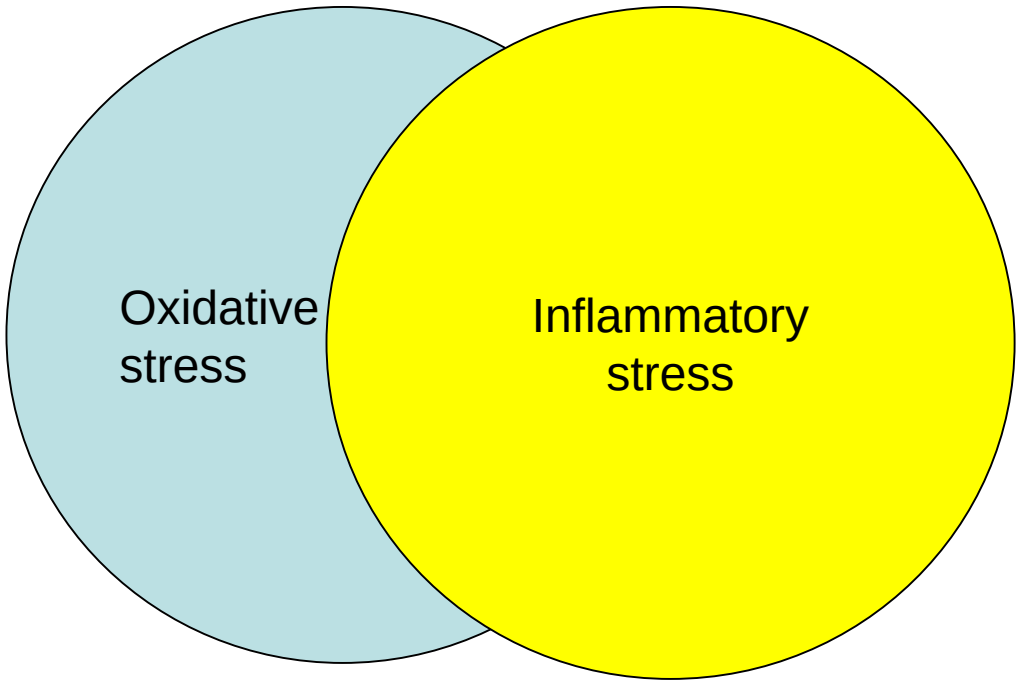
Role of oxidative stress in the atherosclerosis

