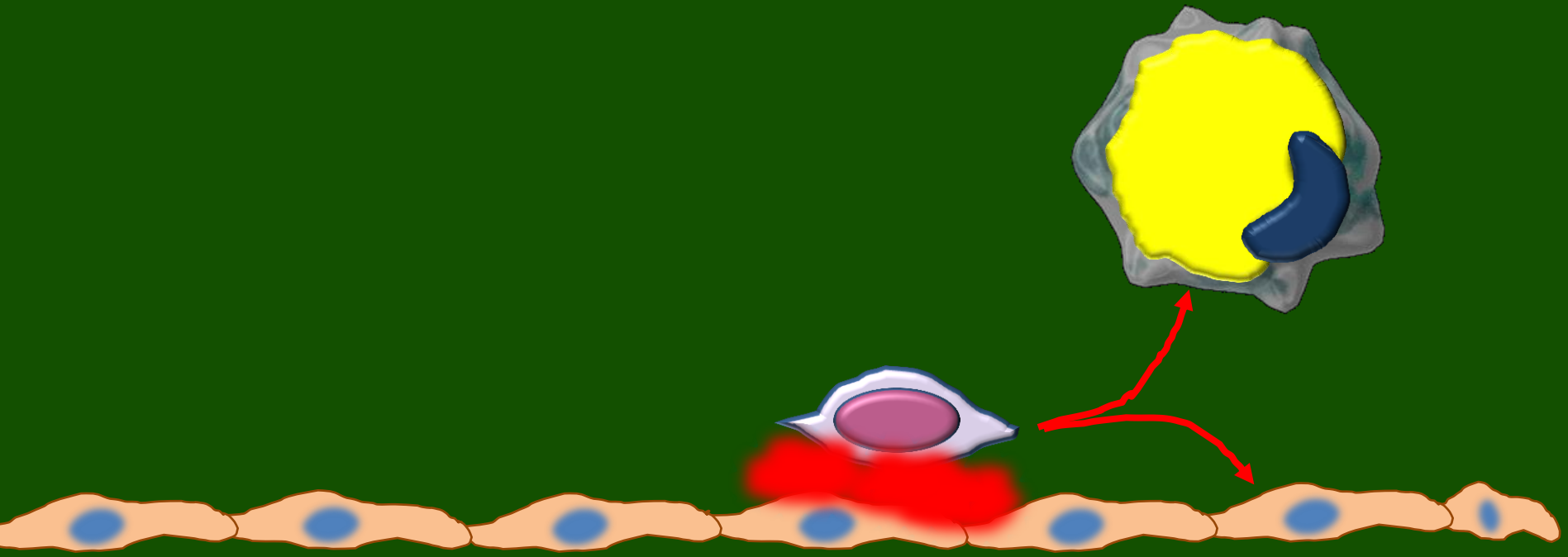
Platelet and endothelial progenitor cells Two different ways of differentiation



Daub K, FASEB, 2006
Langer H, Circ Res, 2006

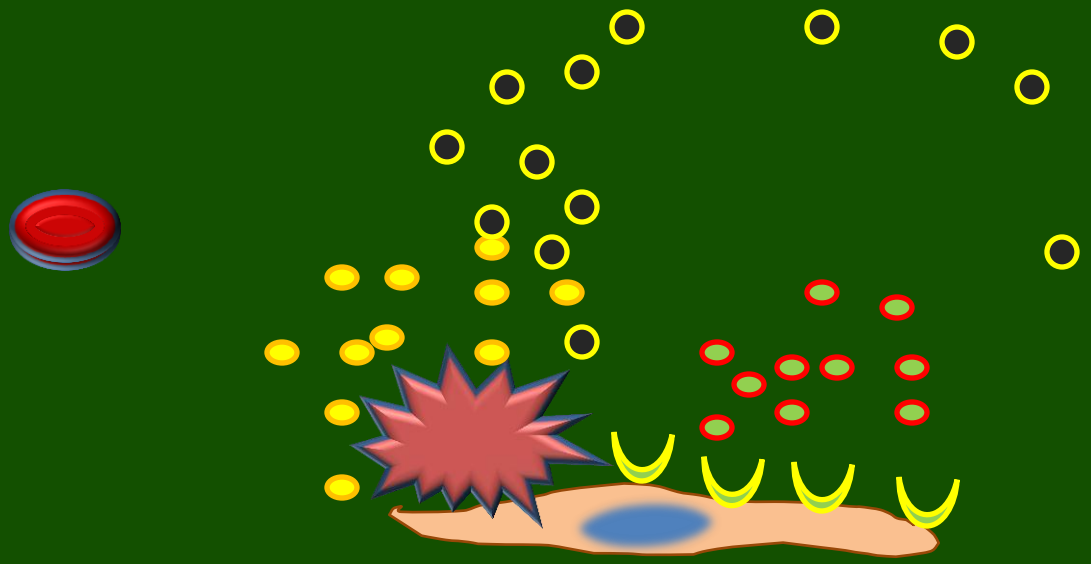
PLTs + EPCs → CD68+ (foam cells)

