C-reactive protein

; *Friend or Foe ?*

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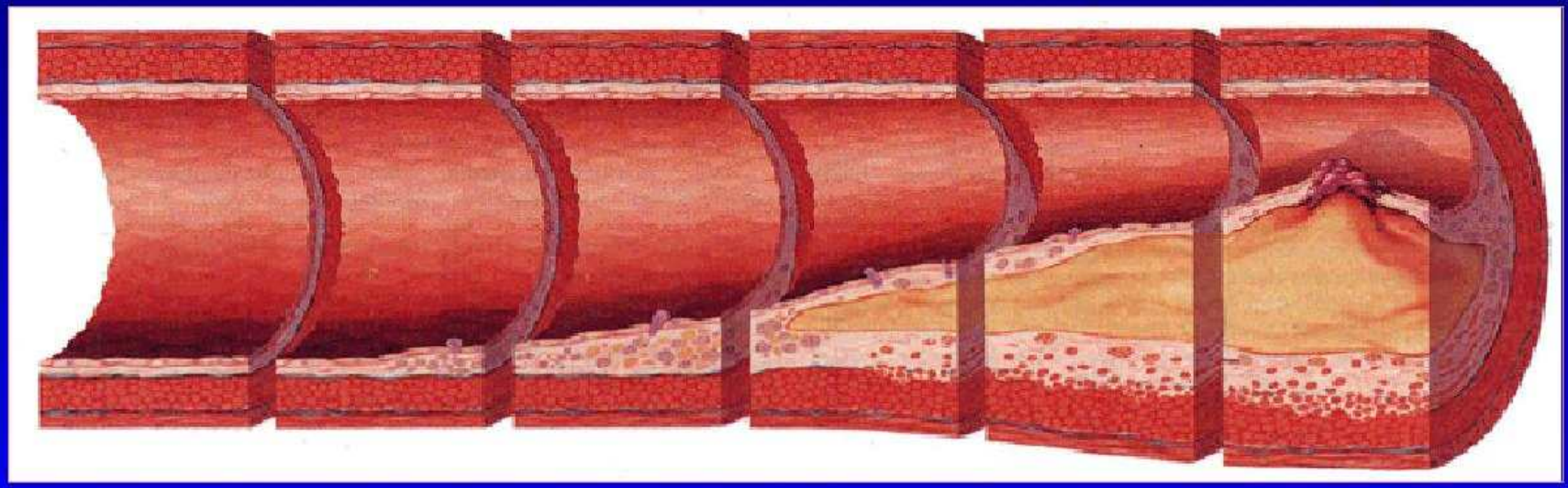
I. Inflammation and atherogenesis

"Atherosclerosis is an inflammatory disease"

Atherosclerosis Timeline

Endothelial Dysfunction

Foam Cells	Fatty Streak	Intermediate Lesion	Atheroma	Fibrous Plaque	Complicated Lesion/Rupture

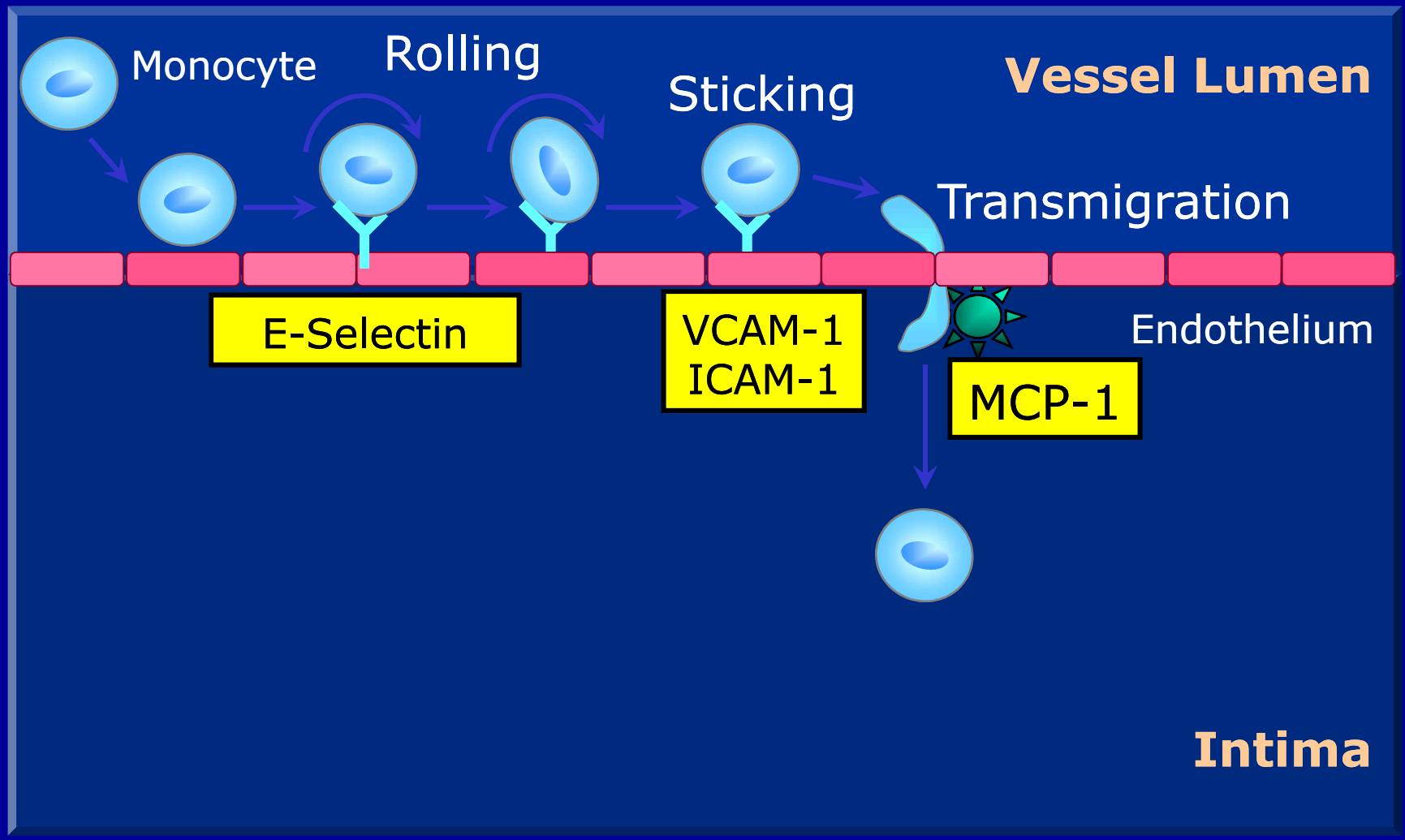


From first decade	From third decade	From fourth decade

Growth mainly by lipid accumulation	Smooth muscle and collagen	Thrombosis, hematoma

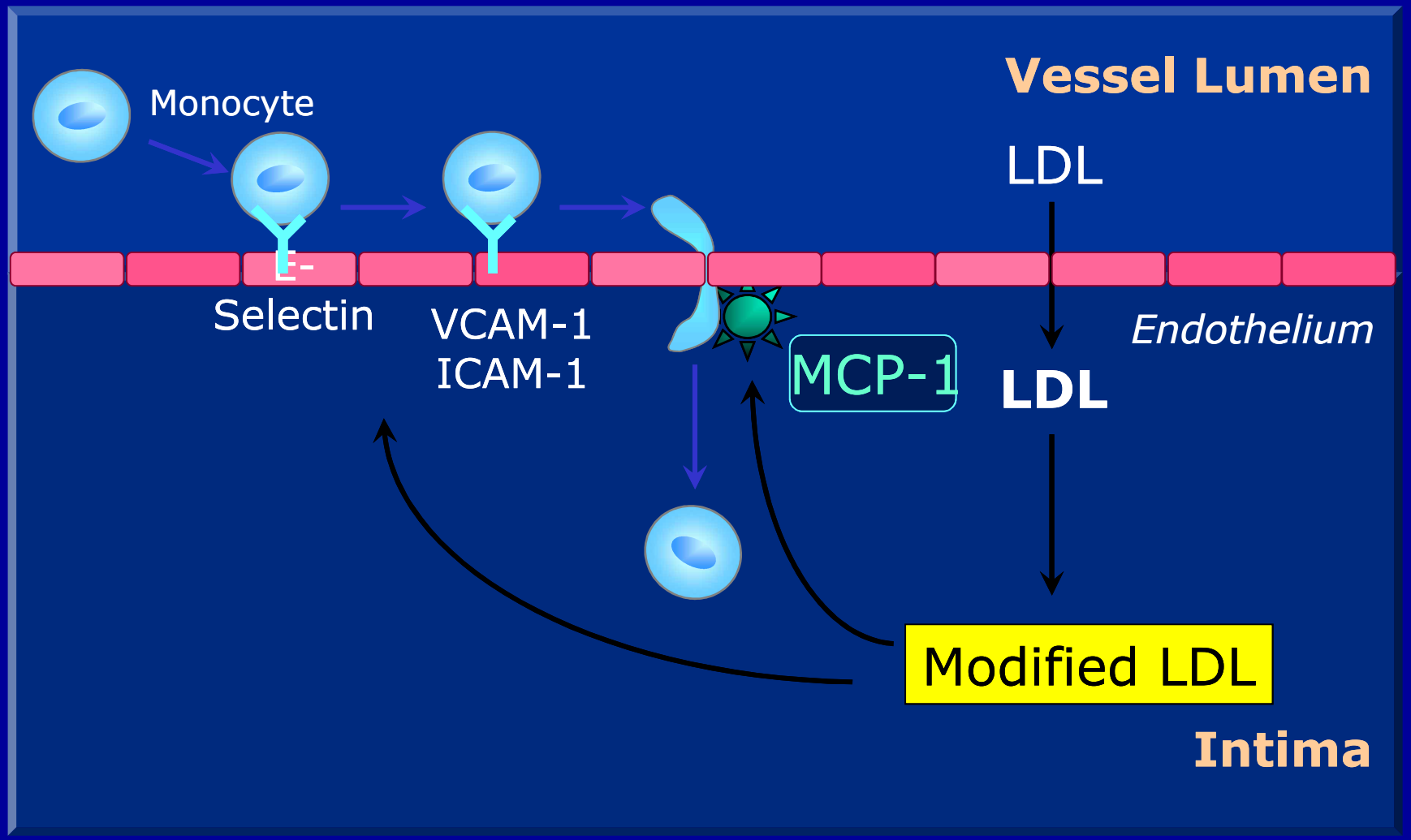
Stary et al. *Circulation*. 1995;92:1355-1374.

Recruitment of Blood Monocytes by Endothelial Cell Adhesion Molecules



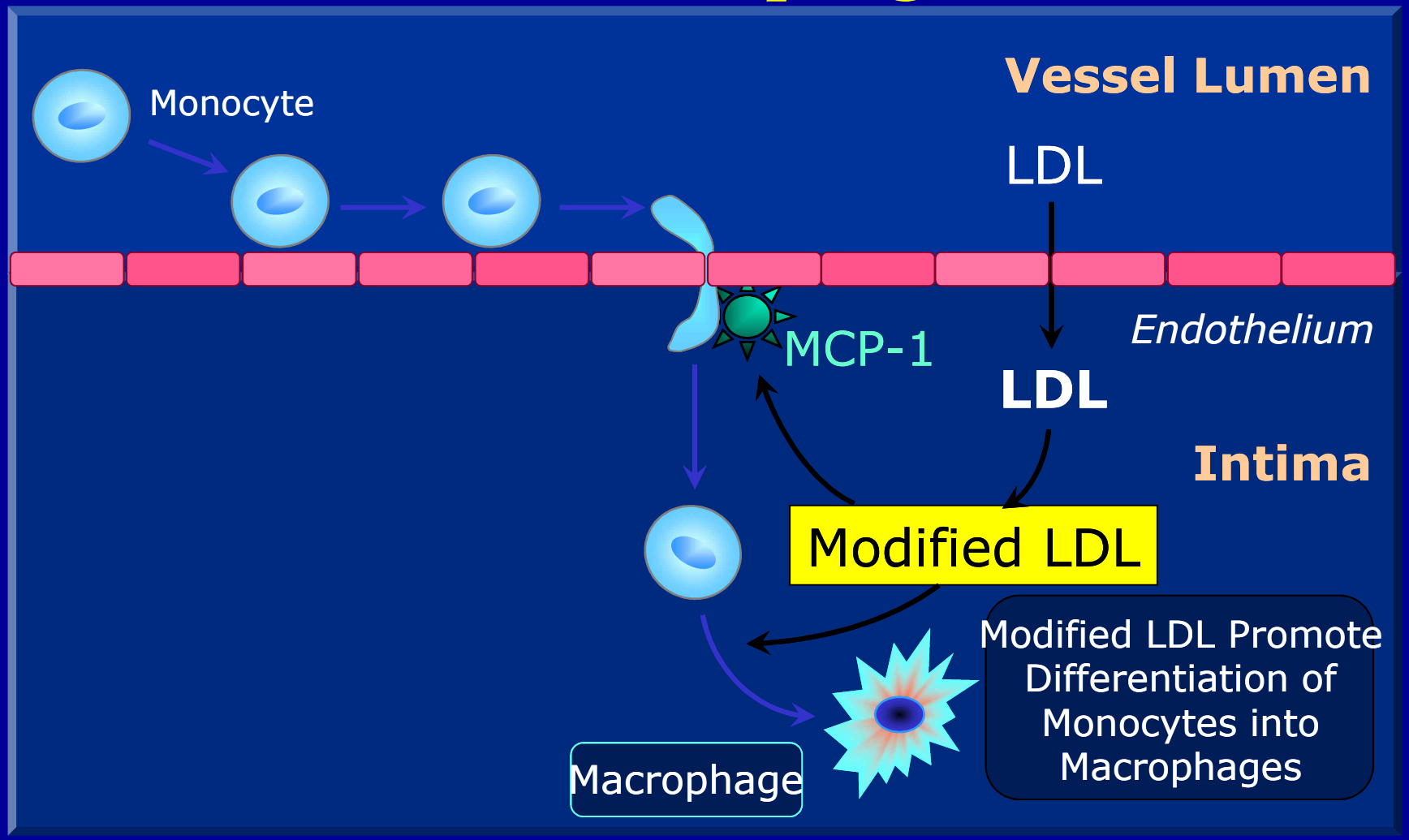
Charo IF. *Curr Opin Lipidol* 1992;3:335-343.

Modified LDL Stimulates Expression of MCP-1/adhesion molecules in Endothelial Cells



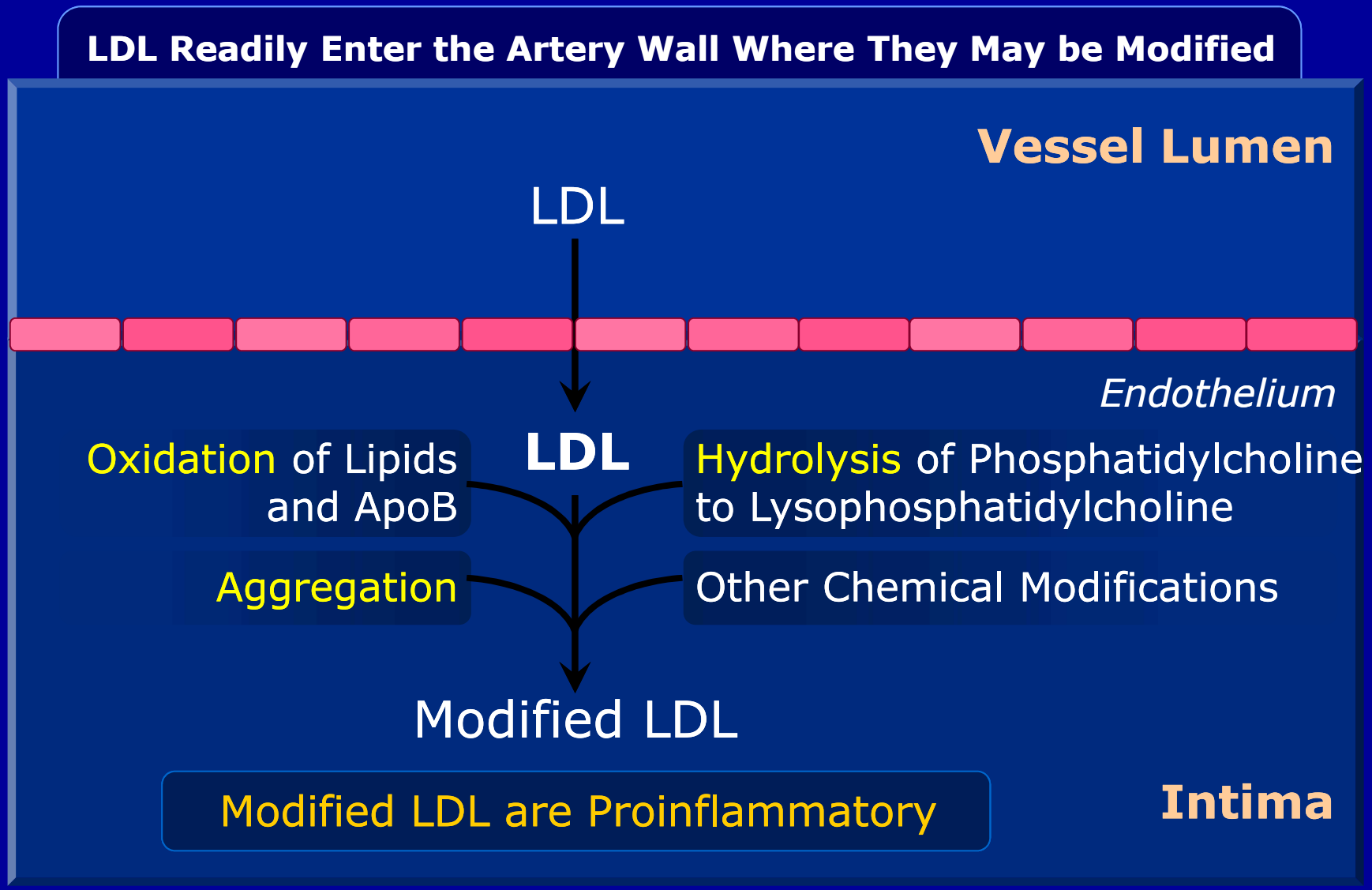
Navab M et al. *J Clin Invest* 1991;88:2039-2046.

Differentiation of Monocytes into Macrophages



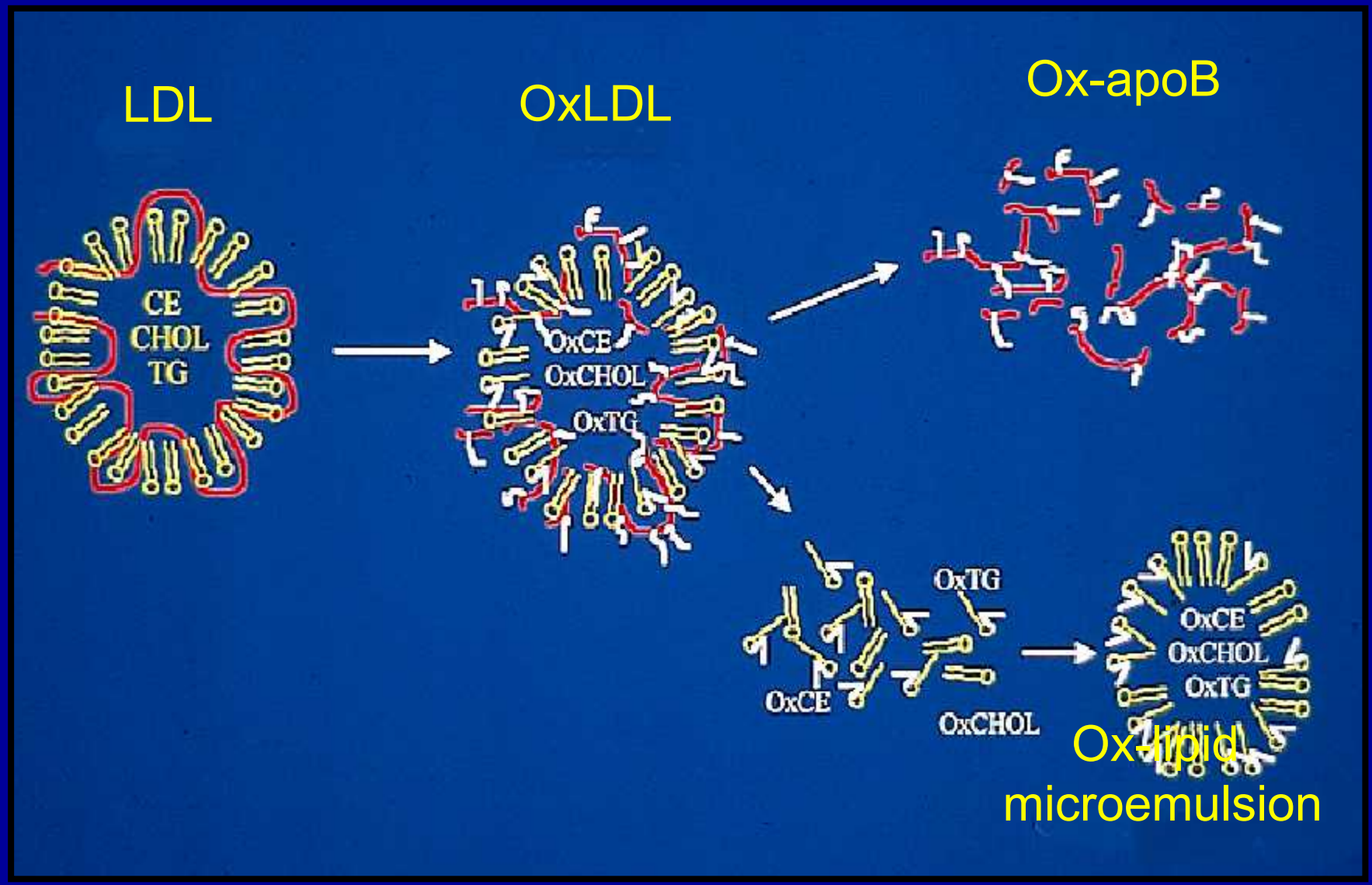
Steinberg D et al. *N Engl J Med* 1989;320:915-924.