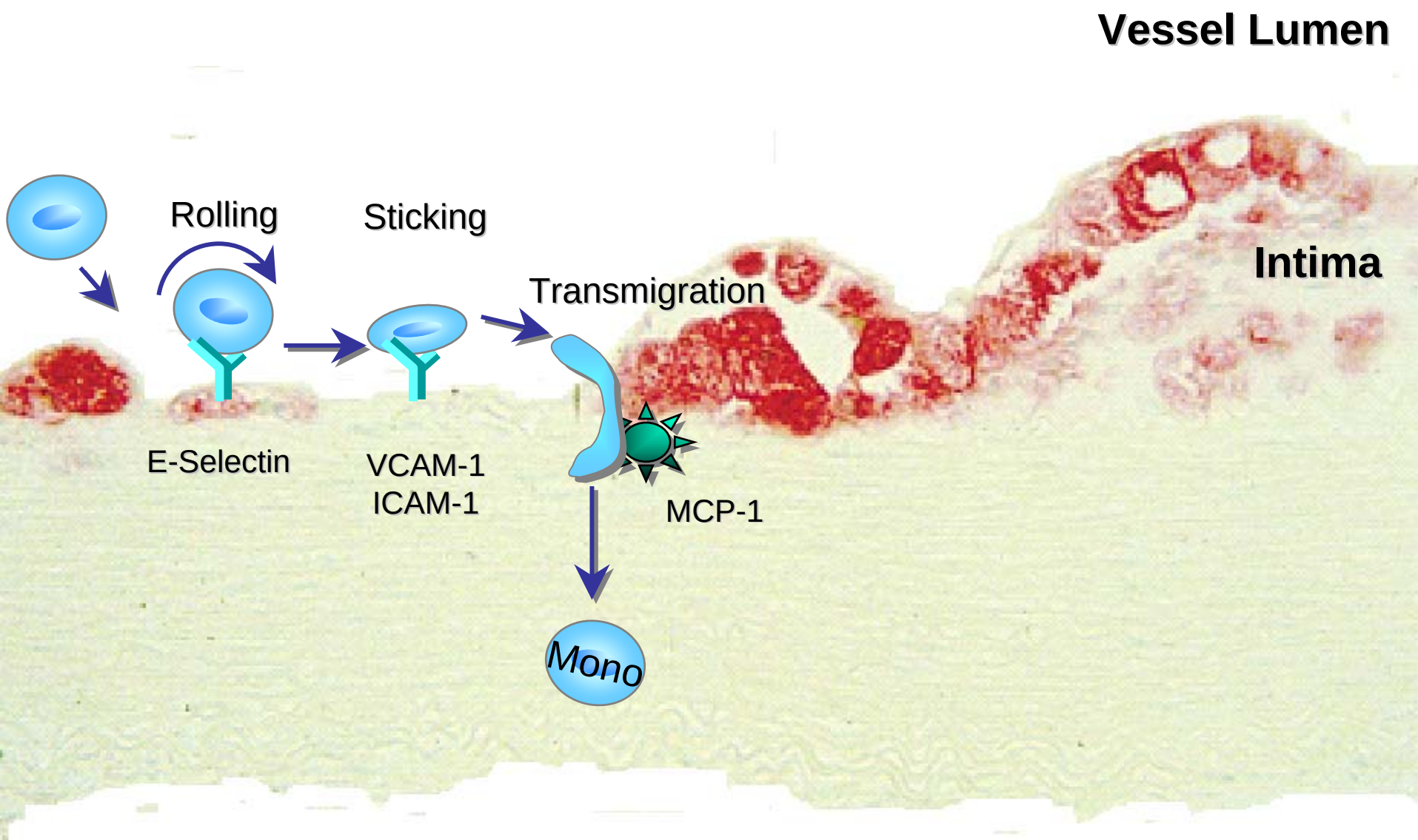
Ki Hoon Han, MD

Asan Medical Center

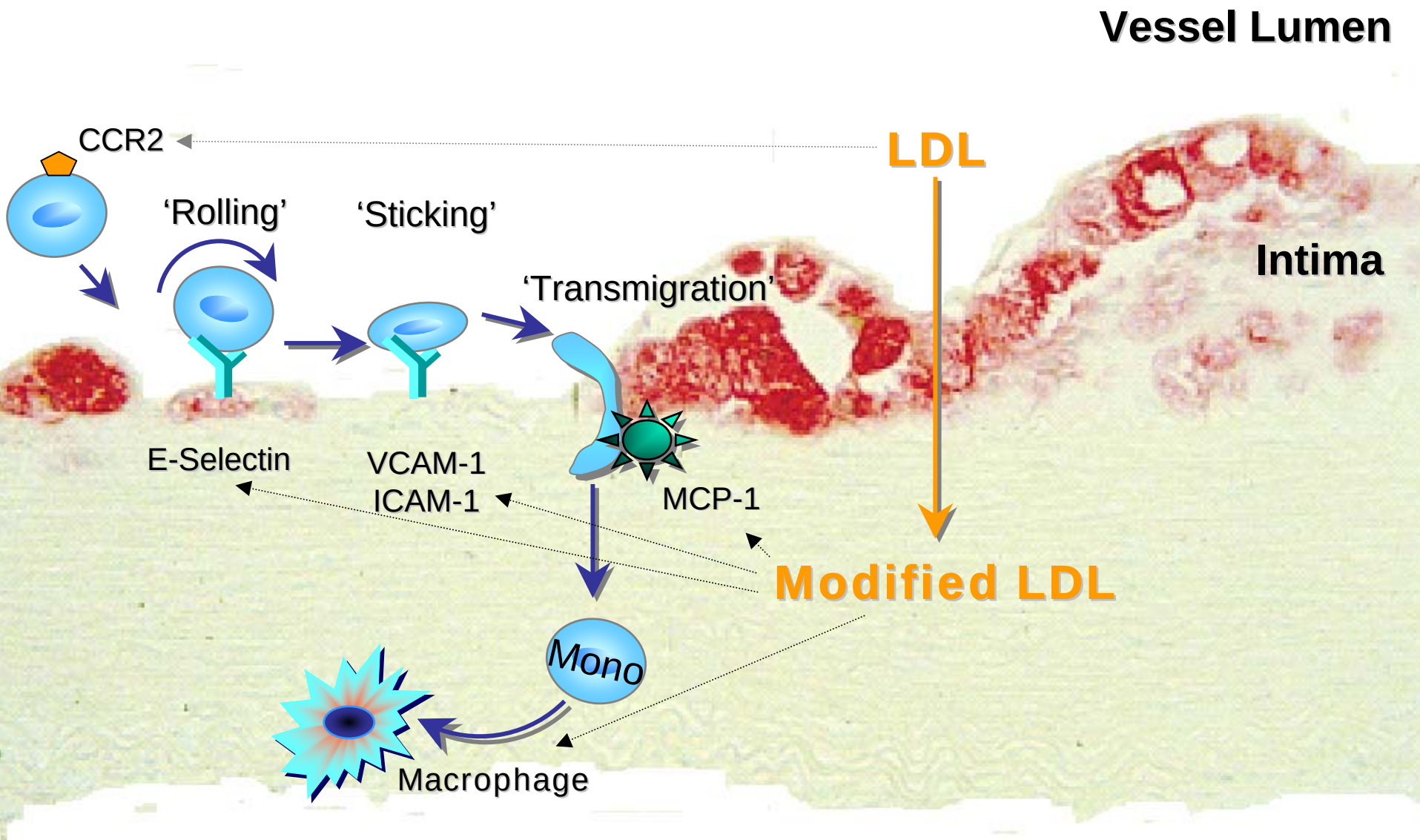
Seoul South Korea


Oxidative
stress
Inflammatory
stress

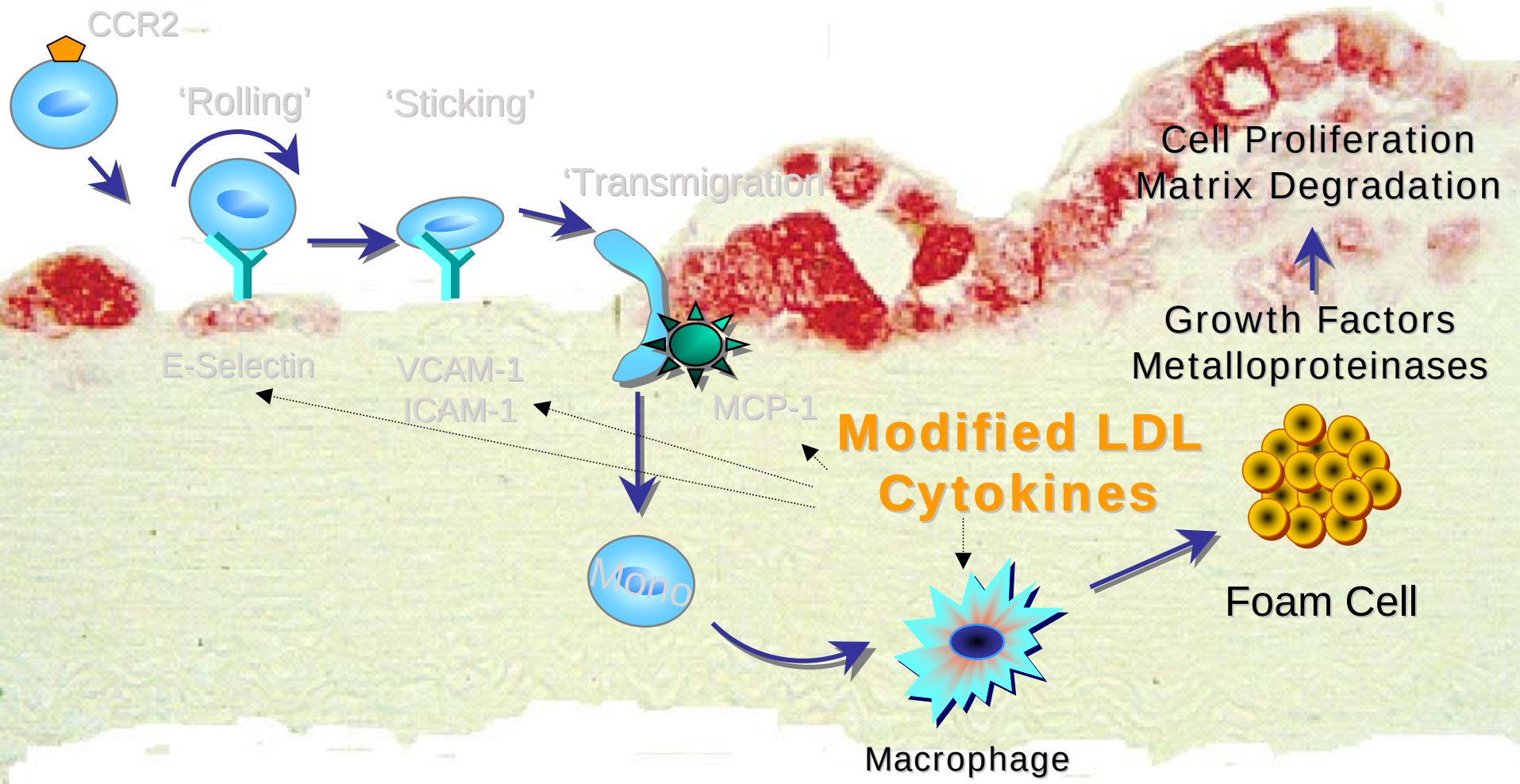
Monocyte Recruitment



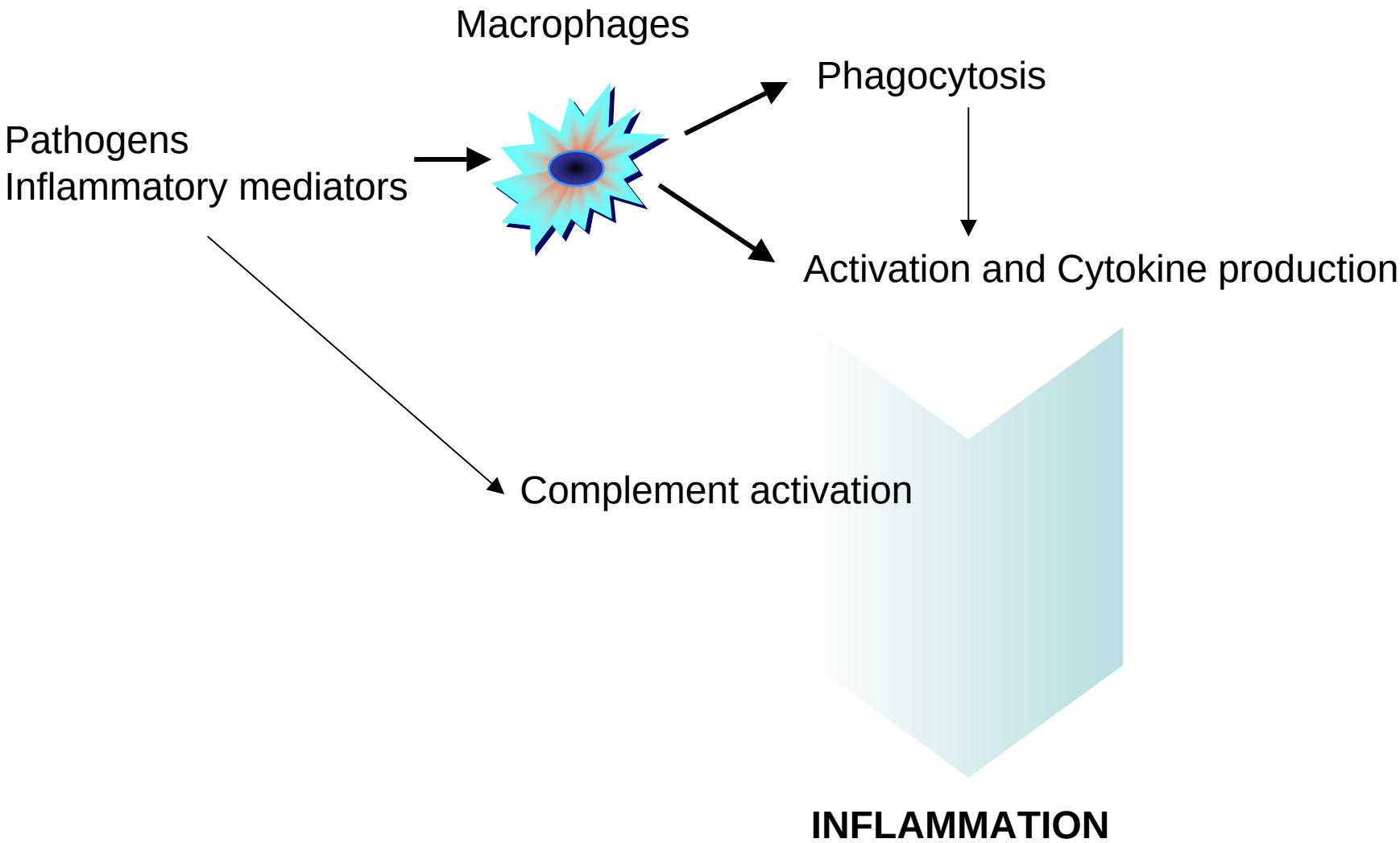
Differentiation to Macrophages

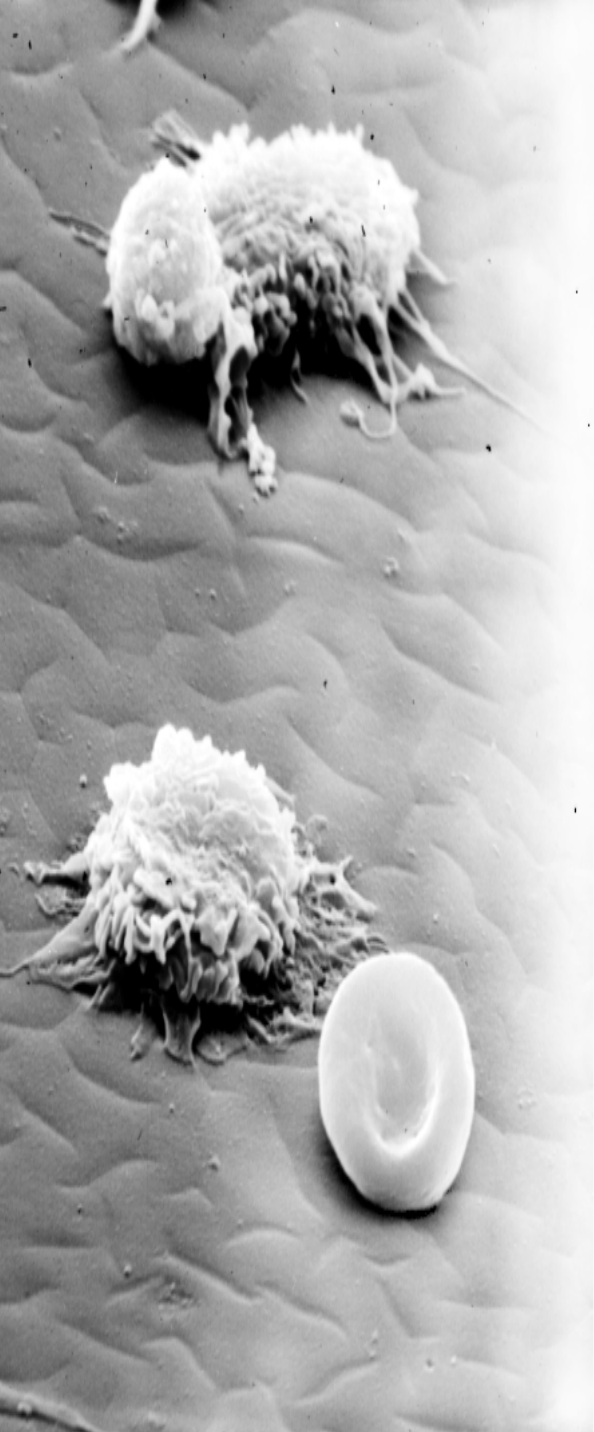


Foam Cell Formation



Scheme of Innate Immunity





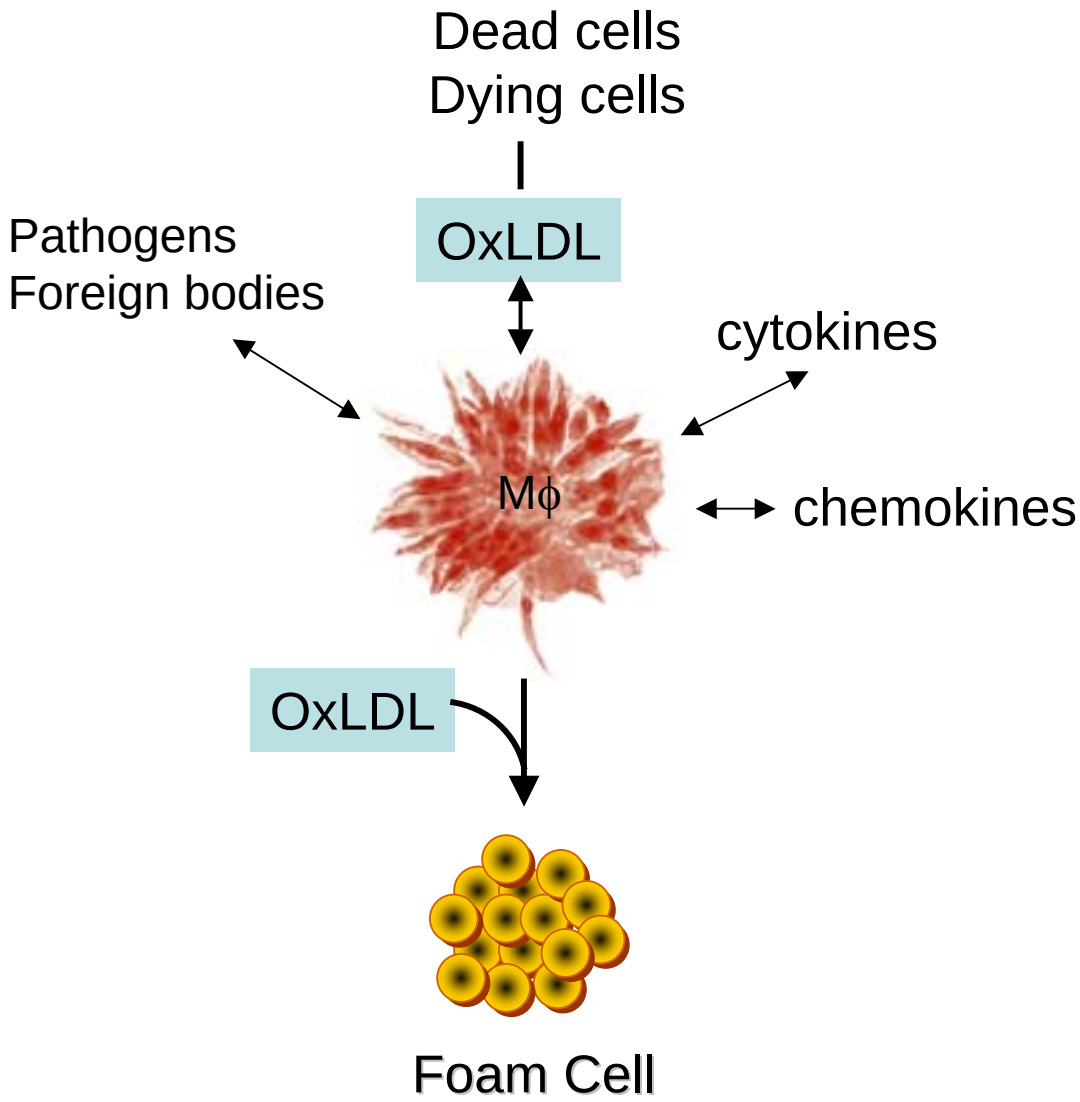

Dead cells
Dying cells
OxLDL
Pathogens
Foreign bodies
cytokines
Mϕ
chemokines
OxLDL
Foam Cell

Table 2. Examples of Genes that Influence Development of Atherosclerosis in Hypercholesterolemia Mice

Gene	Experiment	Genetic Background	Effects on Lesion Area	Proposed Mechanism	Reference
Atherogenic Genes					
12/15-LO	Knockout	apoE$^{-/-}$	↓↓	Decreased LDL oxidation	Cyprus et al., 1999;