PLTs + EPCs → Ecs (repair)



Conclusions

Platelet adhesion to Endothelium→ Promotes Cell “Inter-activation”



Adhesion molecules
Cytokines
Chemoattractants
Cell interactions

End of presentation click to exit

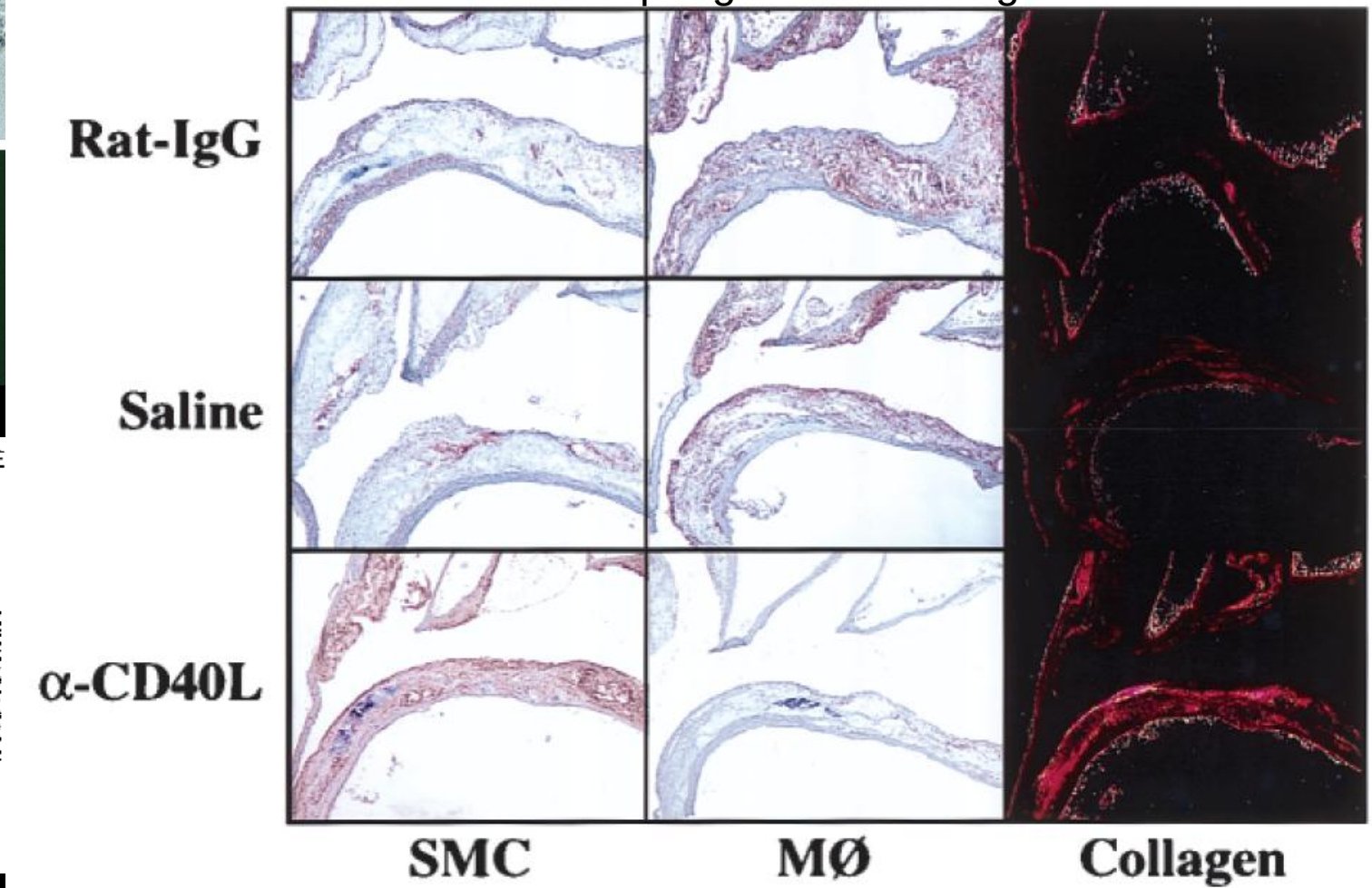
Can these data be transferred to human situation?

If platelets initiate atherosclerosis…

Will long-term antiplatelet / anti-inflammatory therapy be used as primary prevention at early stages of atherosclerosis, at least in high risk individuals ?

Clinical implications

Fewer macrophages more collagen more stable



Since atherosclerosis involves ECs, leukocytes, PLTs and their interactions, then targeting all players simultaneously may be more effective than only one (platelets) as we do now

If indeed platelets are inflammatory cells, then targeting their pro-inflammatory activity rather than PLT aggregation will be an attractive alternative area of research for limiting CVD

Thank you for your attention