The process of LDL modification

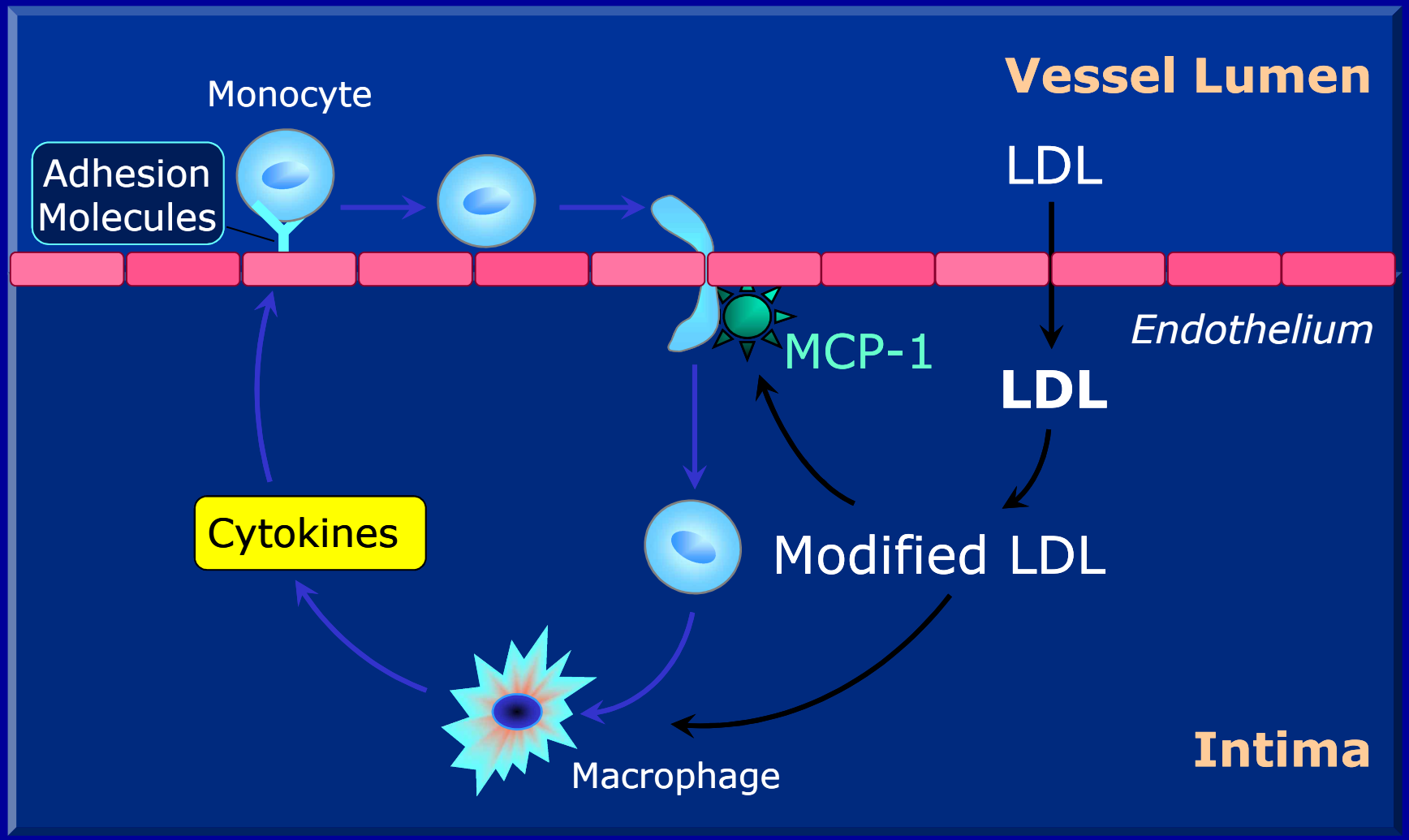


Steinberg D et al. *N Engl J Med* 1989;320:915-924.

Oxidation changes components of LDL particles

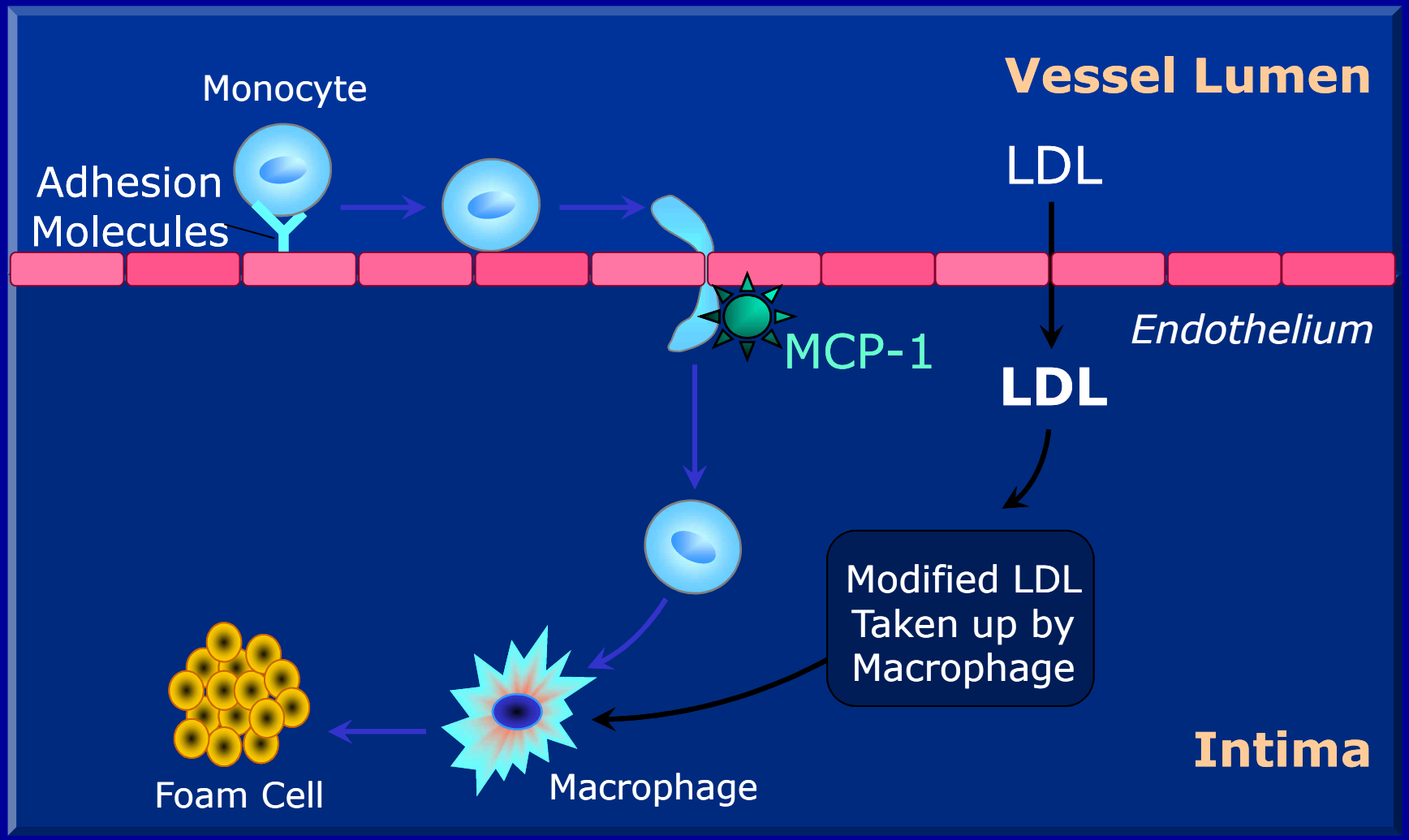


Modified LDL Induces Macrophages to Release Cytokines That Stimulate Adhesion Molecule Expression in Endothelial Cells



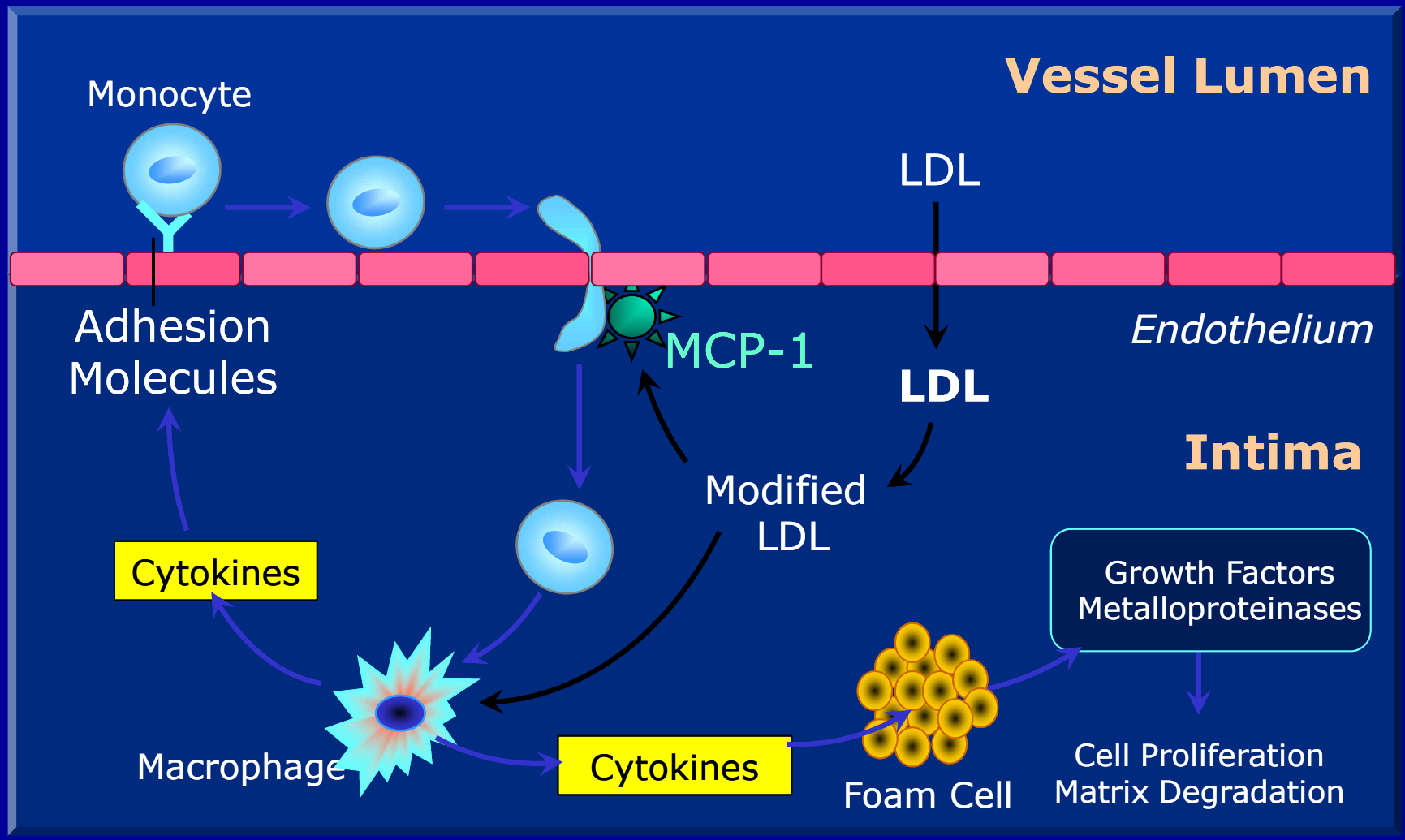
Nathan CF. *J Clin Invest* 1987;79:319-326.

Macrophages Express Receptors That Take up Modified LDL



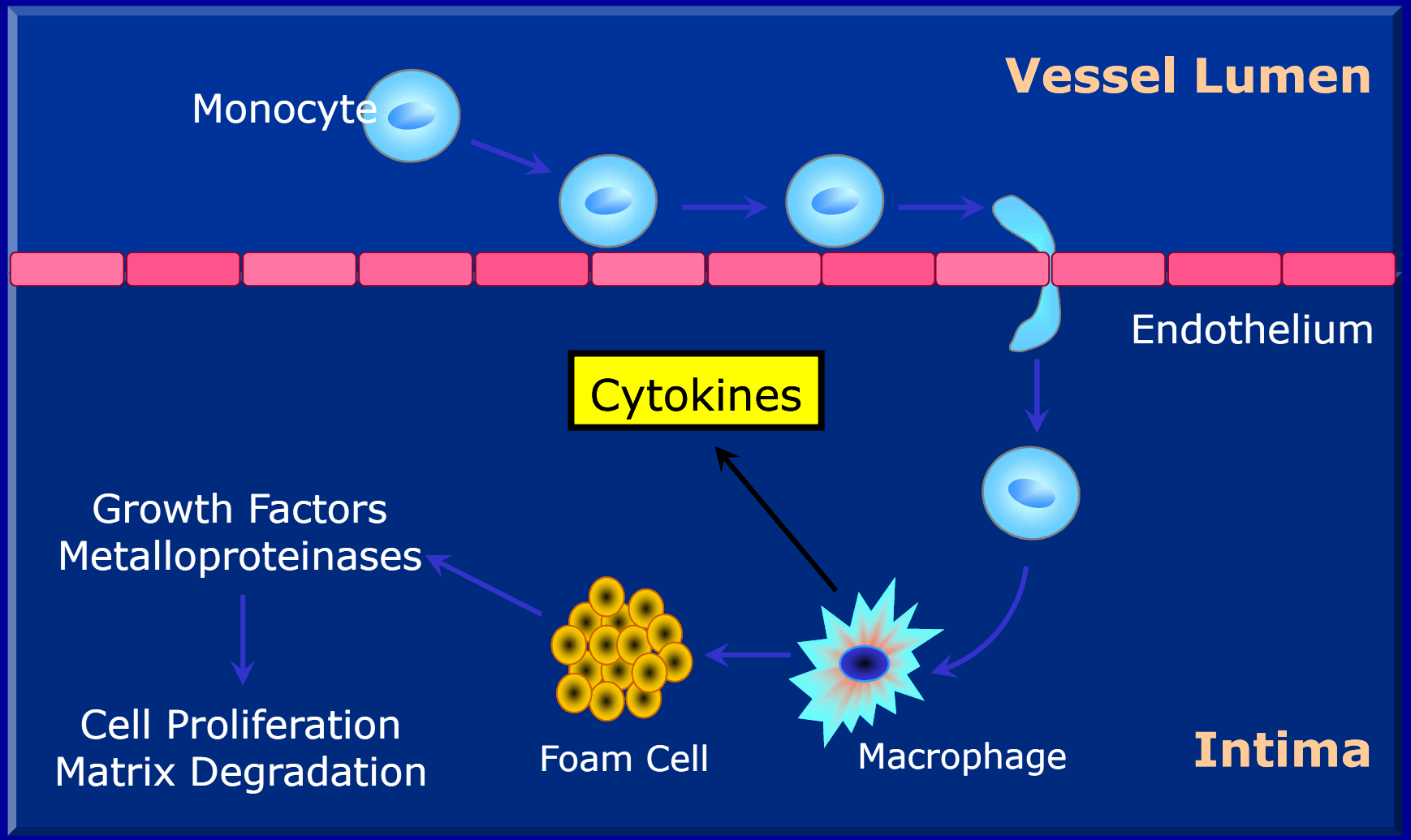
Steinberg D et al. *N Engl J Med* 1989;320:915-924.

Macrophages and Foam Cells Express Growth Factors and Proteinases

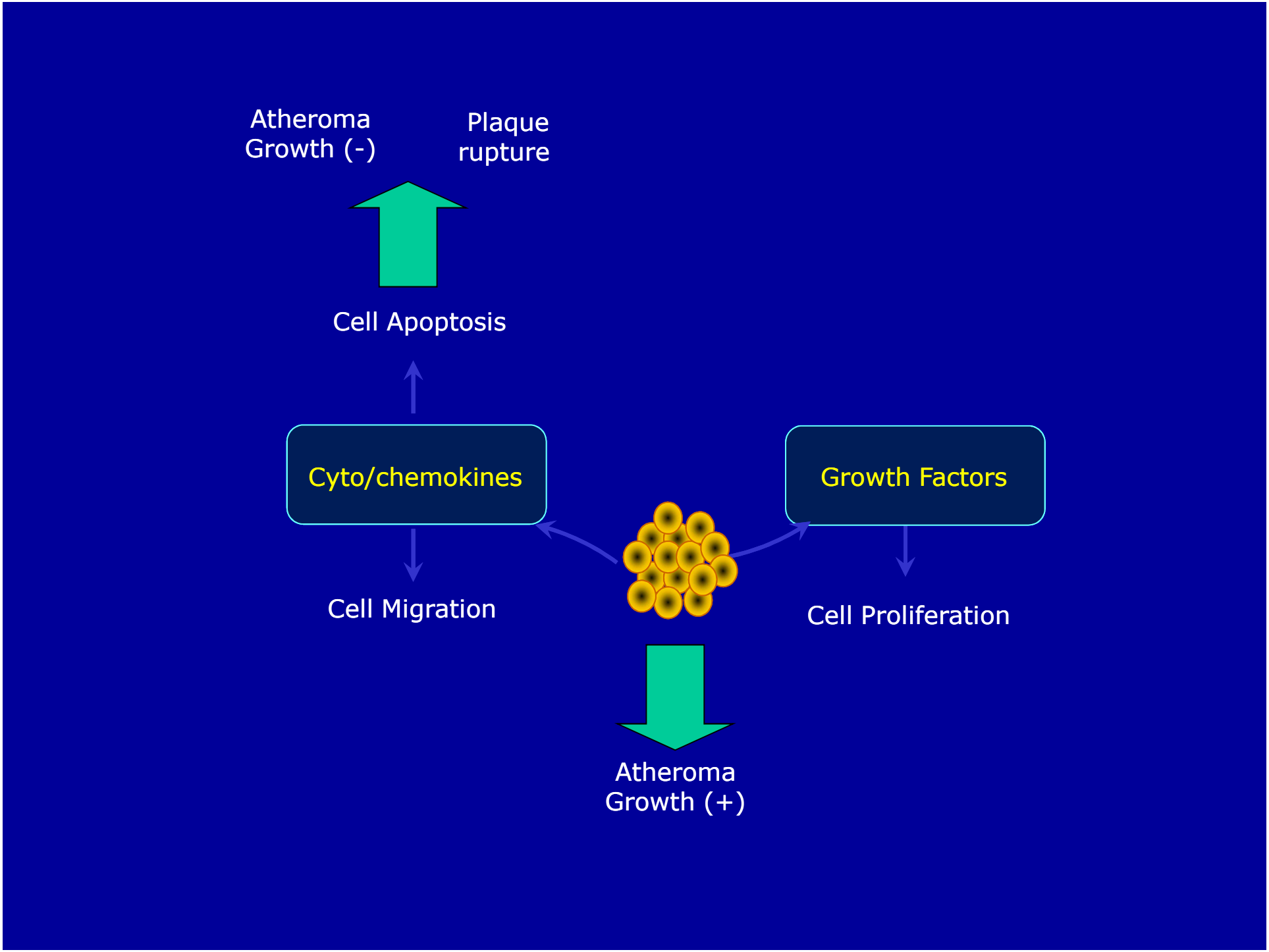


Ross R. *N Engl J Med* 1999;340:115-126.

Atherosclerosis is an Inflammatory Disease



Ross R. *N Engl J Med* 1999;340:115-126.