	Overexpression	LDL R$^{-/-}$	↑	Increased LDL oxidation	Harats et al., 2000
					Detmer et al., 2000;
iNOS	Knockout	apoE$^{-/-}$	↓	Decreased LDL oxidation	Behr-Roussel et al., 2000
M-CSF	Knockout	apoE$^{-/-}$	↓↓	Decreased macrophage infiltration	Smith et al., 1995;
					Qiao et al., 1997
MCP-1	Knockout	LDL R$^{-/-}$	↓↓	Decreased macrophage infiltration	Gu et al., 1999;
		apoE$^{-/-}$			Gosling et al., 1999
CCR2	Knockout	apoE$^{-/-}$	↓↓	Decreased macrophage infiltration	Boring et al., 1998
P- and E-selectin	Combined knockout	LDL R$^{-/-}$	↓	Decreased monocyte adherence	Dong et al., 1998
CXCR-2	Knockout	LDL R$^{-/-}$	↓	Decreased macrophage residence	Boisvert et al., 1998
SR-A	Knockout	apoE$^{-/-}$	↓	Decreased uptake of oxLDL	Suzuki et al., 1997
CD36	Knockout	apoE$^{-/-}$	↓↓	Decreased uptake of oxLDL	Febraio et al., 2000
IFNγ receptor (R0)	Knockout	apoE$^{-/-}$	↓	Decreased inflammatory responses, increased apoAIV	Gupta et al., 1997
CD154	Knockout	apoE$^{-/-}$	↓	Decreased CD40 signaling	Lutgens et al., 1999
IL-10	Knockout	C57 BL/6J	↑	Increased inflammatory responses	Pinderski et al., 1999;
					Mallat et al., 1999
Antiatherogenic Genes					
Paraoxinase	Knockout	apoE$^{-/-}$	↑	Reduced clearance of oxidized lipids	Shih et al., 2000
apo A-I	Knockout	h apoB transgene	↑	Decreased reverse cholesterol transport	Voyaziakis et al., 1998
	Overexpression	apo E$^{-/-}$	↓	Increased reverse cholesterol transport	Benoit et al., 1999;
		LDL R$^{-/-}$			Tangirala et al., 1999
PPARγ	Knockout	LDL R$^{-/-}$	↑	Altered macrophage function	Chawla et al., 2001;
	Agonist	LDL R$^{-/-}$	↓		Lie et al., 2000
SR-B1	Knockout	LDL R$^{-/-}$	↑	Decreased reverse cholesterol transport	Huszar et al., 2000
	Overexpression	LDL R$^{-/-}$	↓	Increased reverse cholesterol transport	Kozavsky et al., 2000