Atheroma
Growth (-)
Plaque
rupture
Cell Apoptosis
Cyto/chemokines
Growth Factors
Cell Migration
Cell Proliferation
Atheroma
Growth (+)

II. C-reactive protein and inflammation

C - reactive protein

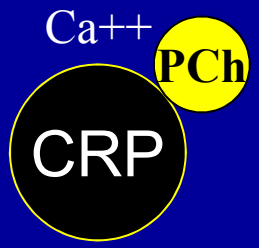
Known for 70 years (discovered in 1930)

Binds with phosphocholine (calcium dependent) in bacterial and fungal polysaccharides

C - reactive protein

Known for 70 years

Binds with phosphocholine



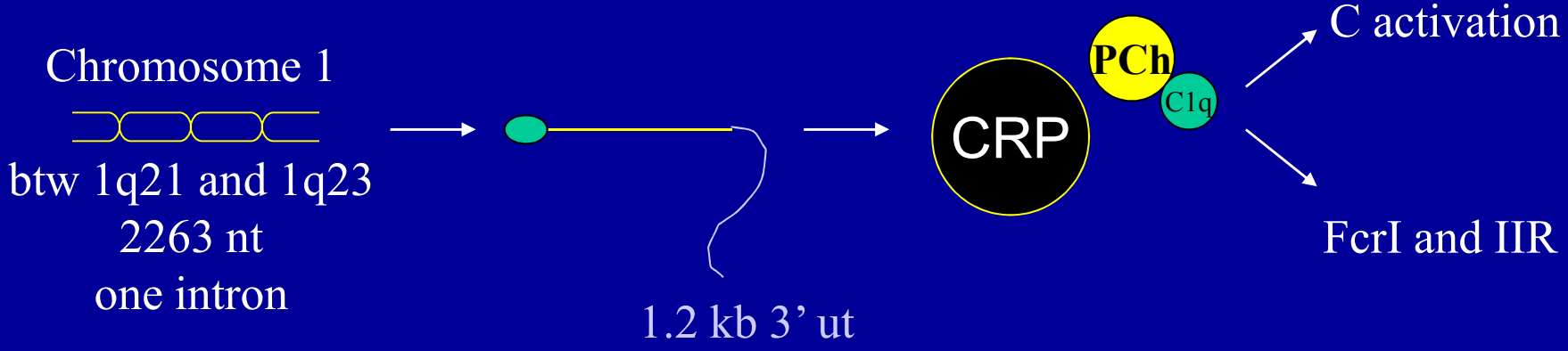
Recognized by C1q - complement activation
Phagocytosed thru FcrI and IIR

C - reactive protein

Known for 70 years

Binds with phosphocholine

Recognized by C1q - complement activation and
Phagocytosed thru FcrI and IIR

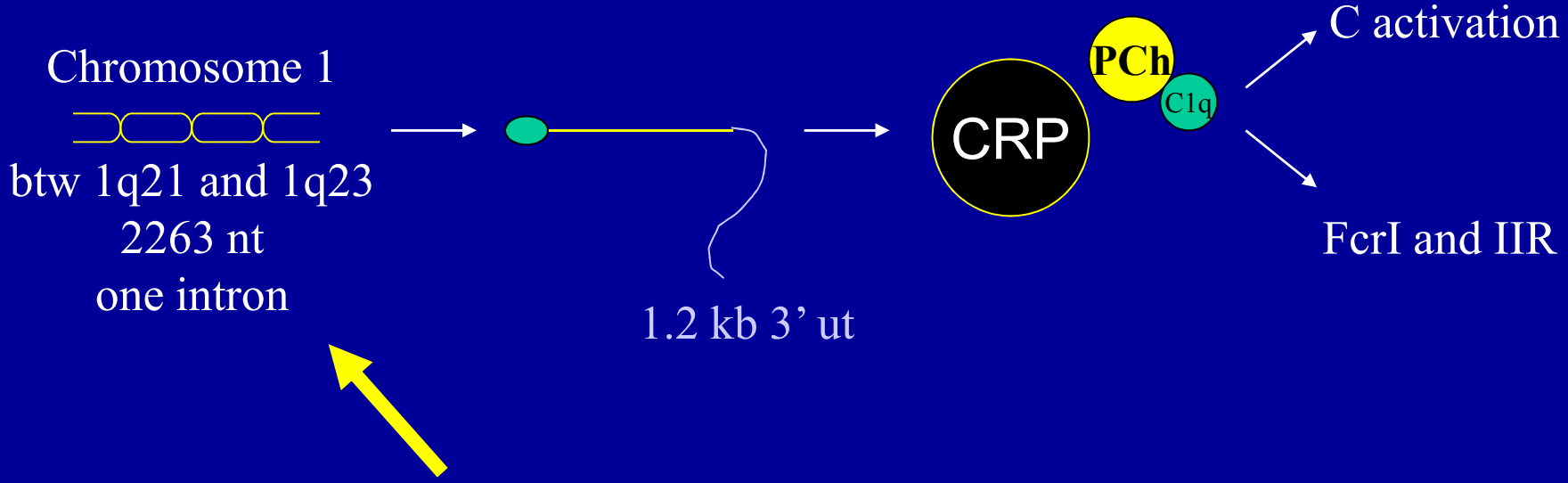


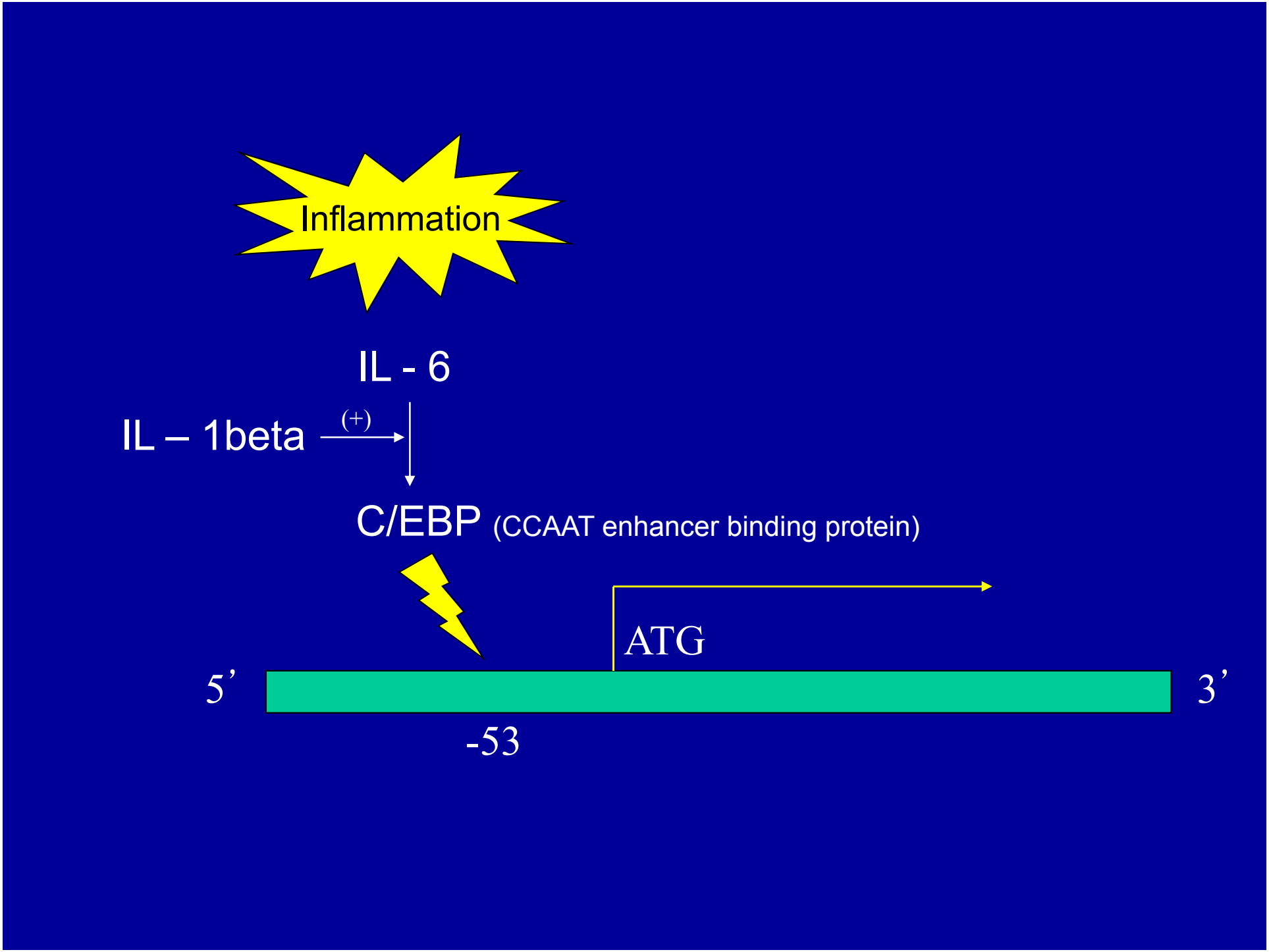
C - reactive protein

Known for 70 years

Binds with phosphocholine

Recognized by C1q - complement activation and
Phagocytosed thru FcrI and IIR



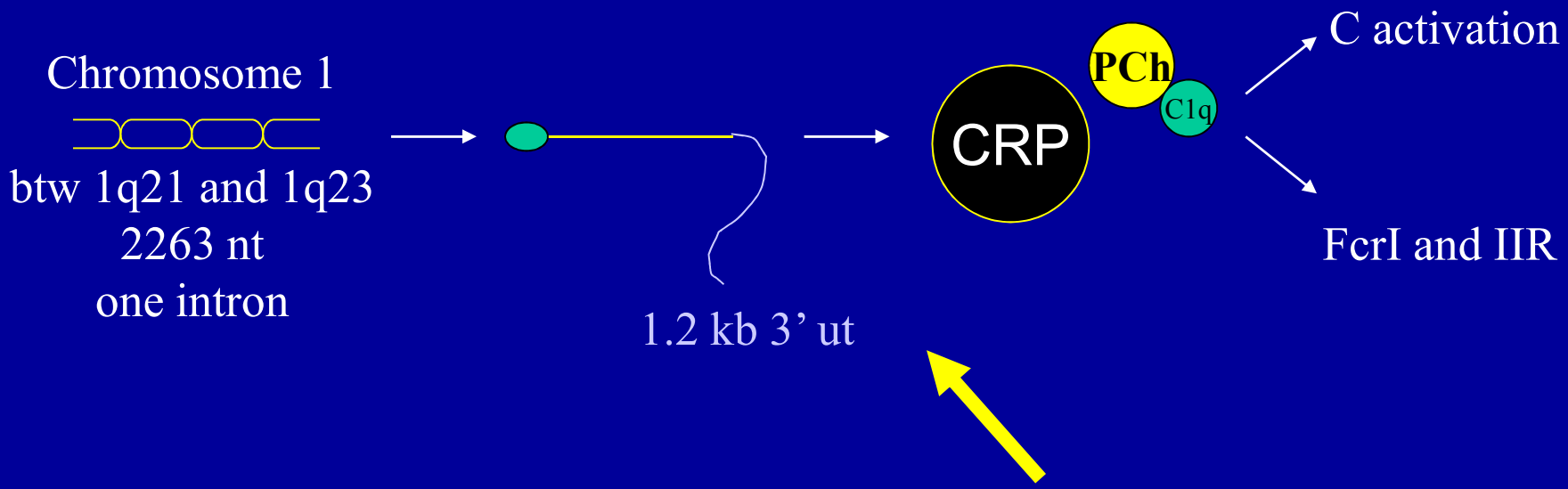

Inflammation
IL - 6
IL – 1beta
(+)
C/EBP (CCAAT enhancer binding protein)
ATG
5’
3’
-53

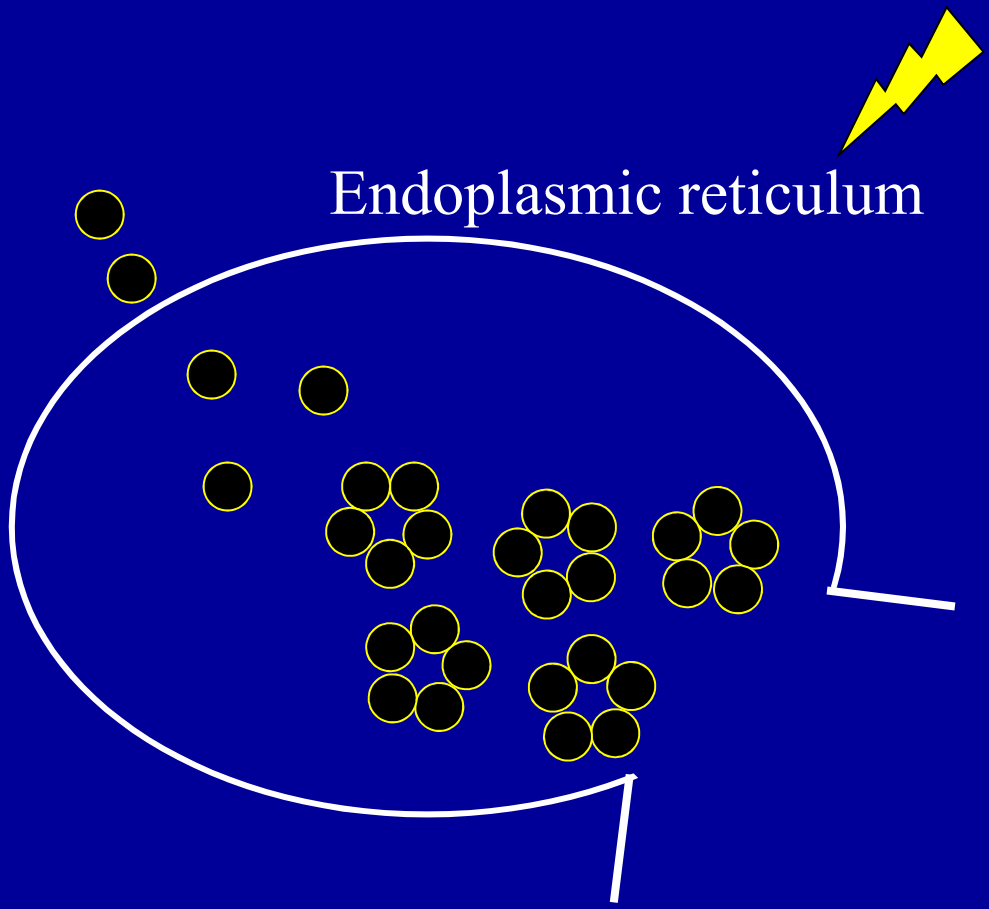
C - reactive protein

Known for 70 years

Binds with phosphocholine

Recognized by C1q - complement activation and
Phagocytosed thru FcrI and IIR



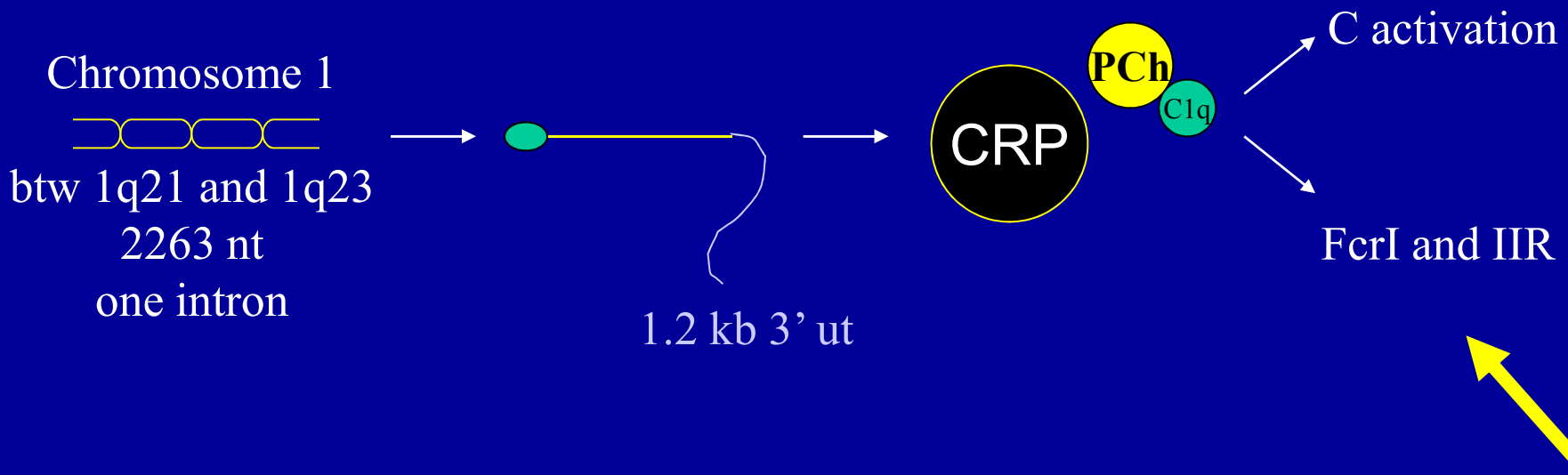

Endoplasmic reticulum

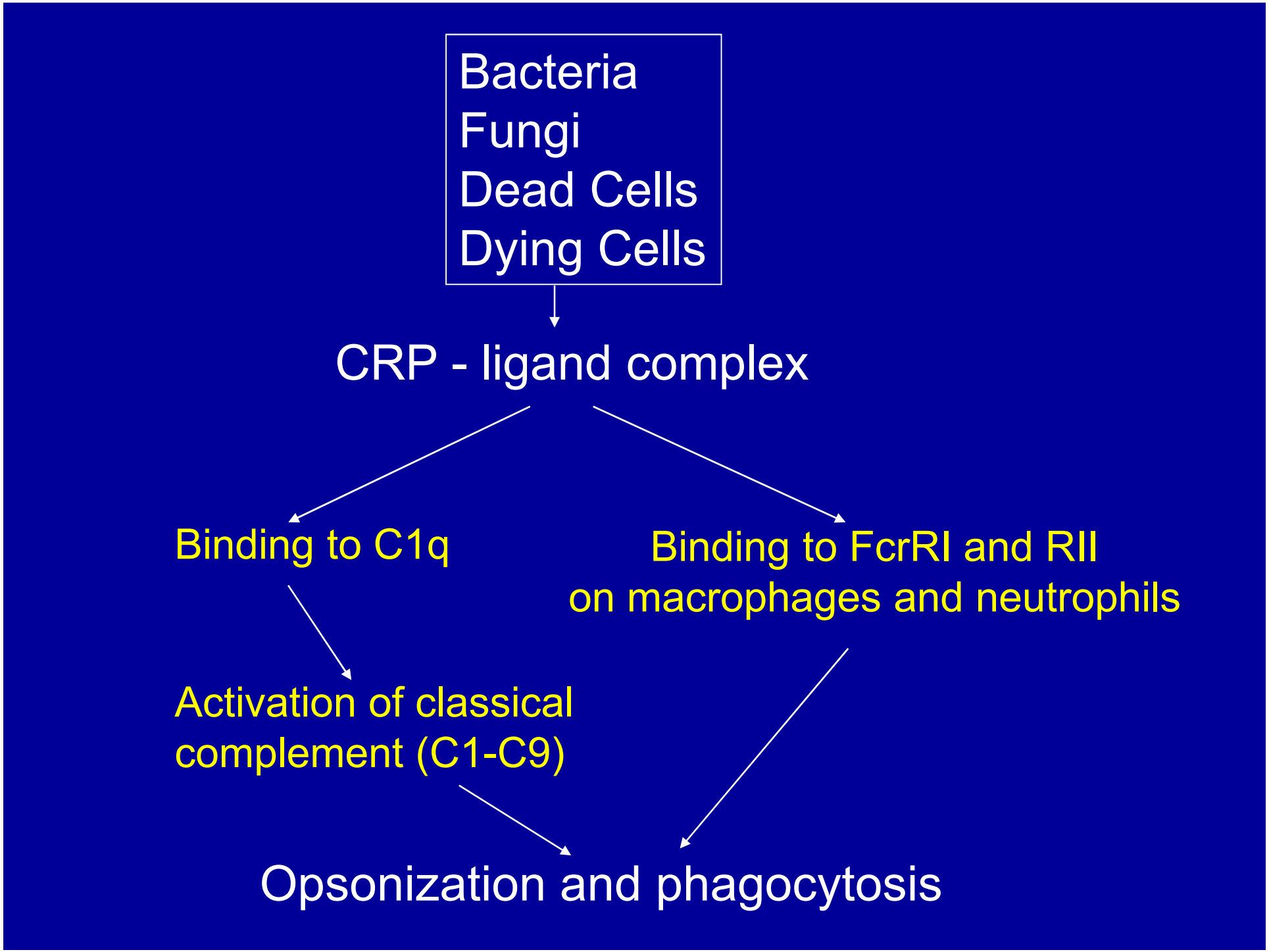
C - reactive protein

Known for 70 years

Binds with phosphocholine

Recognized by C1q - complement activation and
Phagocytosed thru FcrI and IIR




Bacteria
Fungi
Dead Cells
Dying Cells
CRP - ligand complex
Binding to C1q
Binding to FcrRI and RII
on macrophages and neutrophils
Activation of classical
complement (C1-C9)
Opsonization and phagocytosis

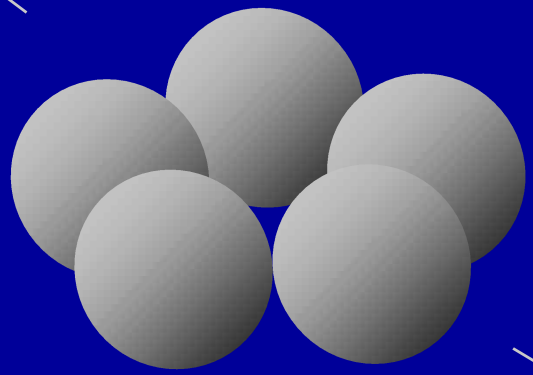
III. C-reactive protein is a good marker for the Atherosclerotic disease

ATHEROSCLEROSIS

Infection

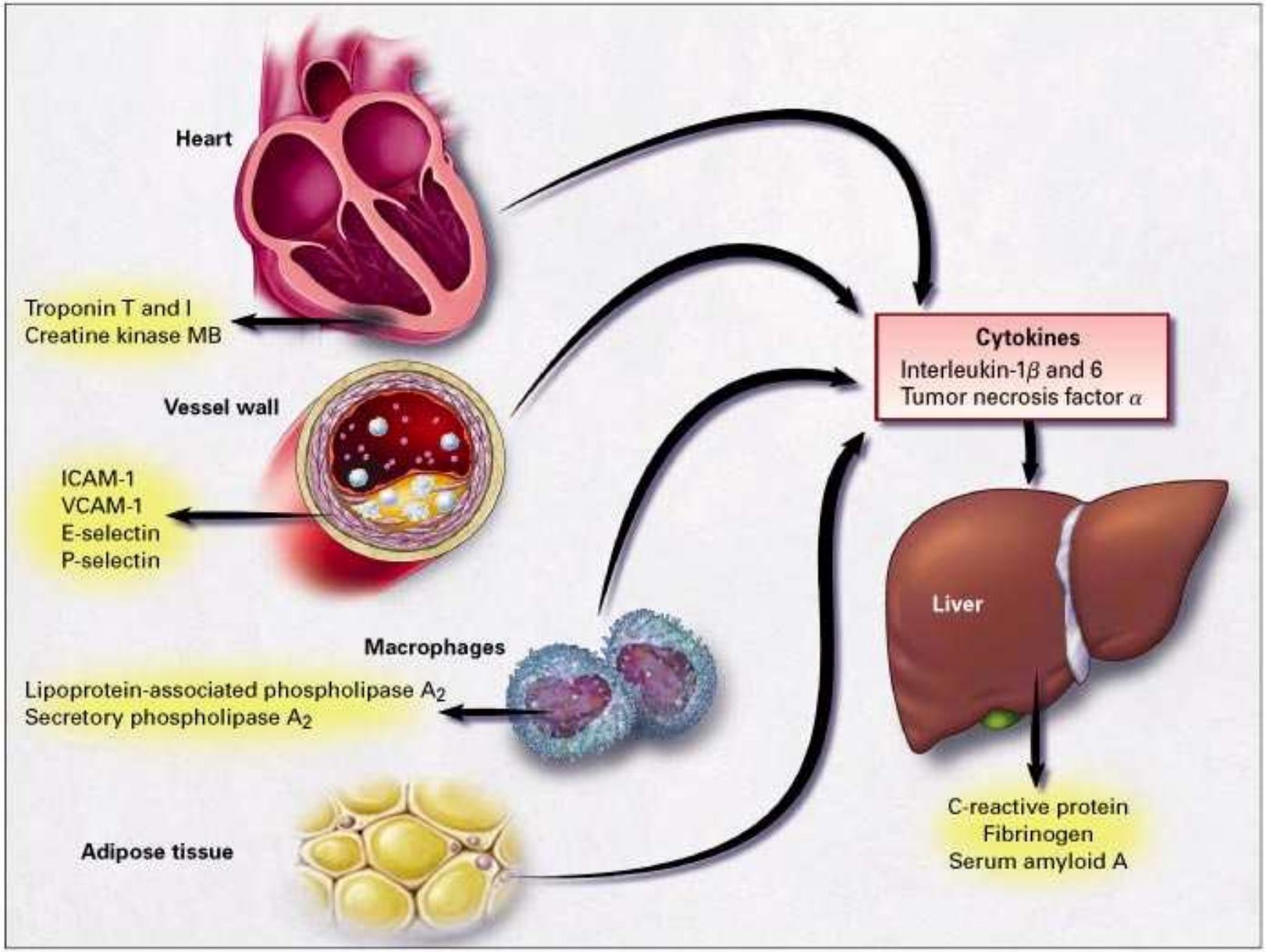
Rheumatoid arthritis

CRP



Crohn's disease

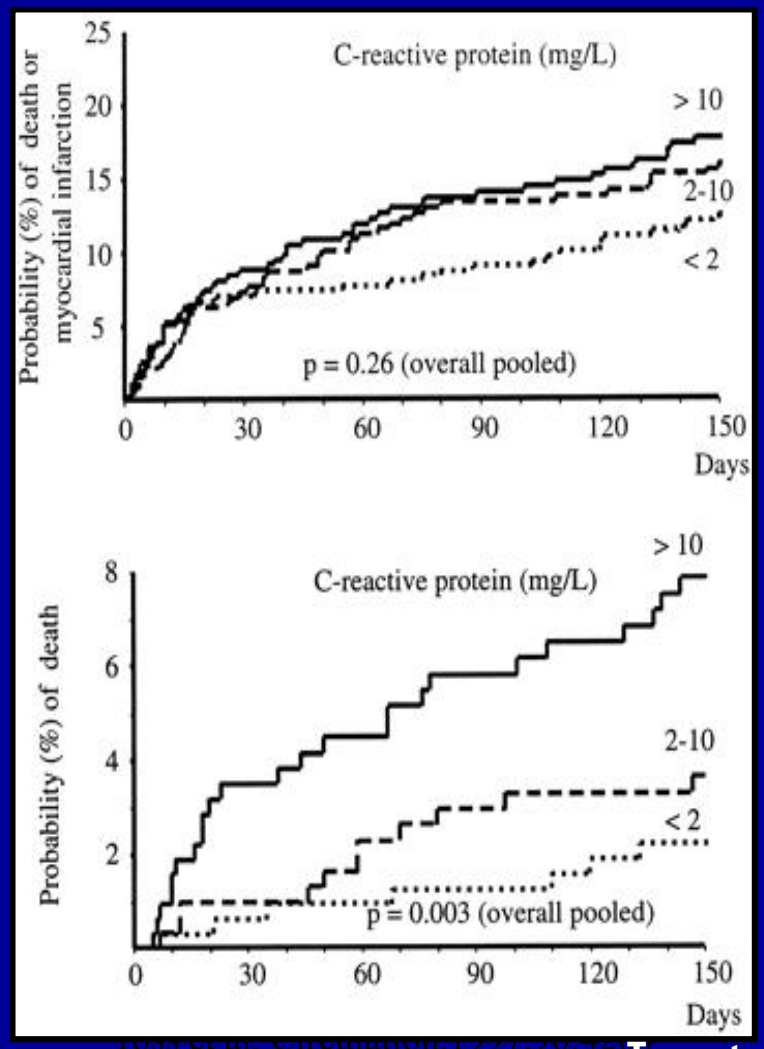
SLE
polymyositis
acute leukemia
ulcerative colitis


Heart
Troponin T and I
Creatine kinase MB
Vessel wall
ICAM-1
VCAM-1
E-selectin
P-selectin
Macrophages
Lipoprotein-associated phospholipase A_2
Secretory phospholipase A_2
Adipose tissue
Cytokines
Interleukin-1β and 6
Tumor necrosis factor α
Liver
C-reactive protein
Fibrinogen
Serum amyloid A

C-reactive protein

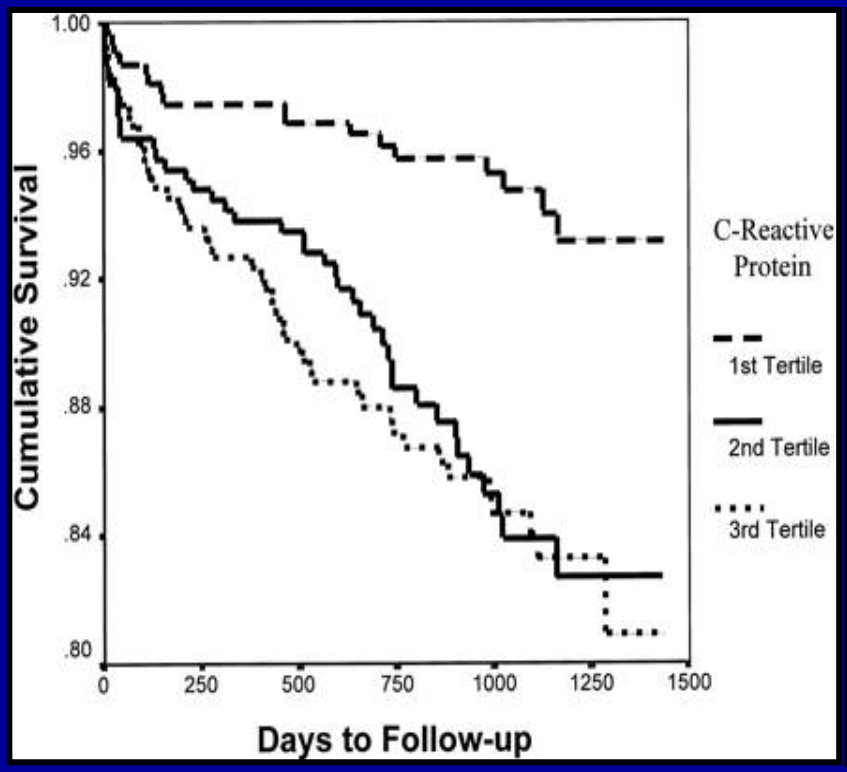
“ FROM PAUPER TO PRINCE ”
“ REVIVAL !!! “

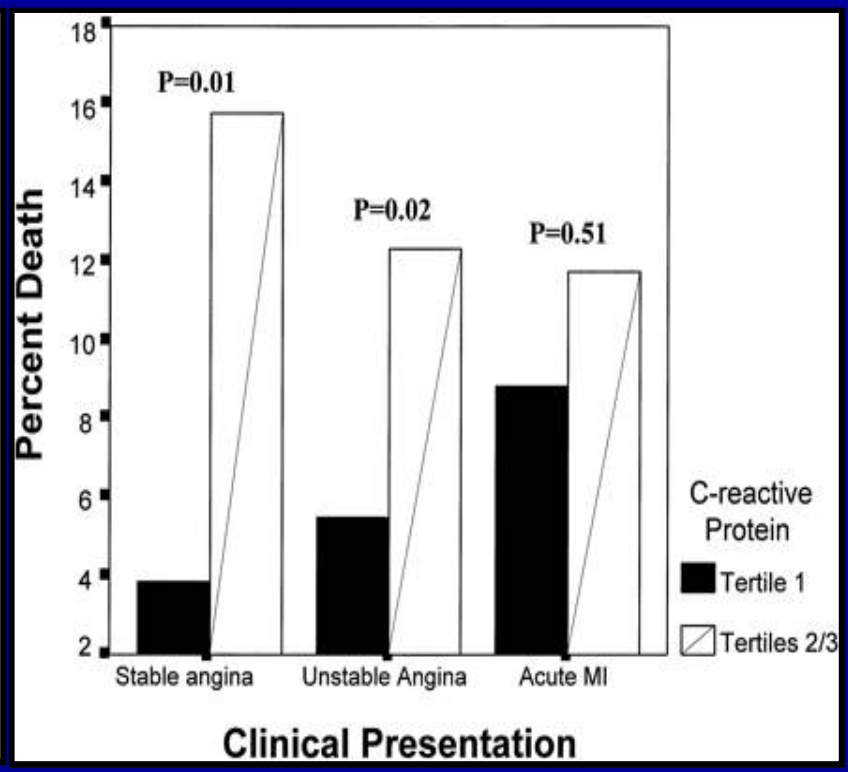
Prognostic Influence of Increased CRP in unstable CAD



Toss et.al. Circulation 1997,96:4204

Prognostic Value of Baseline CRP According to Initial Clinical Presentation

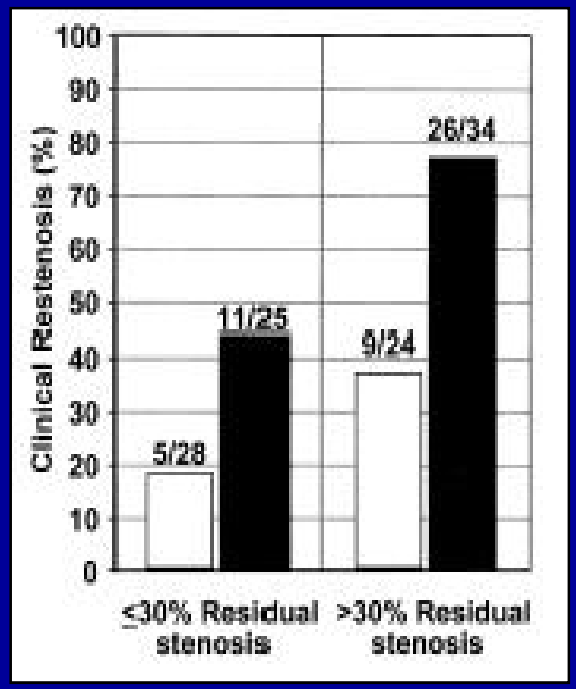


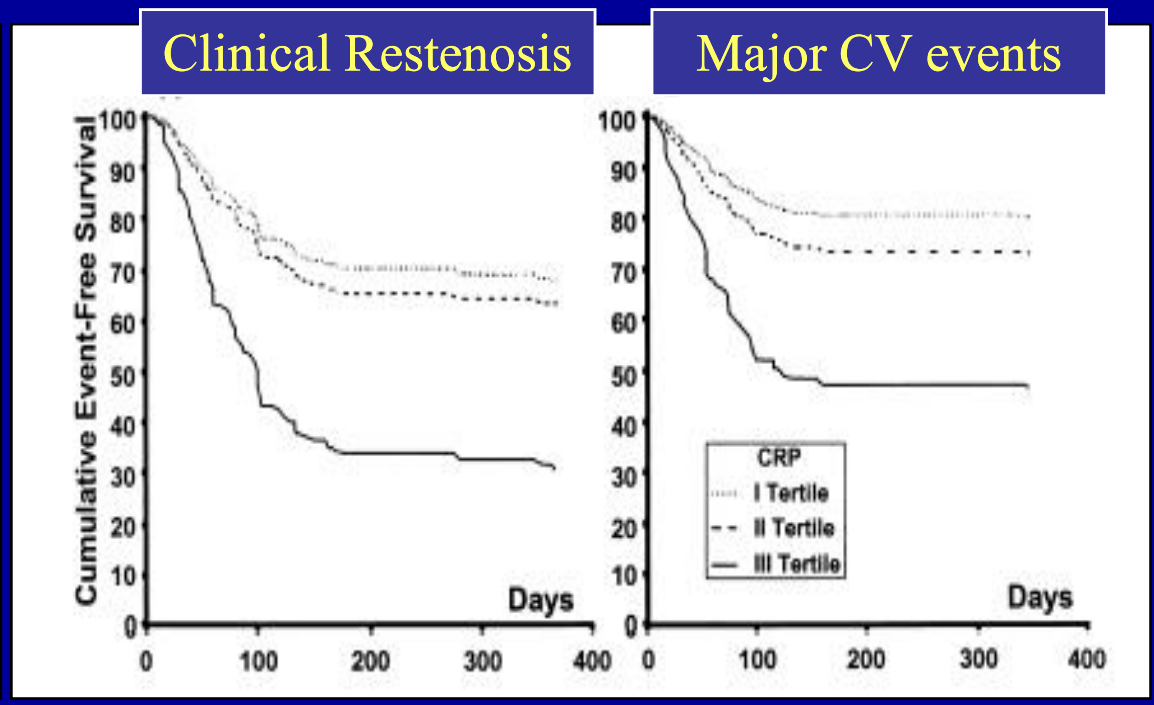


Mulhlestein et.al.
Circulation 2000,102:1917

Mulhlestein et al, Circulation, 2000, 102:1917

Pre-procedure serum CRP level can predict
Early Complications & Late Restenosis after PTCA





Open bar ; CRP ≤ 0.3 mg/dl
Black bar ; CRP > 0.3 mg/dl

Buffon et. al. JACC 1999,34:1512

Plasma Levels of CRP after Stent Implantation

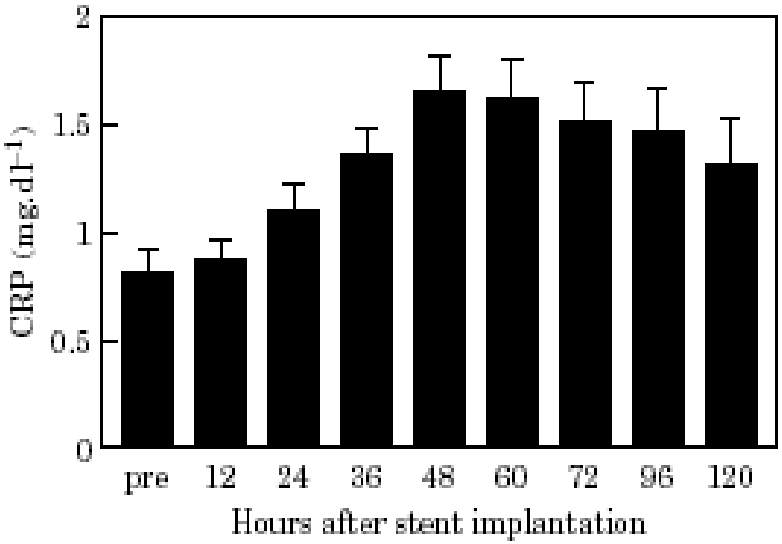


Figure 1 C-reactive protein mean plasma levels (SEM) before stent implantation and during the following 120 h ($P<0{\cdot}0001$).