Glass,K and Witztum, JL Cell 2001 104:513-516

Oxidation of LDL

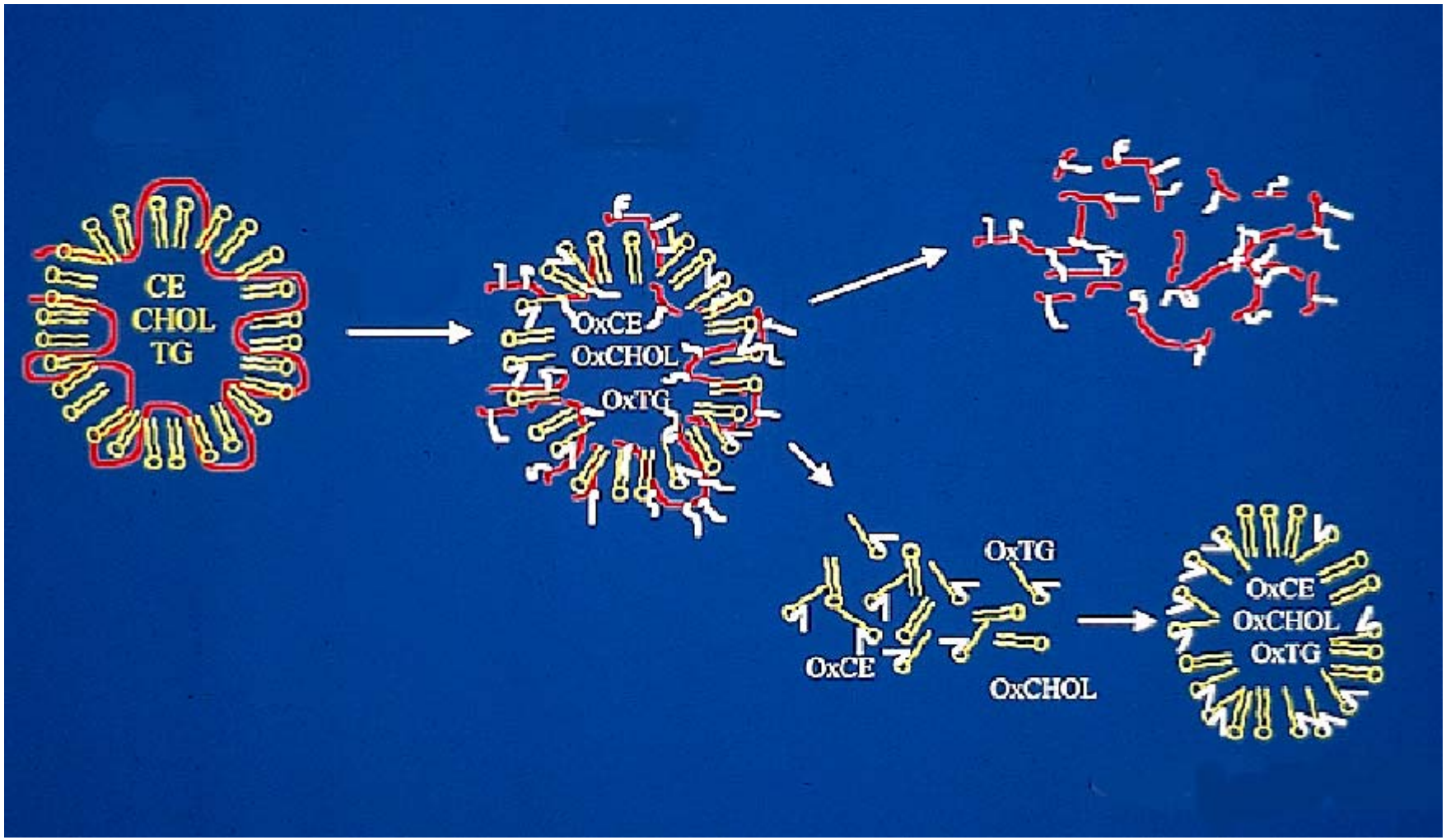


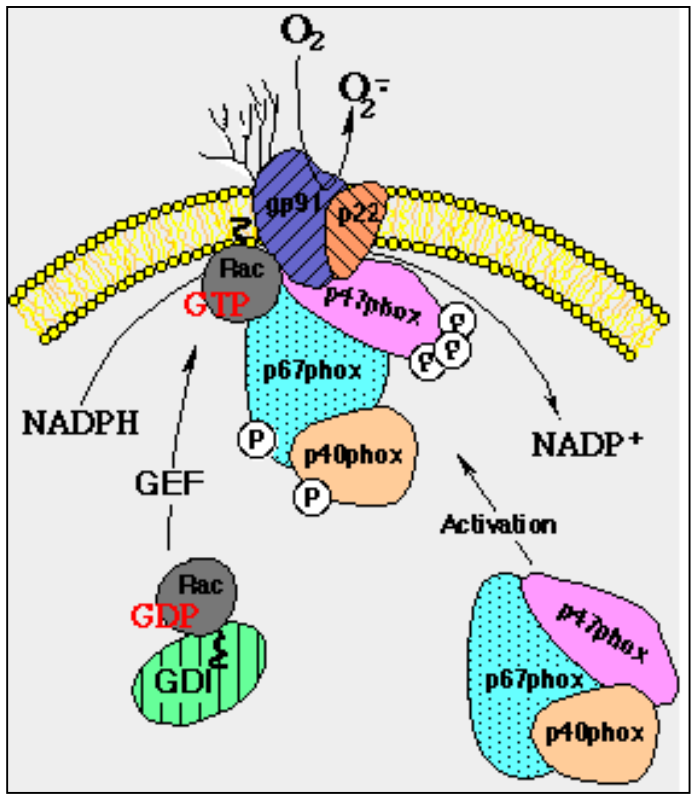
Table 1. Potential mechanisms by which oxidized forms of LDL may influence atherogenesis