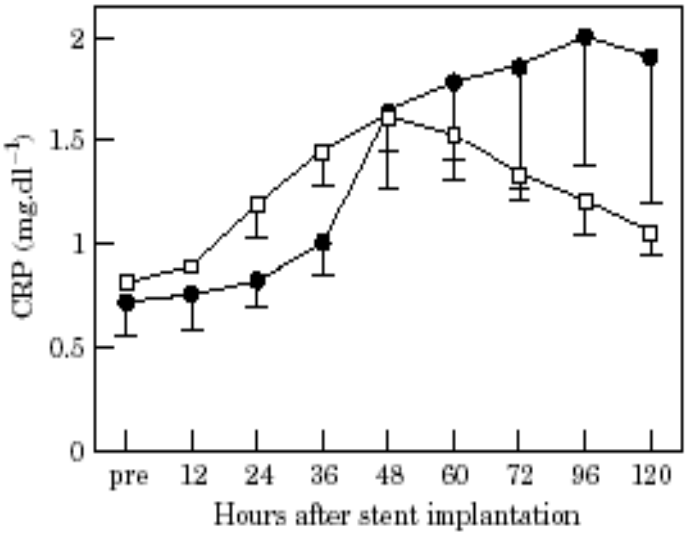
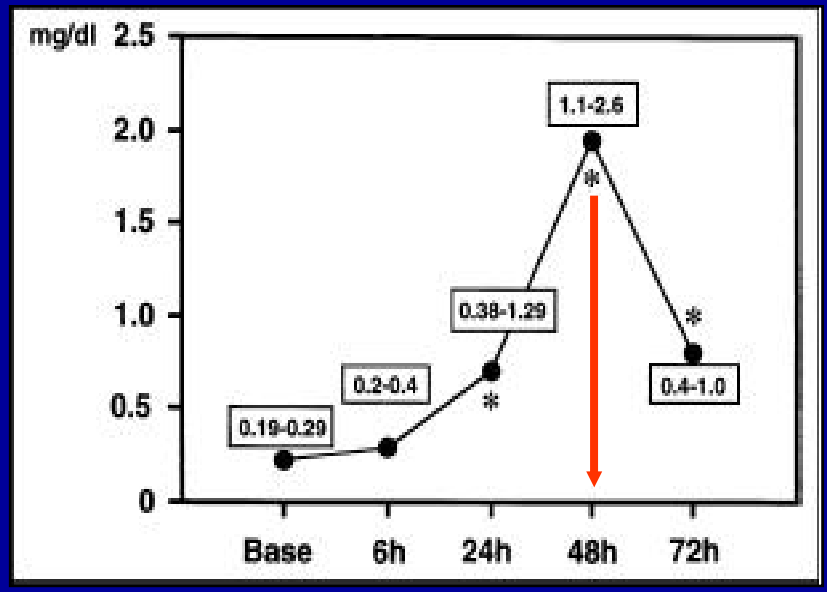
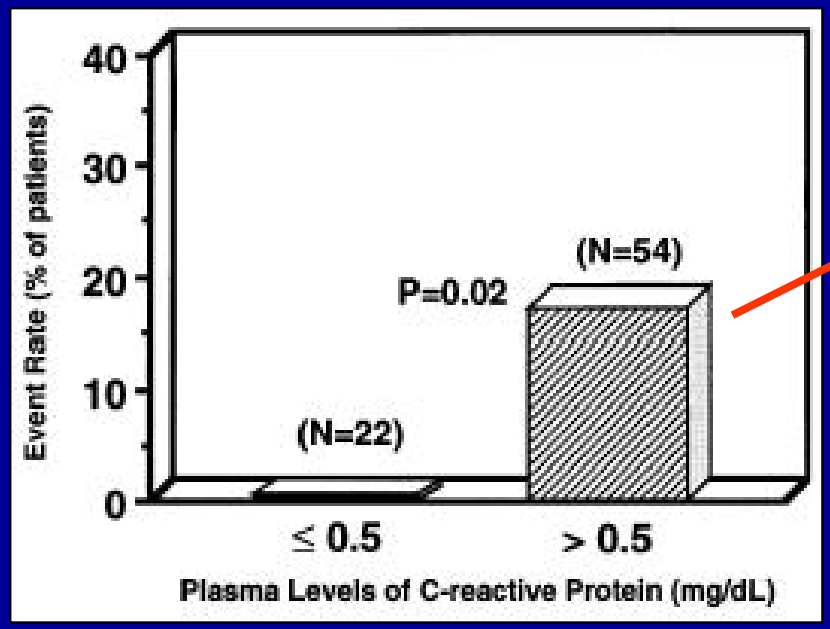


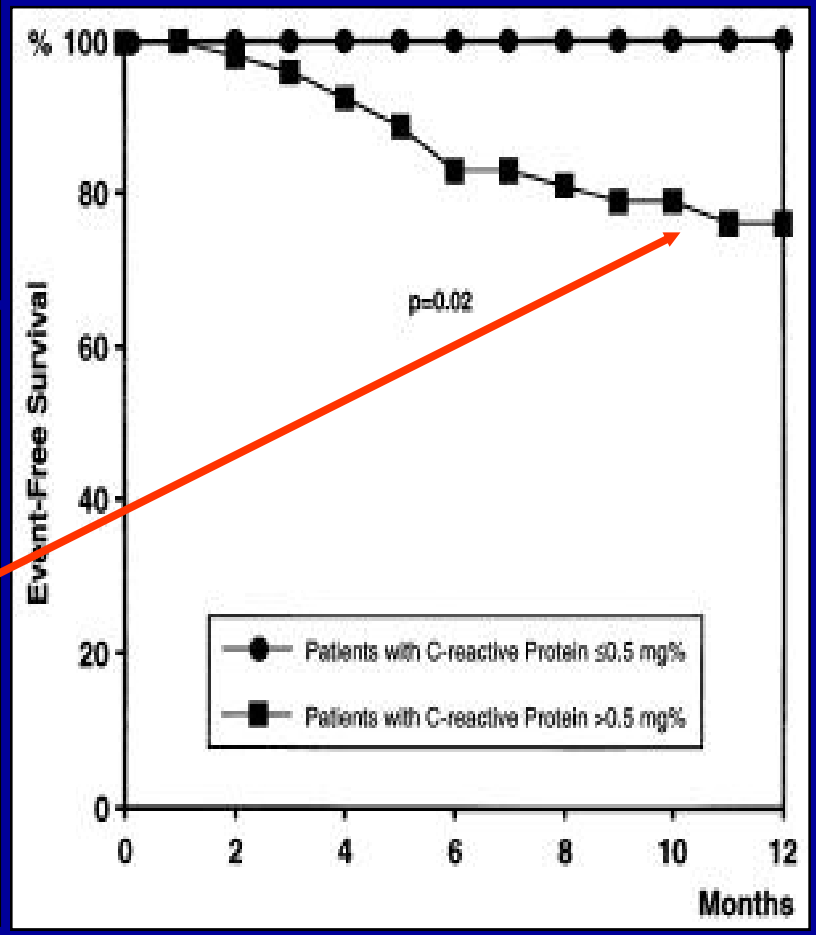
Figure 3 C-reactive protein mean plasma levels (SEM) in patients who remained free from restenosis (○) and patients with restenosis (●) at follow up angiography 6 months after stent implantation ($P=0{\cdot}038$).

Gottsaunder-Wolf et. al. Eur Heart J 2000,21:1152



1. CRP elevated after Stenting.
2. CRP, 72 hours after stenting can predict CV events.





Gasparone et. al. Am J Cardiol 1998,82:515

TABLE 1. DISTRIBUTION OF C-REACTIVE PROTEIN AND LDL CHOLESTEROL LEVELS AMONG 15,745 STUDY PARTICIPANTS WHO WERE NOT TAKING HORMONE-REPLACEMENT THERAPY AT THE TIME OF THE BASE-LINE BLOOD COLLECTION.

AGE GROUP	NO. OF WOMEN	PERCENTILE						
		5TH	10TH	25TH	50TH	75TH	90TH	95TH
		milligrams per liter						
C-reactive protein								
45–54 yr	10,075	0.17	0.25	0.52	1.31	3.18	6.15	8.80
55–64 yr	3,604	0.25	0.39	0.82	1.89	4.12	7.47	9.76
65–74 yr	1,862	0.33	0.46	0.91	1.99	3.92	6.79	8.77
≥75 yr	204	0.29	0.43	0.80	1.52	3.55	7.56	13.33
Total	15,745	0.19	0.29	0.61	1.52	3.48	6.61	9.14
		milligrams per deciliter*						
LDL cholesterol								
45–54 yr	10,075	72.7	82.1	97.6	117.3	139.6	162.5	178.2
55–64 yr	3,604	83.4	94.9	113.4	134.4	158.8	181.9	198.3
65–74 yr	1,862	86.4	97.0	115.1	137.0	157.9	183.5	199.3
≥75 yr	204	91.2	100.4	117.3	139.3	159.6	178.4	189.4
Total	15,745	75.8	85.3	102.4	123.7	147.4	170.5	187.2

*To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

Ridker et al NEJM 2002;347:1557

Distribution of hsCRP in Korean population

Chang JW et. al. Am J Kid Dis 39,2002:1213-1217

healthy volunteers
n=1399
median = 0.64 mg/L (<0.006 to 0.567 mg/L)

25th = 0.34
75th = 1.23
95th = 4.01

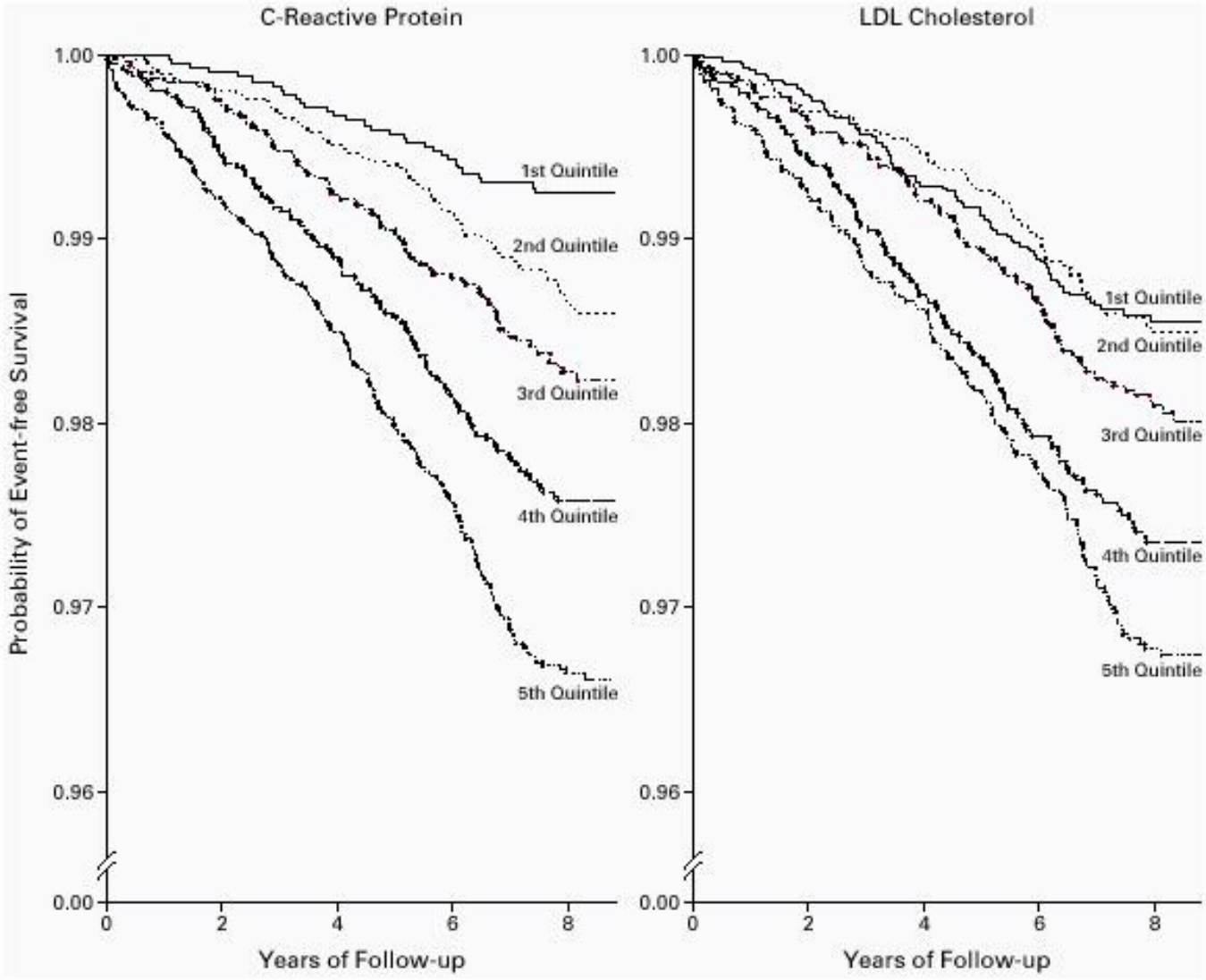


Figure 1. Event-free Survival According to Base-Line Quintiles of C-Reactive Protein and LDL Cholesterol.

The range of values for C-reactive protein was as follows: first quintile, ≤0.49 mg per liter; second quintile, >0.49 to 1.08 mg per liter; third quintile, >1.08 to 2.09 mg per liter; fourth quintile, >2.09 to 4.19 mg per liter; fifth quintile, >4.19 mg per liter. For LDL cholesterol, the values were as follows: first quintile, ≤97.6 mg per deciliter; second quintile, >97.6 to 115.4 mg per deciliter; third quintile, >115.4 to 132.2 mg per deciliter; fourth quintile, >132.2 to 153.9 mg per deciliter; fifth quintile, >153.9 mg per deciliter. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586. Note the expanded scale on the ordinate.

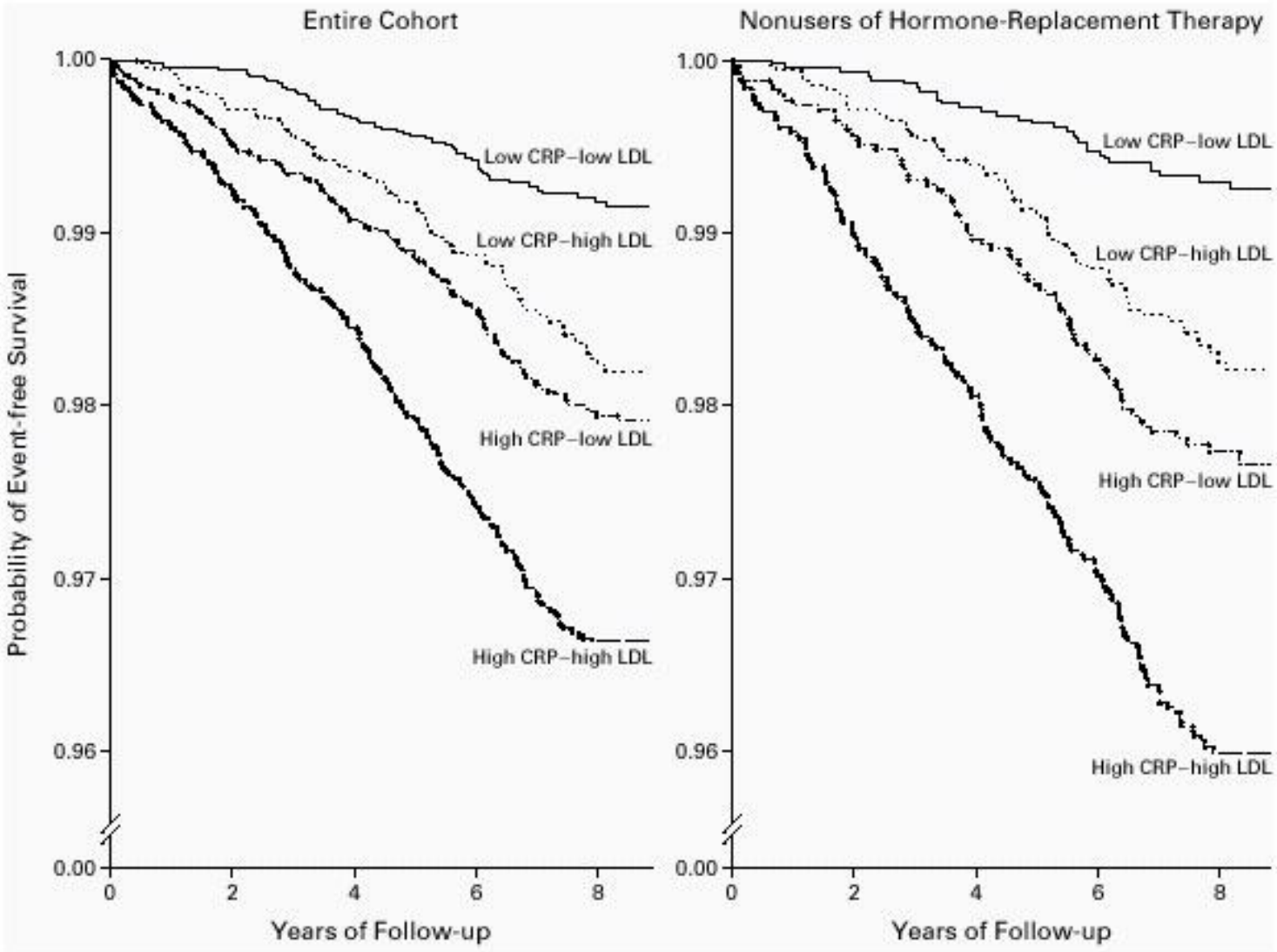
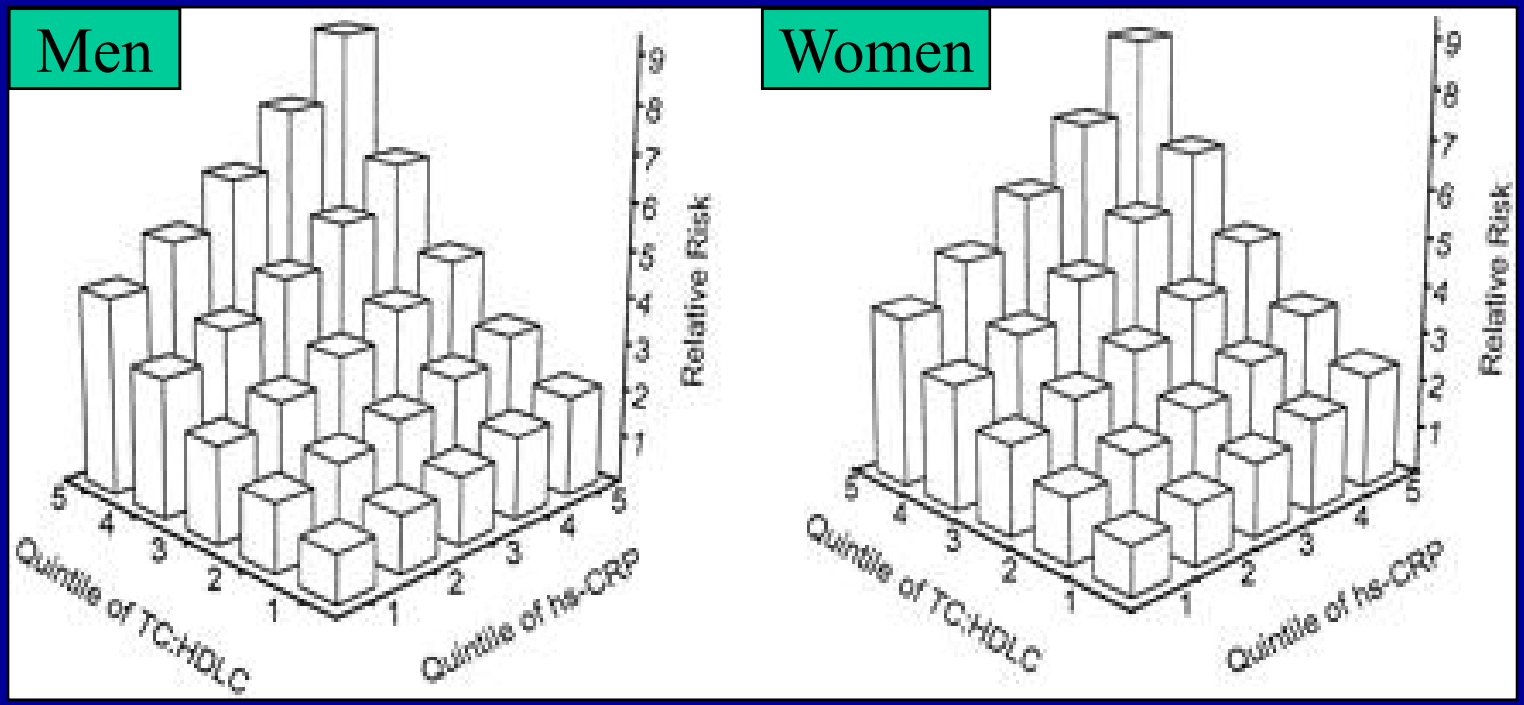


Figure 3. Event-free Survival among Women with C-Reactive Protein (CRP) and LDL Cholesterol Levels above or below the Median for the Study Population.

Data are shown for the entire cohort (27,939 women) and for women who were not taking hormone-replacement therapy at base line (15,745 women). The median values were as follows: for C-reactive protein, 1.52 mg per liter; for LDL cholesterol, 123.7 mg per deciliter (3.20 mmol per liter). Note the expanded scale on the ordinate.

High-Sensitivity CRP and TC/HDL-C
- risk assessment in primary prevention of CVD

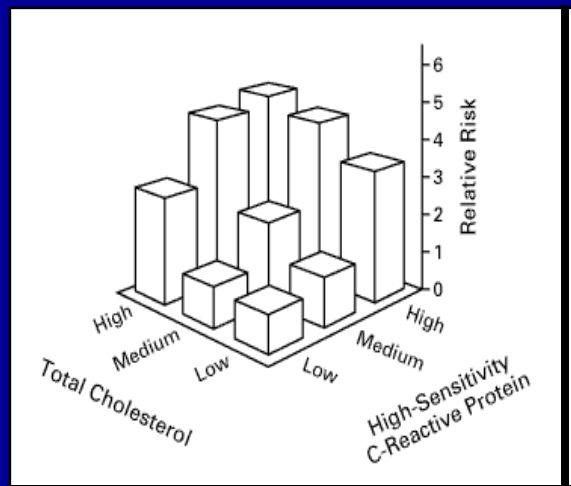


TC: HDLC ratio
for men <3.5, 3.5 to 4.3, 4.4 to 5.0, 5.1 to 6.1, and >6.1
for women <3.1, 3.1 to 3.6, 3.7 to 4.3, 4.4 to 5.2, and >5.2
(Harvey Kaufman, MD, personal communication, 2001).

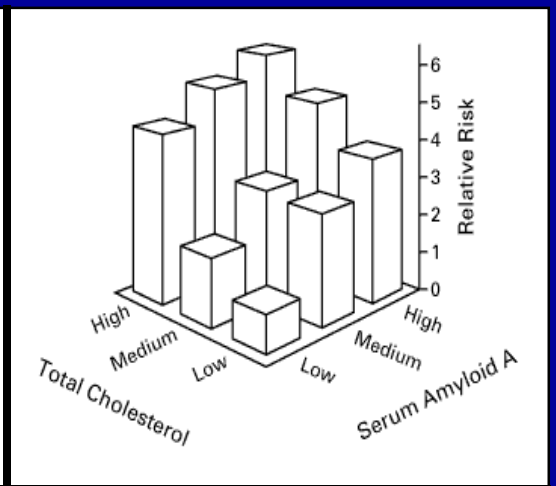
Ridker Circulation 2001,103:1813

Risk of Cardiovascular Events among Healthy Postmenopausal Women

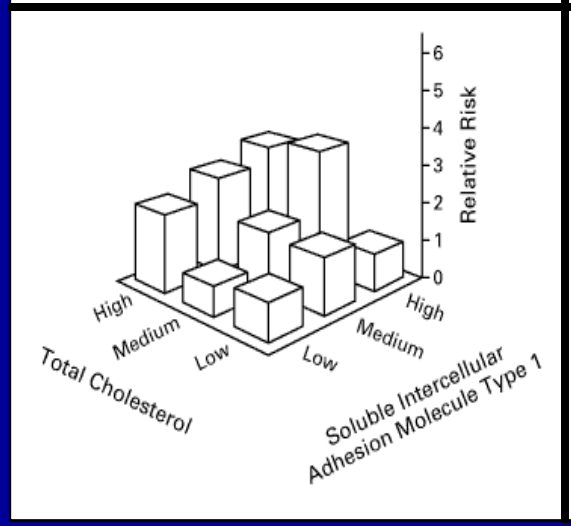
CRP



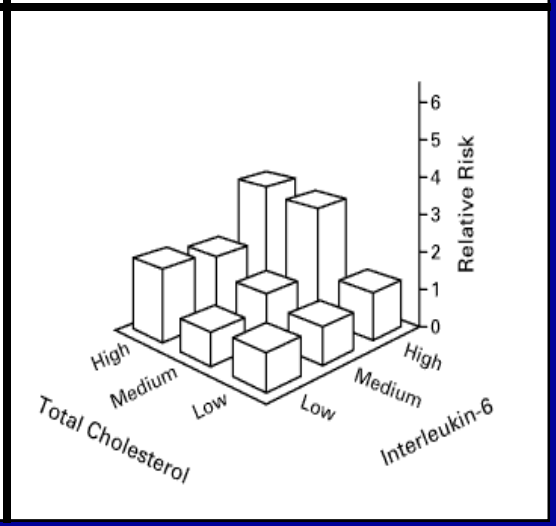
SAA



sICAM-1



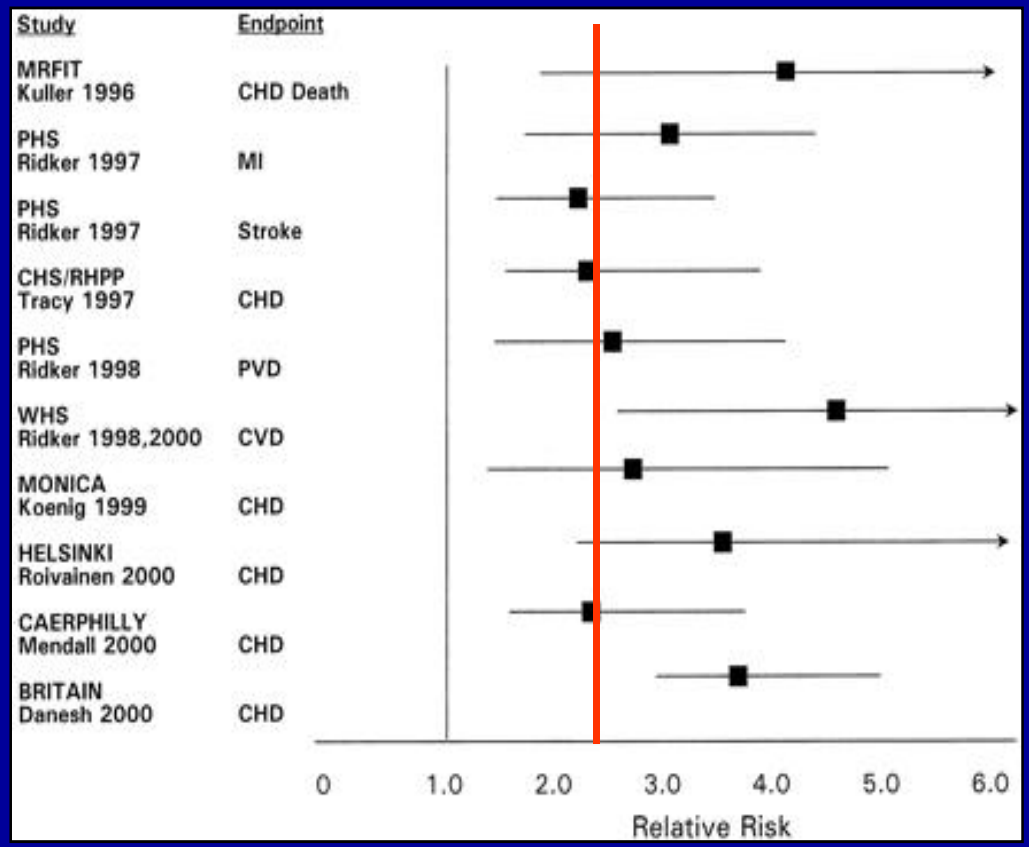
IL-6



Ridker et al, NEJM, 2000,342:863

Ridker et.al.
NEJM 2000,342:863

High-Sensitivity CRP
- risk assessment in primary prevention of CVD -

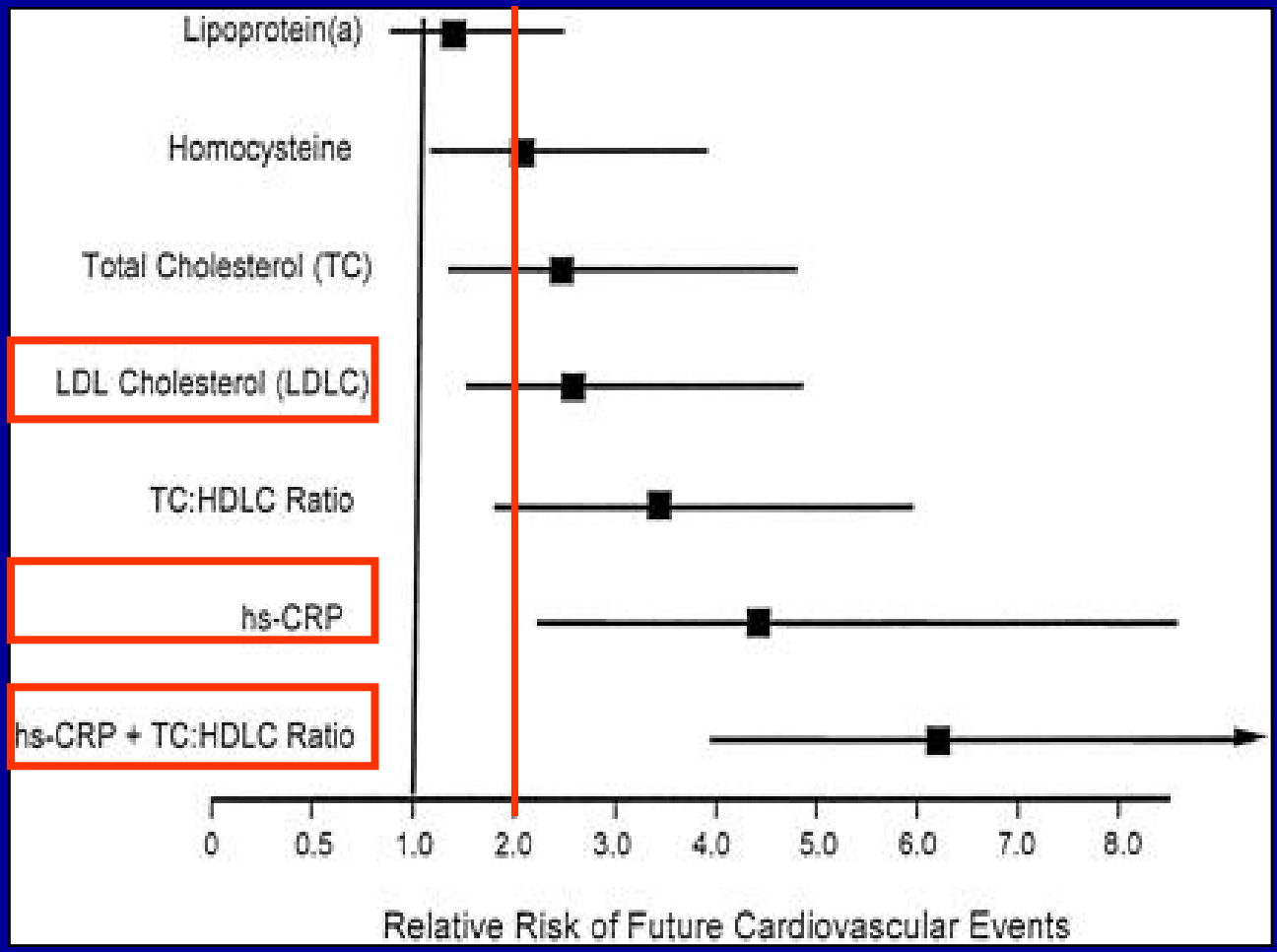


Ridker Circulation 2001,103:1813

Factors related to CRP

인종	B >W
Gender	female > male
Age	+
Hypertension	+
Insulin sensitivity	+
Smoking	++
Glucose tolerance	++
Obesity	+++
Coagulation activity	++
IMT of int. carotid a.	+

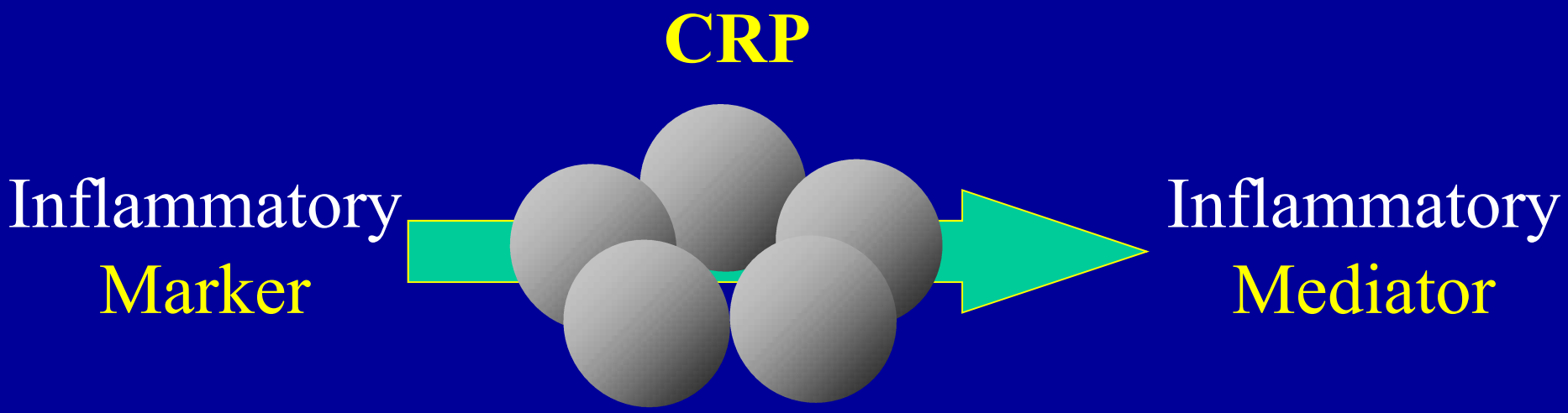
Direct comparison of magnitude of relative risk of future cardiovascular events



Ridker, Circulation, 2001;103:1813

Ridker Circulation 2001,103:1813

III. C-reactive protein may affect the process of Atherosclerosis


CRP
Inflammatory
Marker
Inflammatory
Mediator

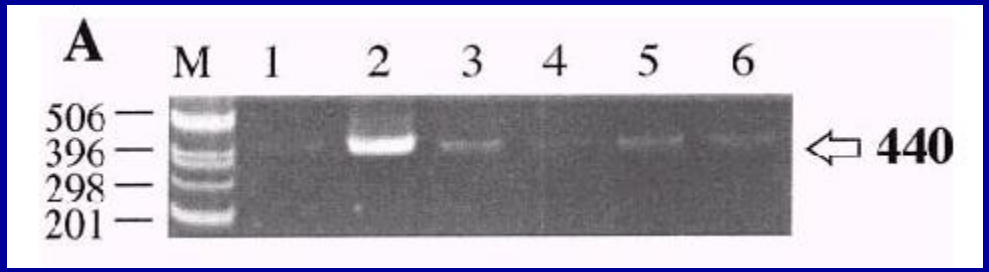
The origin of CRP in the plaque ?

- *De novo* synthesis by VSMCs and macrophages ;
  Circulation. 2003 108(16):1930-2
  Exp Biol Med. 2005 230(10):762-70

- Mainly from blood circulation
  Am J Pathol. 2005 Oct;167(4):1139-48

- Maybe from both
  Am J Pathol. 2001 Mar;158(3):1039-51

CRP antisense probe



Lane 1, normal artery; lane 2, plaque tissue; lane 3, lane 4, heart; lane 5, kidney; lane 6, spleen.

Human autopsy sample
Am J Path 2001;158:1039

C - reactive protein ; proinflammatory ?

- Complement activation

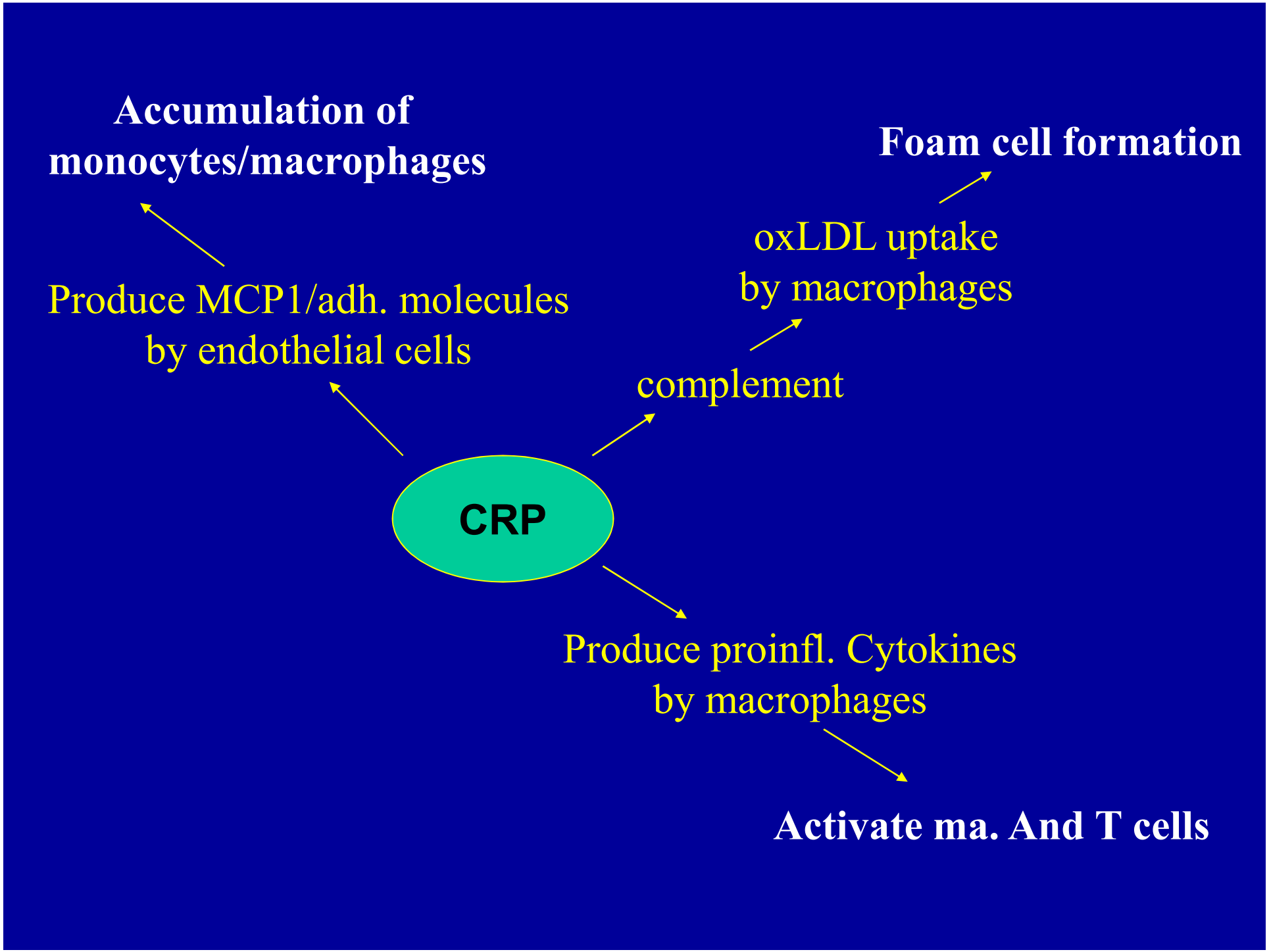
- produced by macrophages and SMCs in plaques

- colocalized with membrane attack complex

- produce IL-1 beta, IL-6 and TNF-alpha by monocytes

- produce ICAM-1 and VCAM-1 and MCP-1 by endothelial cells

Am J Med Sci 2000;319:77
Am J Path 2001;158:1039
Circulation 2000;102:2165
Circulation 2001;103:2531


Accumulation of monocytes/macrophages
Foam cell formation
oxLDL uptake by macrophages
Produce MCP1/adh. molecules by endothelial cells
complement
CRP
Produce proinfl. Cytokines by macrophages
Activate ma. And T cells

Can we reduce blood CRP levels ?
YES

1. Statins (Atherosclerosis 165:361, 157:411)
2. PPAR ligands ; alpha- and gamma (Circulation 106:679)
3. Aspirin (Circulation 100:793)
4. Antibiotics ;
   azithromycin (Circulation 105:1298, 106:1071)
   roxithromycin (Eur Hrt J 120:121)

To prevent cardiovascular events

1. Life style modification
   - lipid lowering can stabilize atheroma
2. Statins
3. PPAR-gamma ligands
4. Aspirin
5. Antibiotics ?

Reduce inflammation

IV. C-reactive protein and Hypertension

CRP elevates Blood Pressure ?

C-Reactive Protein Causes Downregulation of Vascular Angiotensin Subtype 2 Receptors and Systolic Hypertension in Mice

Wanpen Vongpatanasin, MD; Gail D. Thomas, PhD; Randall Schwartz, MD; Lisa A. Cassis, PhD; Sherri Osborne-Lawrence, MS; Lisa Hahner, BS; Linda L. Gibson, BS; Steven Black, PhD; David Samols, PhD; Philip W. Shaul, MD

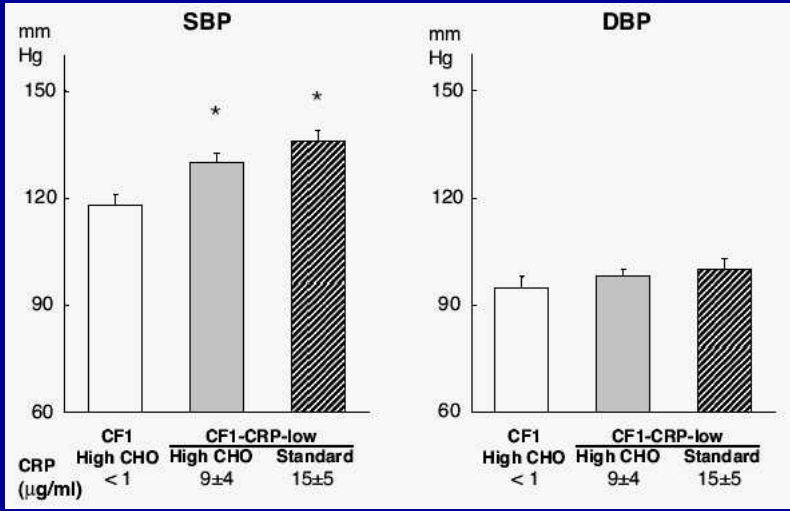
Background—Chronic elevations in circulating C-reactive protein (CRP) are associated with a greater risk of hypertension. Whether elevations in CRP cause hypertension is unknown.