- OxLDL has enhanced uptake by macrophages leading to foam cell formation.
- Products of OxLDL are chemotactic for monocytes and T-cells and inhibit the motility of tissue macrophages.
- Products of OxLDL are cytotoxic, in part due to oxidized sterols, and can induce apoptosis.
- OxLDL, or products, are mitogenic for smooth muscle cells and macrophages.
- OxLDL, or products, can alter gene expression of vascular cells, e.g. induction of MCP-1, colony-stimulating factors, IL-1 and expression of adhesion molecules.
- OxLDL, or products, can increase expression of macrophage scavenger receptors, thereby enhancing its own uptake.
- OxLDL, or products, can induce proinflammatory genes, e.g. hemoxygenase, SAA and ceruloplasmin.
- OxLDL can induce expression and activate PPARγ, thereby influencing the expression of many genes.
- OxLDL is immunogenic and elicits autoantibody formation and activated T-cells.
- Oxidation renders LDL more susceptible to aggregation, which independently leads to enhanced uptake. Similarly, OxLDL is a better substrate for sphyingomyelinase, which also aggregates LDL.
- OxLDL may enhance procoagulant pathways, e.g. by induction of tissue factor and platelet aggregation.
- Products of OxLDL can aversely impact arterial vasomotor properties.

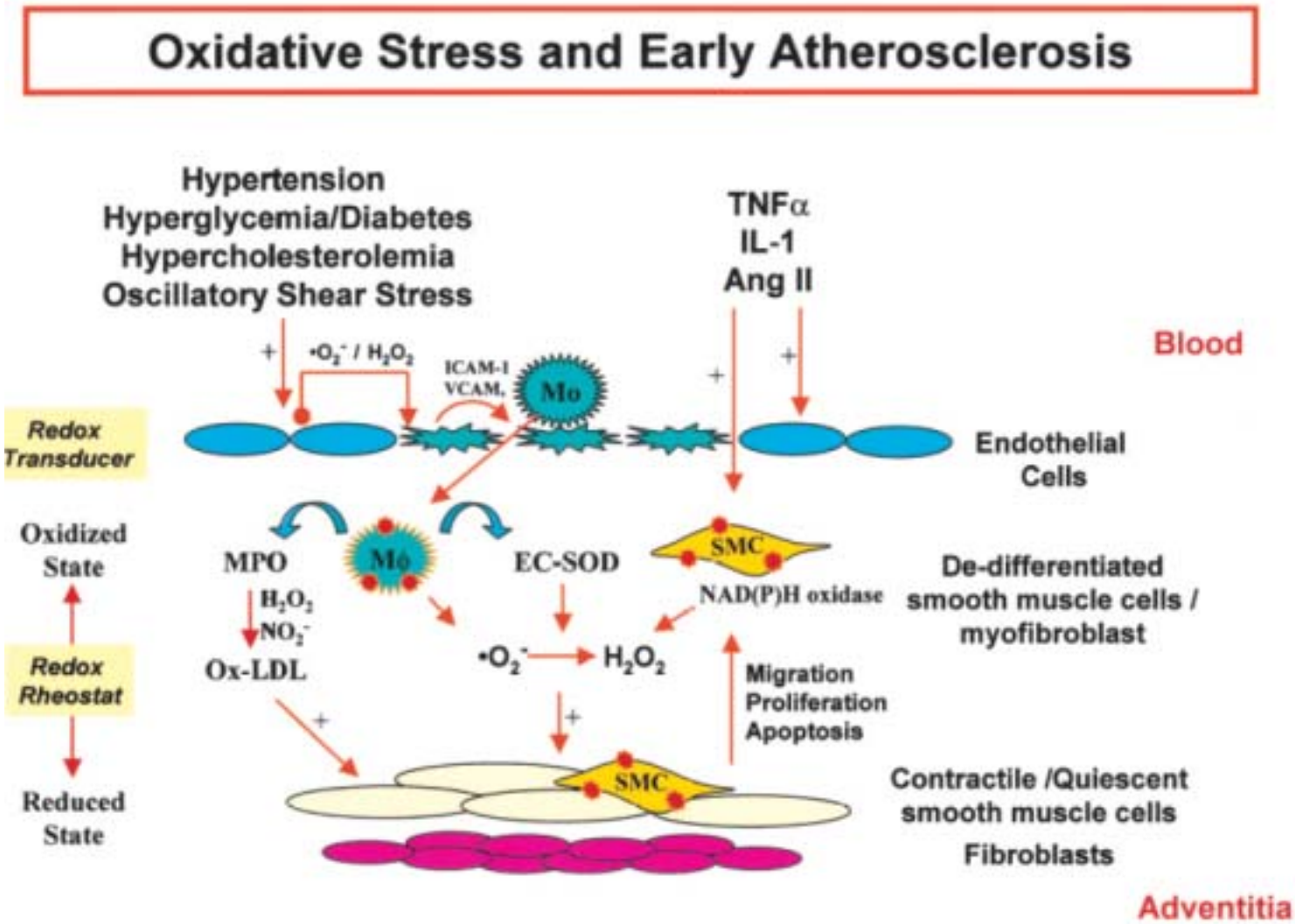
Modified from Steinberg and Witztum (1999).

Who made this oxidized ?

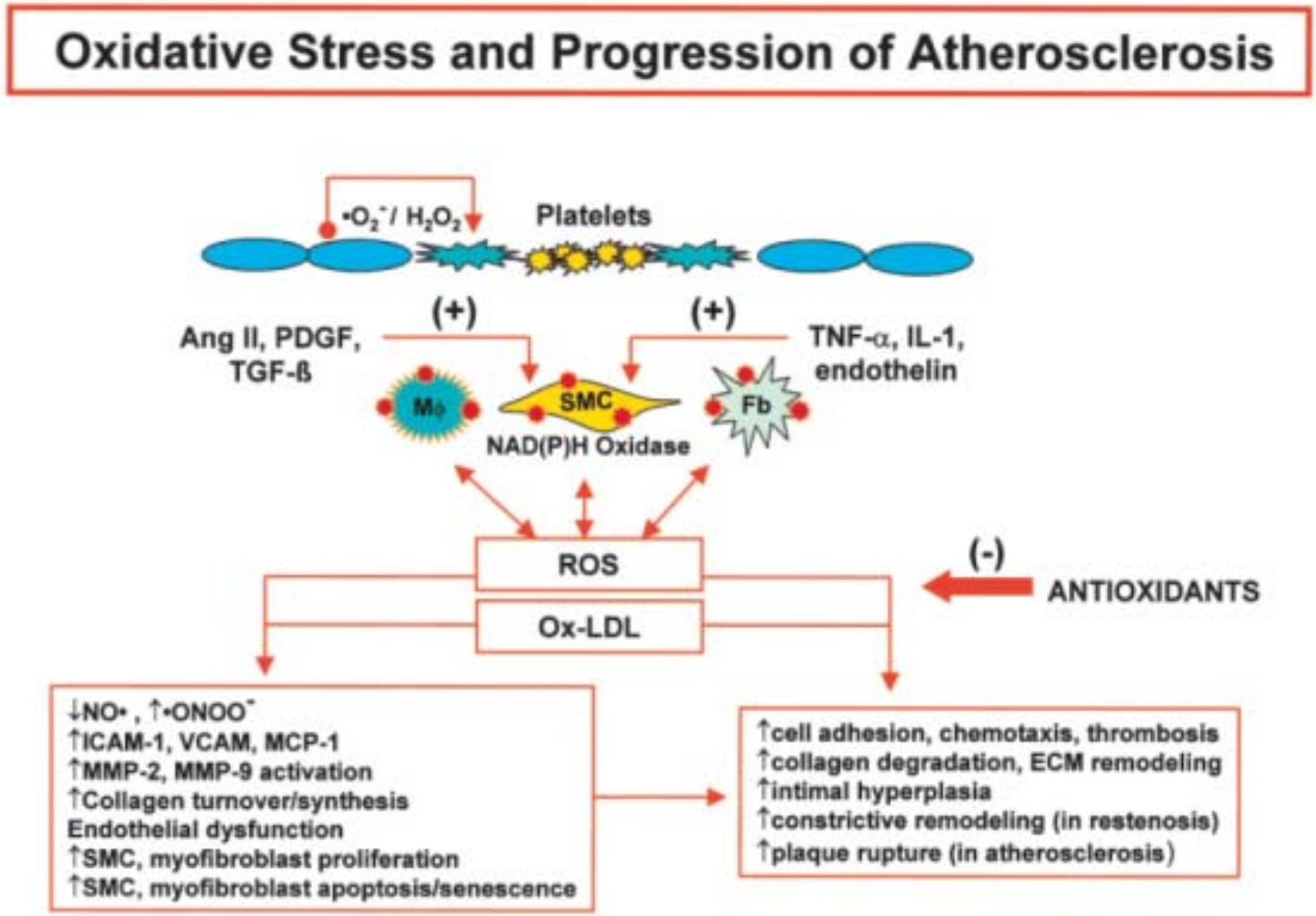
NADPH oxidase in atherogenesis



NADPH oxidase in atherogenesis



NADPH oxidase in atherogenesis



12/15-lipoxygenase

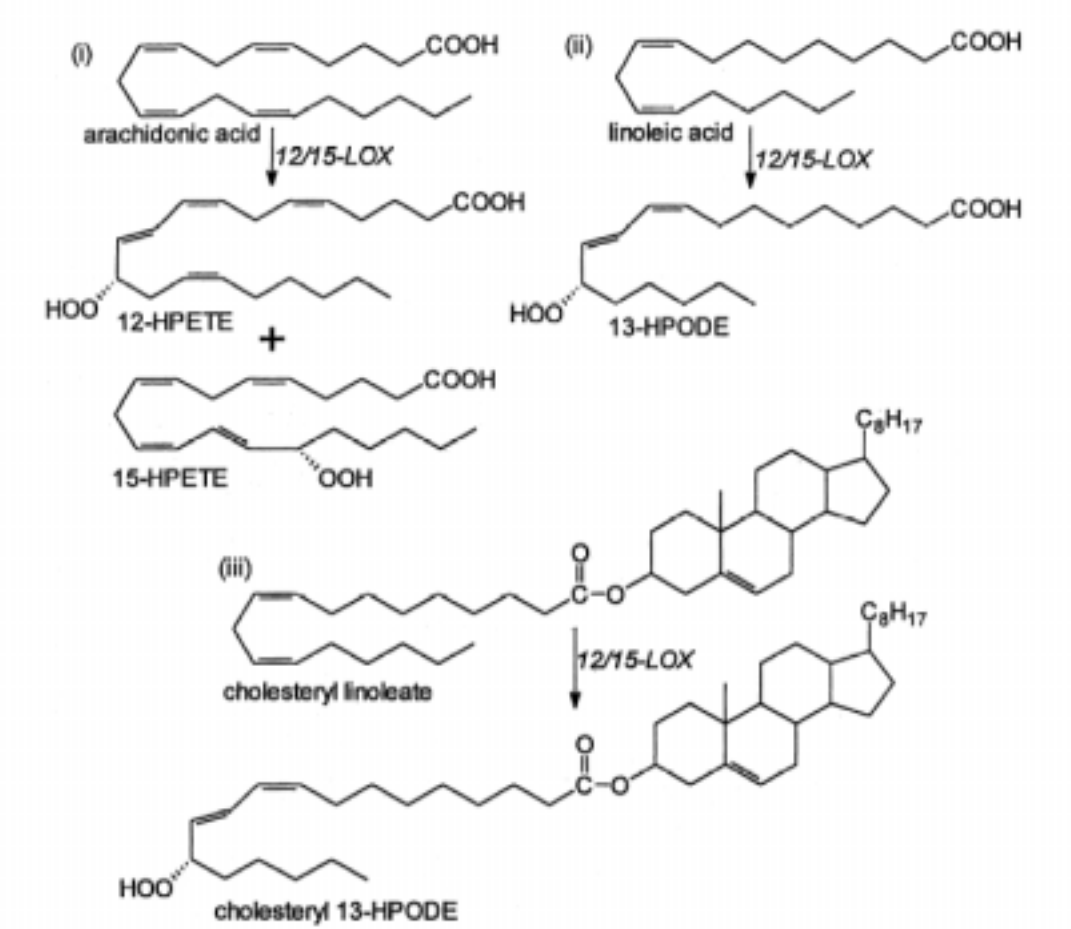


Figure 1. 12/15-Lipoxygenase conversion of (i) arachidonic acid (ii) linoleic acid and (iii) cholesteryl linoleate to various hydroperoxide metabolites.