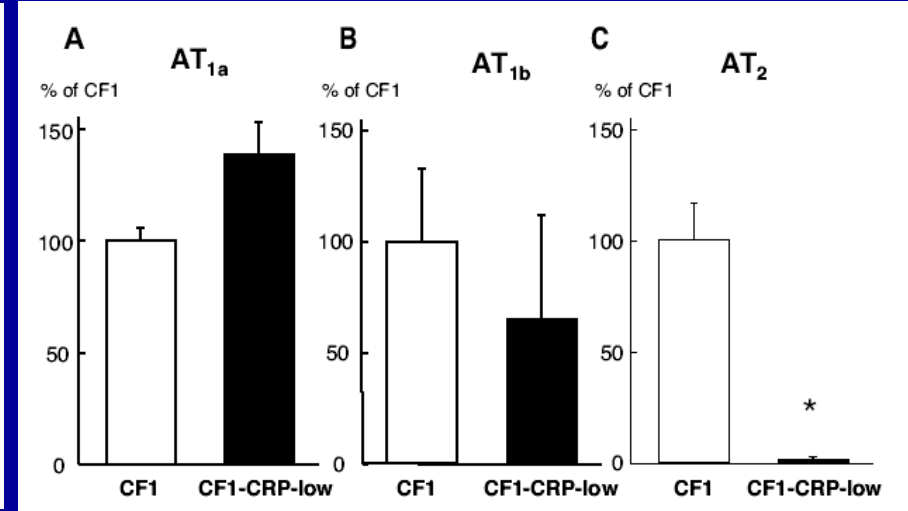
Methods and Results—Chronic, conscious blood pressure (BP) measurements were performed by radiotelemetry in wild-type CF1 control and CF1 transgenic mice expressing rabbit CRP (CF1-CRP) under the regulation of the phosphoenolpyruvate carboxykinase promoter. Compared with controls, CF1-CRP mice had hypertension that was predominantly systolic, and the severity of hypertension varied in parallel with changes in CRP levels modulated by dietary manipulation. Mice that were hemizygous for the transgene with CRP levels of 9 μg/mL were also hypertensive, indicating that modest elevations in CRP are sufficient to alter BP. CRP transgenic mice had exaggerated BP elevation in response to angiotensin II and a reduction in vascular angiotensin receptor subtype 2 (AT_2) expression. In contrast, the decline in BP with angiotensin receptor subtype 1 (AT_1) antagonism and vascular AT_1 abundance were unaltered, which indicates a selective effect of CRP on AT_2. Ex vivo experiments further showed that the CRP-induced decrease in AT_2 is a direct effect on the vascular wall, not requiring systemic responses, and that it is reversed by an NO donor, which indicates a role for NO deficiency in the process. In parallel, the chronic inhibition of NO synthase in wild-type mice attenuated vascular AT_2 expression without affecting AT_1.

Conclusions—These findings provide direct evidence for CRP-induced hypertension, and they further identify a novel underlying mechanism involving downregulation of AT_2 related to NO deficiency. (*Circulation*. 2007;115:1020-1028.)

Key Words: angiotensin ■ C-reactive protein ■ endothelium ■ hypertension ■ nitric oxide ■ receptors

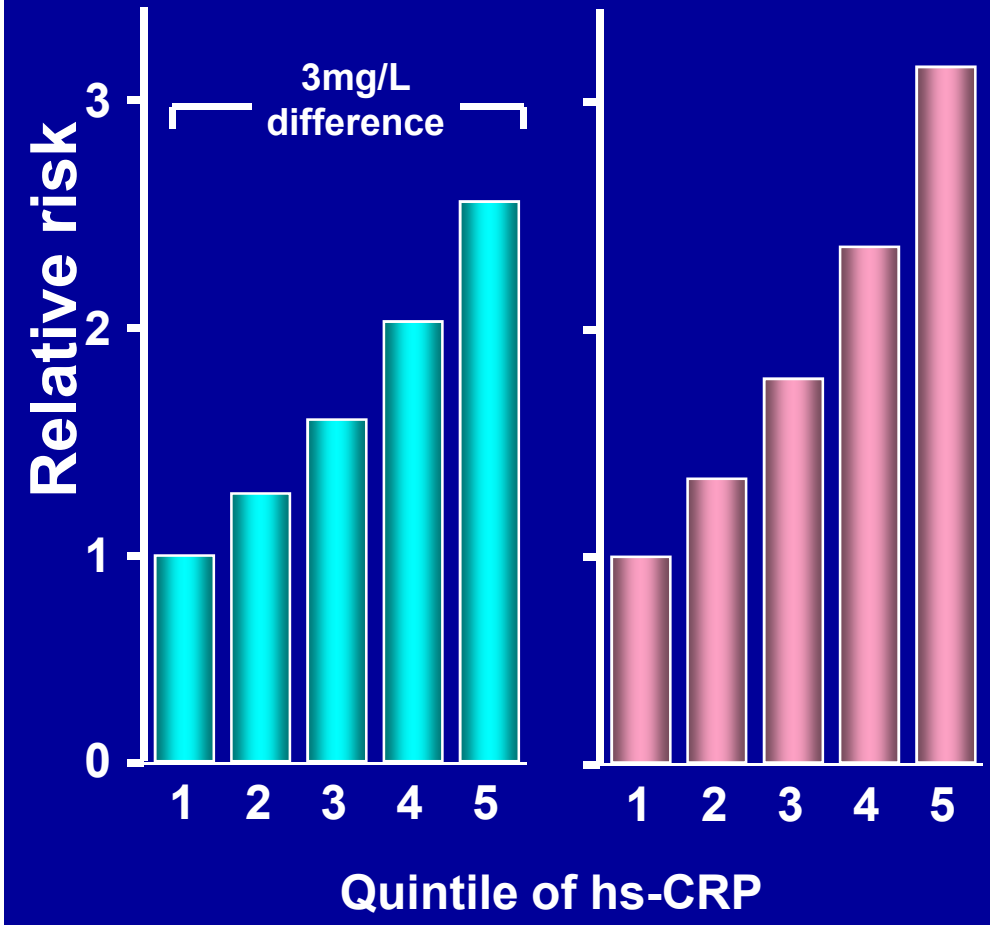
CRP elevates Blood Pressure through AT2R downregulation





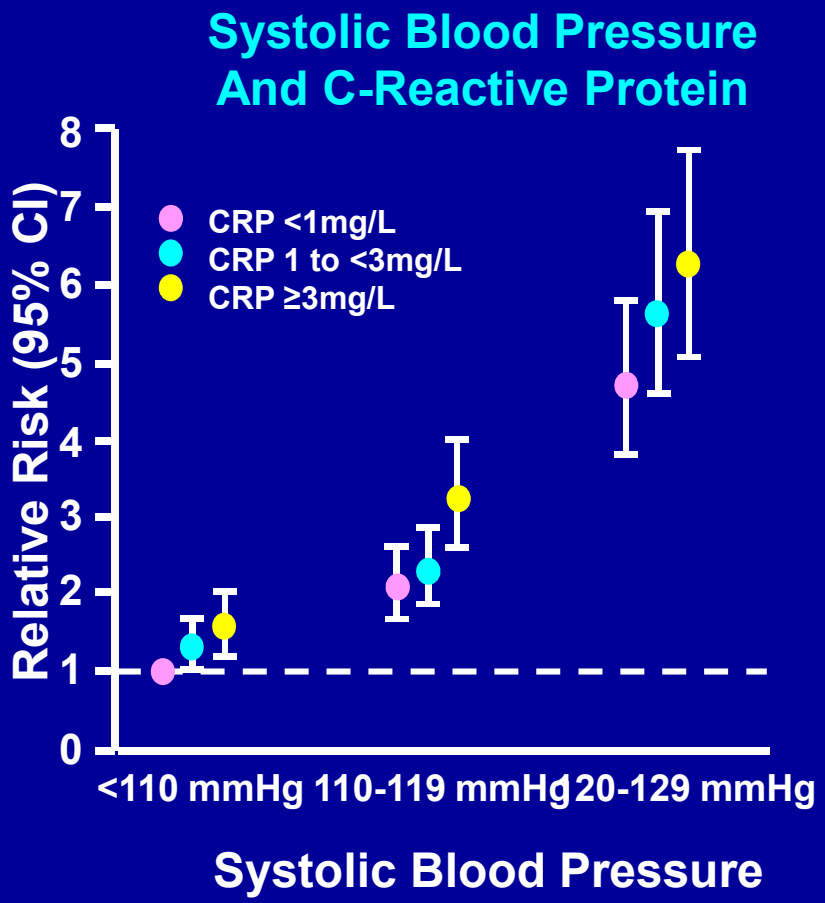
Circulation 2007;115:1020-1028

CRP and Coronary Disease



Relative risk of future coronary events according to quintiles of CRP.

Ridker P. Circulation 2001; 103 (13): 1813-8.



Association between increasing CRP concentration and systolic blood pressure.

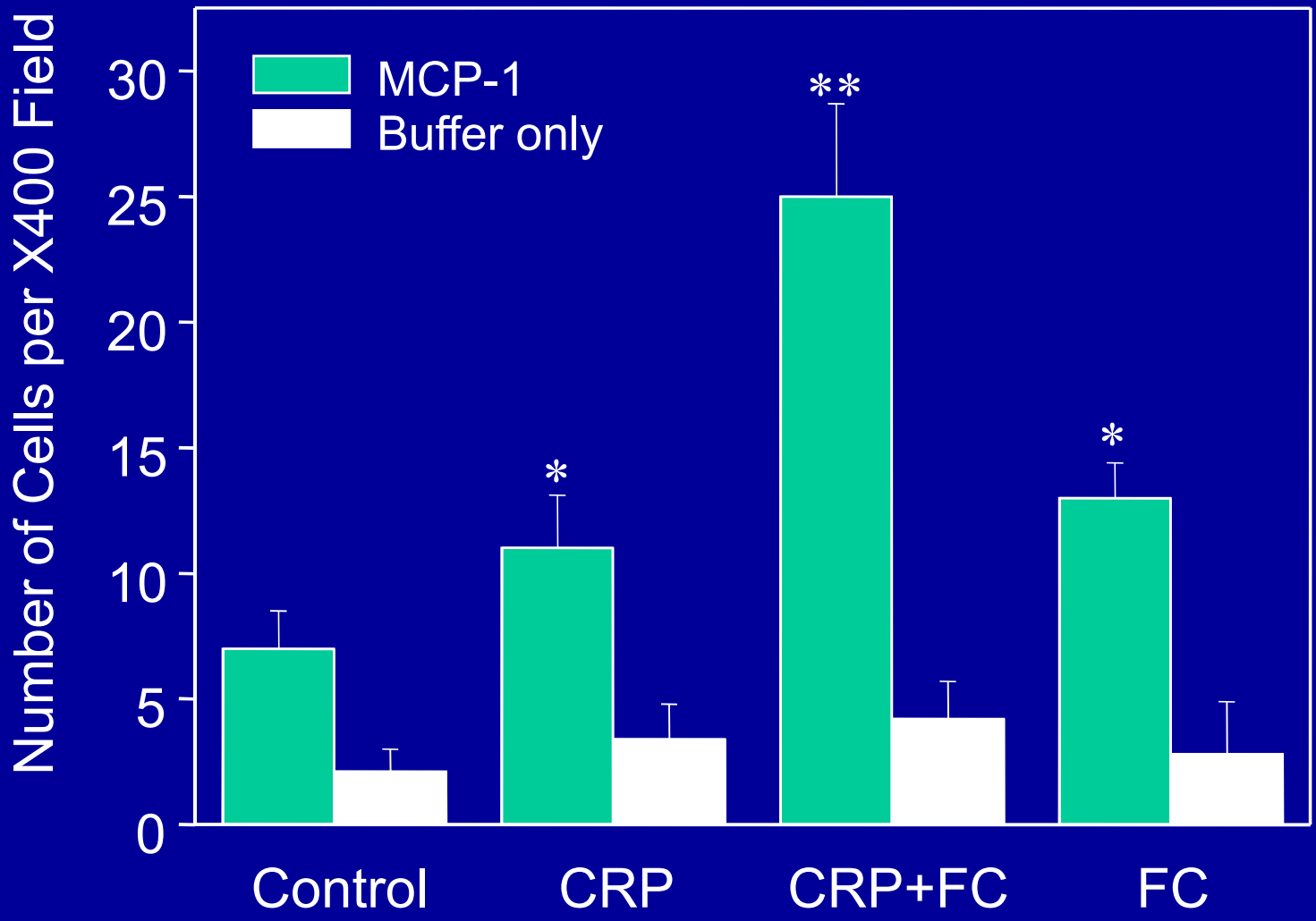
Sesso et al. JAMA 2003; 290 (22):2945-51.

V. C-reactive protein Controversies

CRP is nothing more than a marker ?

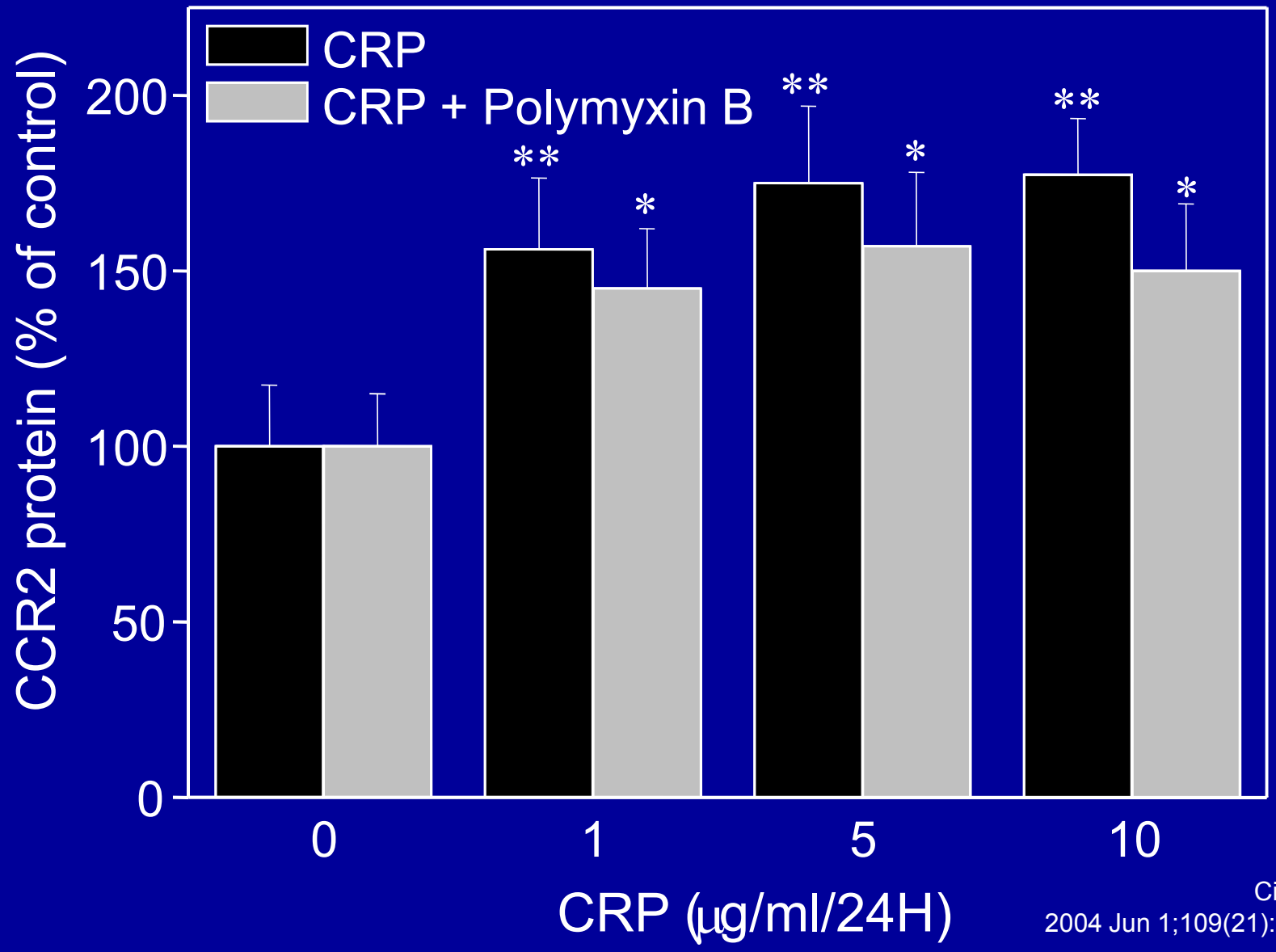
- Reported CRP effects are not really from CRP
- May be due to sodium azide, endotoxin, or/and immunoglobulin.

CRP enhances MCP-1 mediated chemotaxis of monocytes



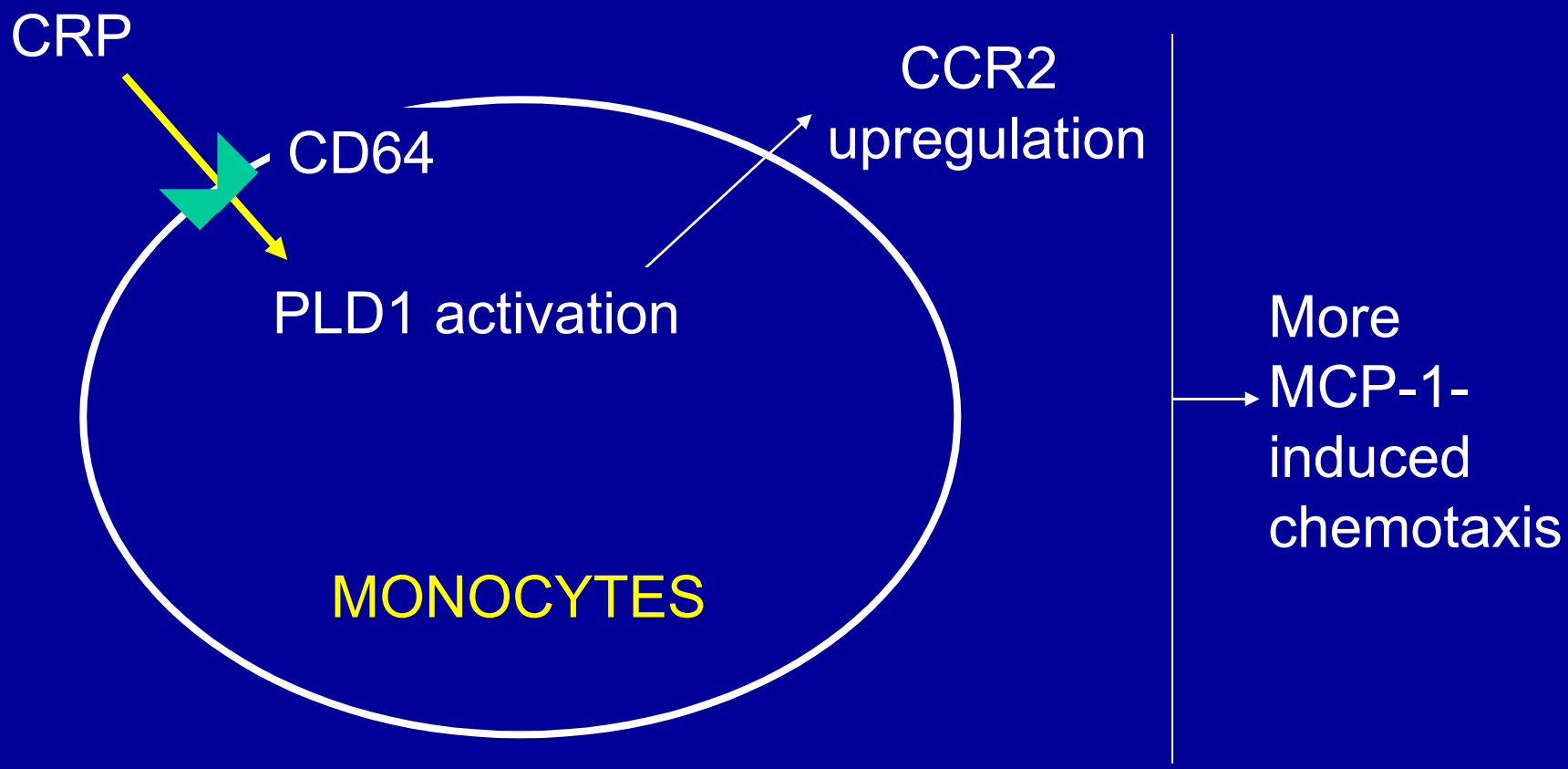
Circulation
2004 Jun 1;109(21):2566-71

CRP upregulates monocyte chemotaxis receptor for MCP-1, CCR2



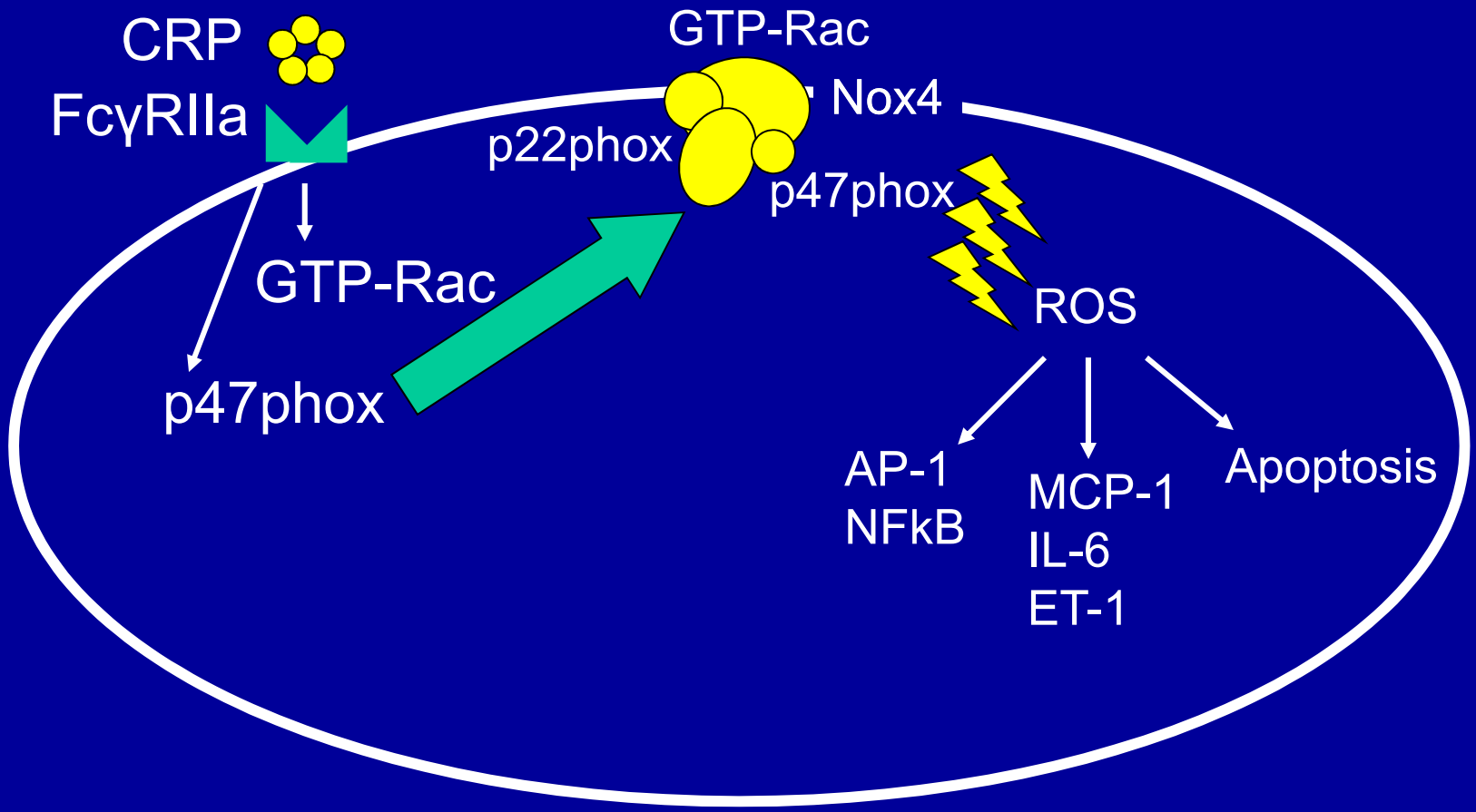
Circulation
2004 Jun 1;109(21):2566-71

CRP enhances CCR2 expression by monocytes and MCP-1-induced chemotaxis



Circulation
2004 Jun 1;109(21):2566-71

CRP induces apoptosis of VSMCs and cytokine release from VSMCs



Cardiovasc Res. 2007 Aug 1;75(3):555-65

Evidences that CRP is atherogenic

Table 1. Proatherogenic effects of CRP.

Endothelial cells	Monocyte-macrophages	Smooth muscle cells
Increased VCAM, ICAM-1, E-selectin, MCP-1, monocyte adhesion	Increased tissue factor	Increased AT-1 and VSMC migration and proliferation
Increased PAI-1, IL-8, CD40/CD40L, MMP-1, ET-1, M-CSF	Increased superoxide and myeloperoxidase	Increased neointimal formation in vivo
Decreased tPA	Increased proinflammatory cytokines and decreased IL-10	Increased iNOS
Decreased prostacyclin	Increased CD11b, CCR2	Increased ROS
Increased superoxide, iNOS	Promoted OxLDL uptake and decreases cholesterol efflux	Increased tissue factor
Decreased eNOS (uncoupling)	Increased MMPs, HMGB1	
Promoted endothelial dysfunction in vivo	Increased M-CSF and proliferation	
Impaired EPC number and function in vitro		

Devaraj S, Singh U, Jialal I. Clin Chem. 2009 Feb;55(2):229-38

Is CRP always bad ?

CRP and foam cell formation

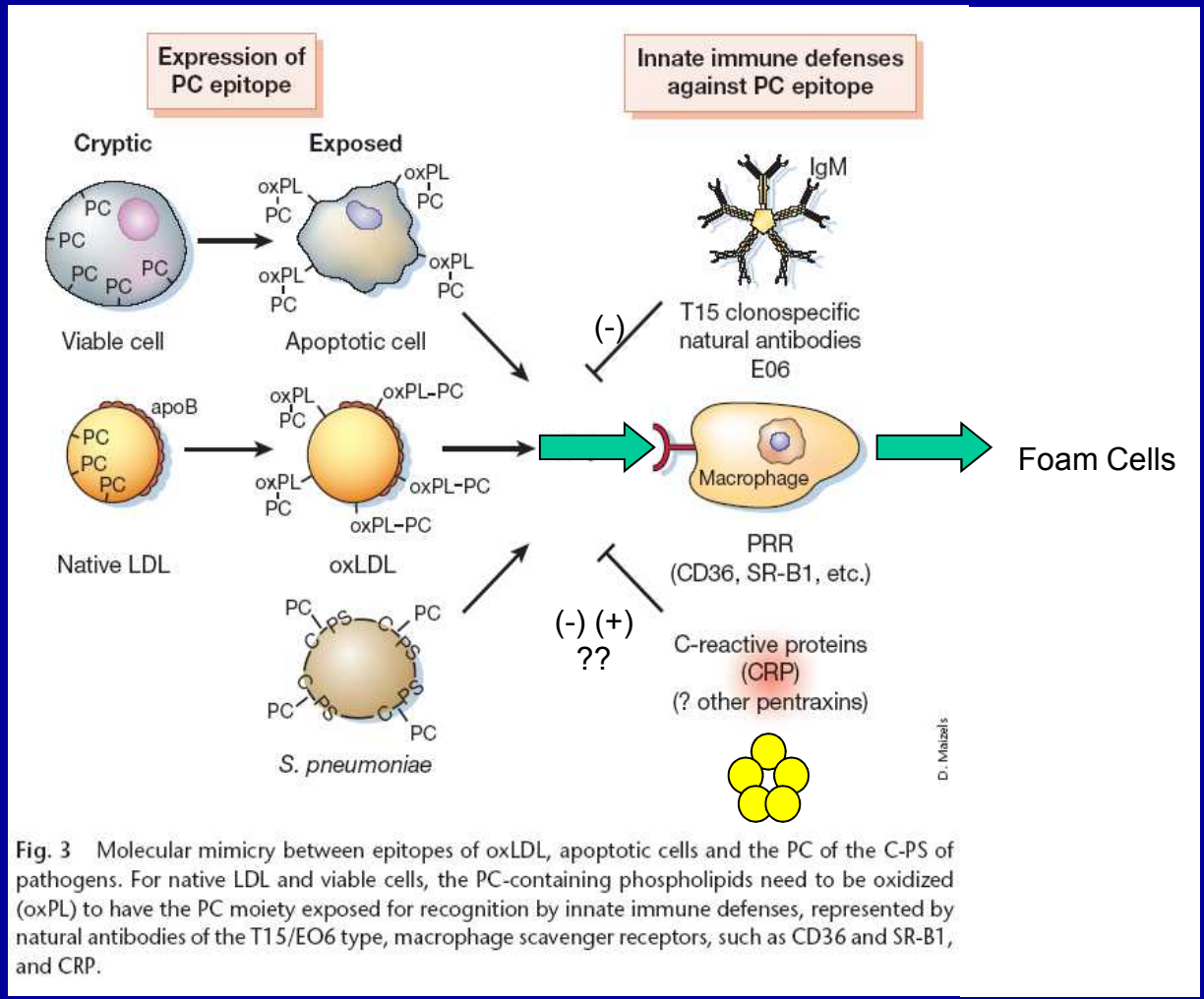


Fig. 3 Molecular mimicry between epitopes of oxLDL, apoptotic cells and the PC of the C-PS of pathogens. For native LDL and viable cells, the PC-containing phospholipids need to be oxidized (oxPL) to have the PC moiety exposed for recognition by innate immune defenses, represented by natural antibodies of the T15/EO6 type, macrophage scavenger receptors, such as CD36 and SR-B1, and CRP.

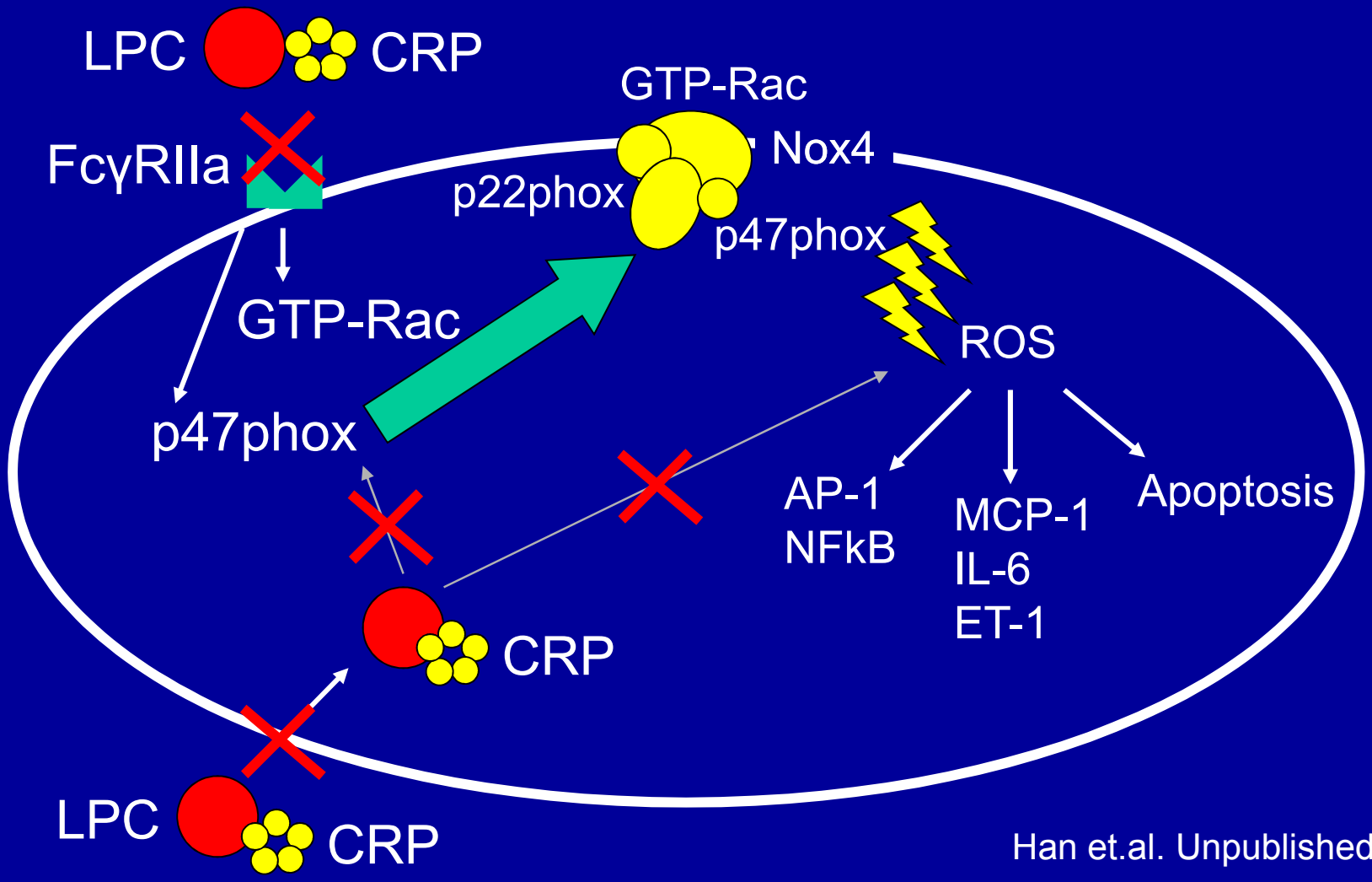
Witztum et.al.

C-reactive protein under oxidative condition ; anti-atherogenic ?

" CRP inhibits the binding of OxLDL to scavenger receptor and subsequent uptake of OxLDL "

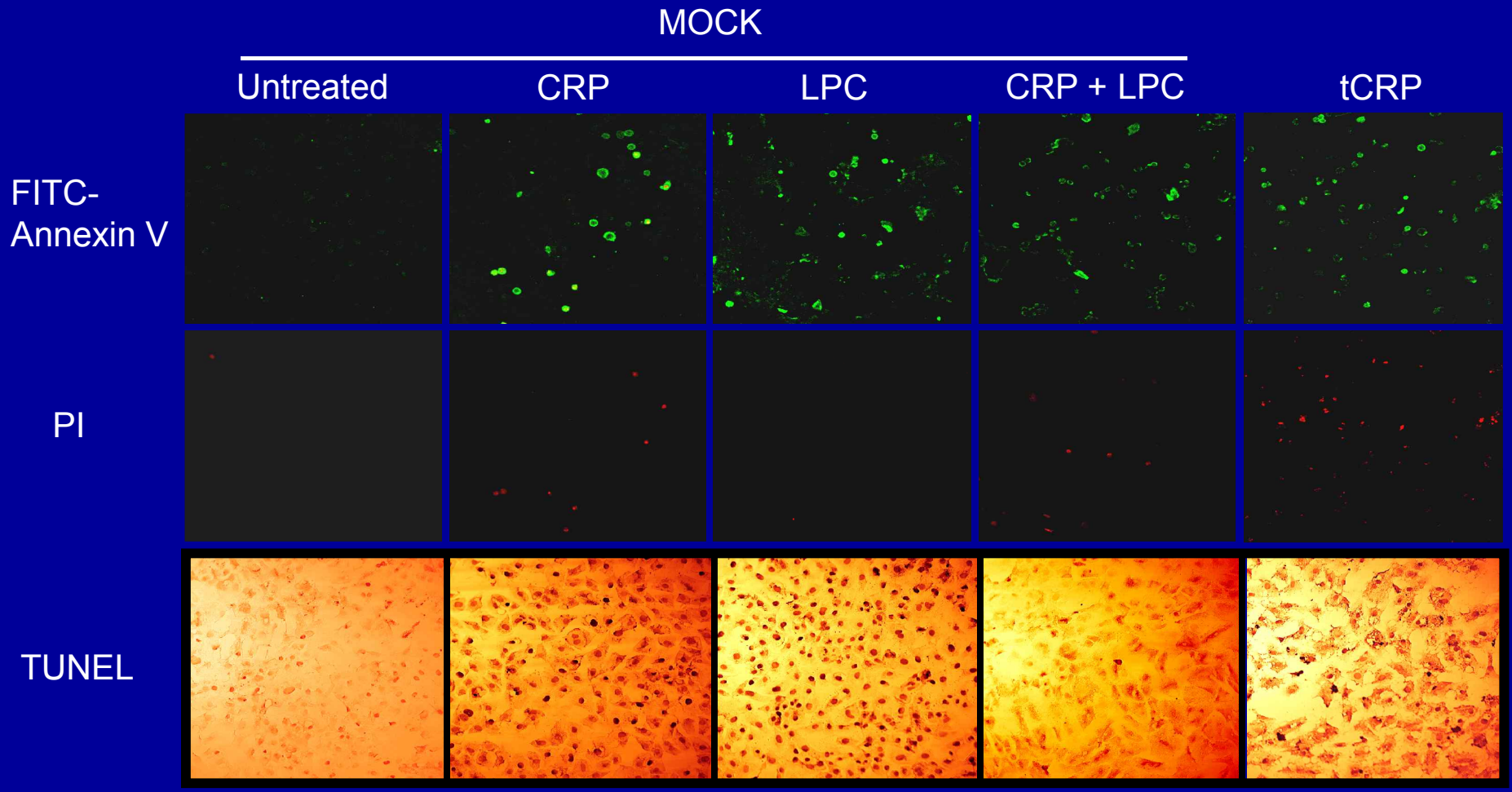
" CRP inhibits the lysoPC-induced NF*k*B activation "

How can CRP-LPC less activate VSMCs ?



Han et.al. Unpublished

CRP-induced apoptosis becomes less potent in the presence of LPC



Han et.al. Unpublished

CRP has another face

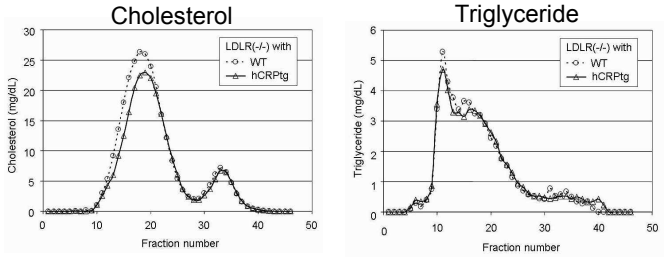
- CRP prevented the formation of membrane attack complex induced by enzymatically modified LDL (E-LDL).

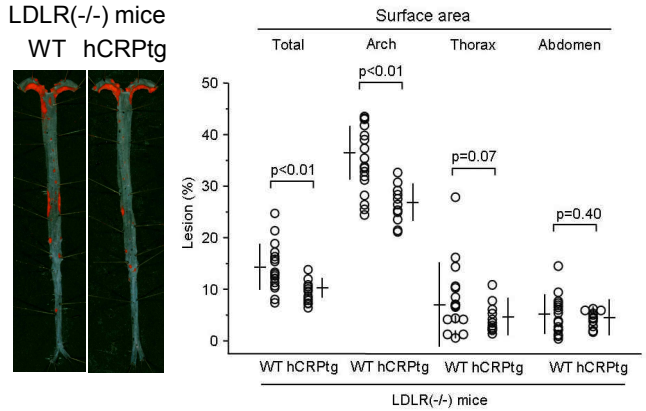
  Bhakdi,S, M Torzewski, K Paprotka, S Schmitt, H Barsoom, P Suriyaphol, S Han, K J Lackner, M Husmann, 2004, Possible protective role for C-reactive protein in atherogenesis: Circulation, v. 109, p. 1870-1876.

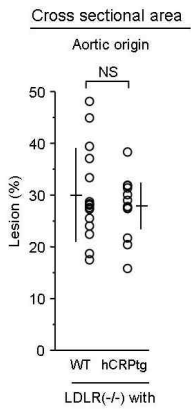
- CRP-induced complement activation did not occur when CRP bound to apoptotic cells, while production of tumor growth factor alpha, an anti-inflammatory cytokine, was sustained.

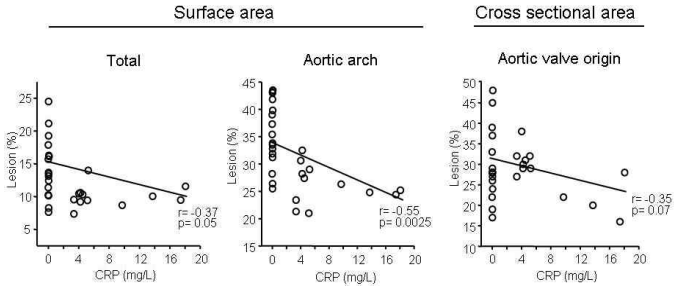
  Gershov,D, S Kim, N Brot, K B Elkon, 2000, C-reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implication for symmetric autoimmunity: J Exp Med, v. **192**, p. 1353-1364.

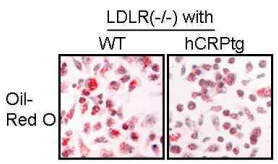
CRP tends to retard atherogenesis and inhibit foam cell formation in CRP/LDLR double KO mice

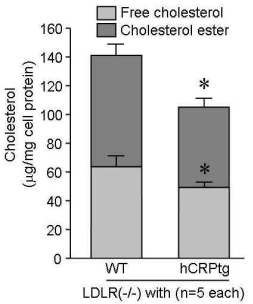












Han et.al. Unpublished