Phospholipase A2 ; PLA2

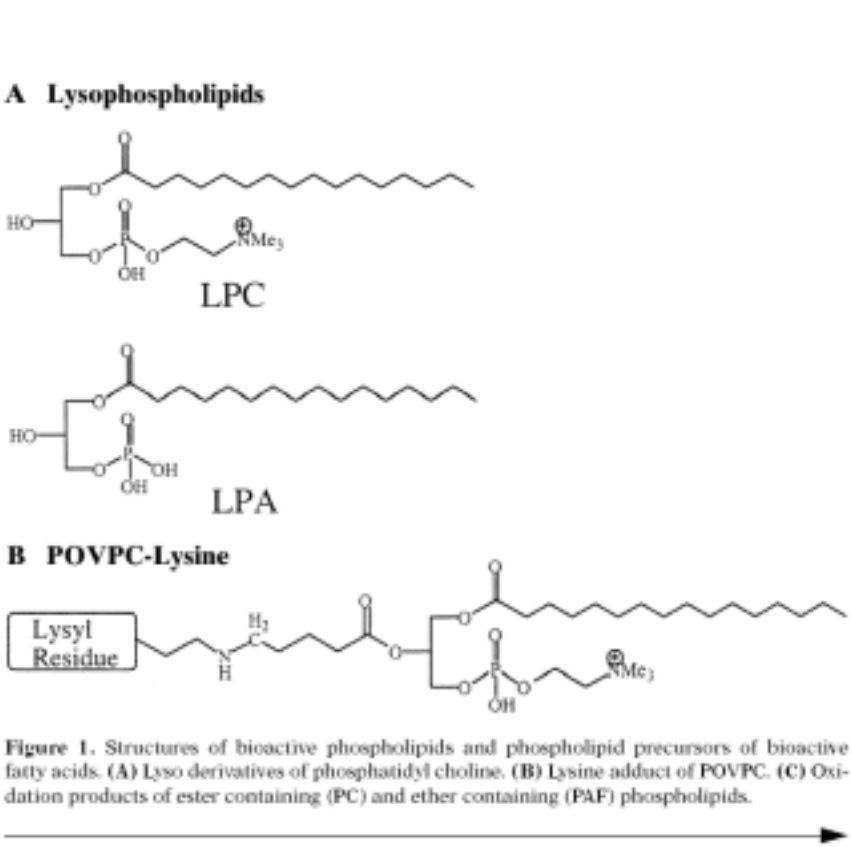


Figure 1. Structures of bioactive phospholipids and phospholipid precursors of bioactive fatty acids. **(A)** Lyso derivatives of phosphatidyl choline. **(B)** Lysine adduct of POVPC. **(C)** Oxidation products of ester containing (PC) and ether containing (PAF) phospholipids.

Table 1. In vitro evidence for importance of phospholipid oxidation products in atherogenesis

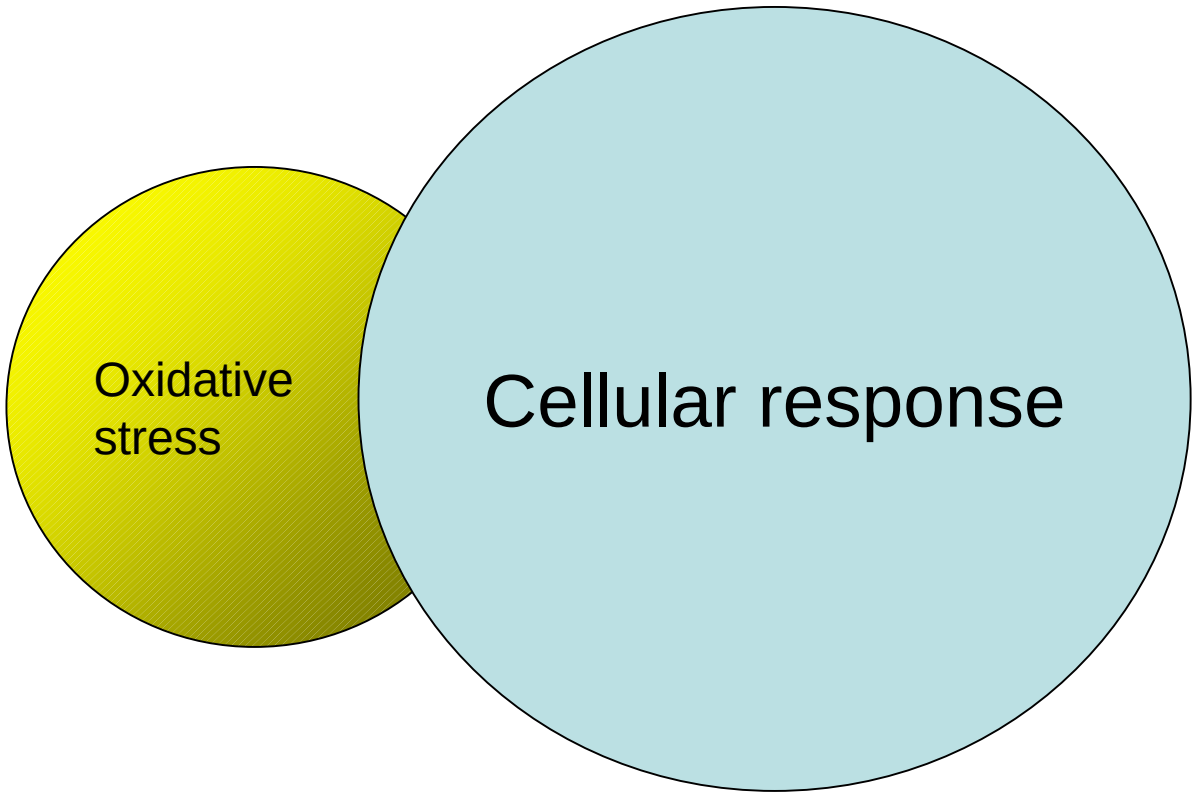
Active lipid	*Effect*
Fatty Streak	
LPC	Inhibition of vascular relaxation (Mangin et al. 1993, Fukao et al. 1996)
LPA	E-Selectin, VCAM-1 (Rizza et al. 1999)
LPA	Actin reorganization (Siess et al. 1999)
LPC	VCAM-1, ICAM-1 (Kume et al. 1992, Kita et al. 1999)
OVPCs	CS-1 fibronectin (Shih et al. 1999, Leitinger et al. 1999)
GPCs, EIPCs, MM-LDL	VCAM-1, E-Selectin (Subbanagounder et al. 2001)
OxPAPC, POVPC, MM-LDL	IL-8, MCP-1 (Lee et al. 2001)
OxPAPC, POVPC	Inhibition of PMN binding (Leitinger et al. 1999)
Fibrous Plaque	
LPC	Smooth muscle migration (Kita et al. 1999, Kohno et al. 1998)
LPA	Smooth muscle migration (Tokumura et al. 1994)
PAF-like lipids	Smooth muscle proliferation (Marathe et al. 1999, Heery et al. 1995)
Thrombosis	
LPA	Platelet activation (Siess et al. 1999)
PAF-like lipids	Platelet activation (Kern et al. 1998)
LPC	UPA and UPAR synthesis (Oka et al. 2000)
MM-LDL	MMP synthesis (Rajavashisth et al. 1999)
MM-LDL	Macrophage fibronectin deposition (Engelmann et al. 1999)
LPC	Suppression of tissue factor expression (Lewis et al. 1995)

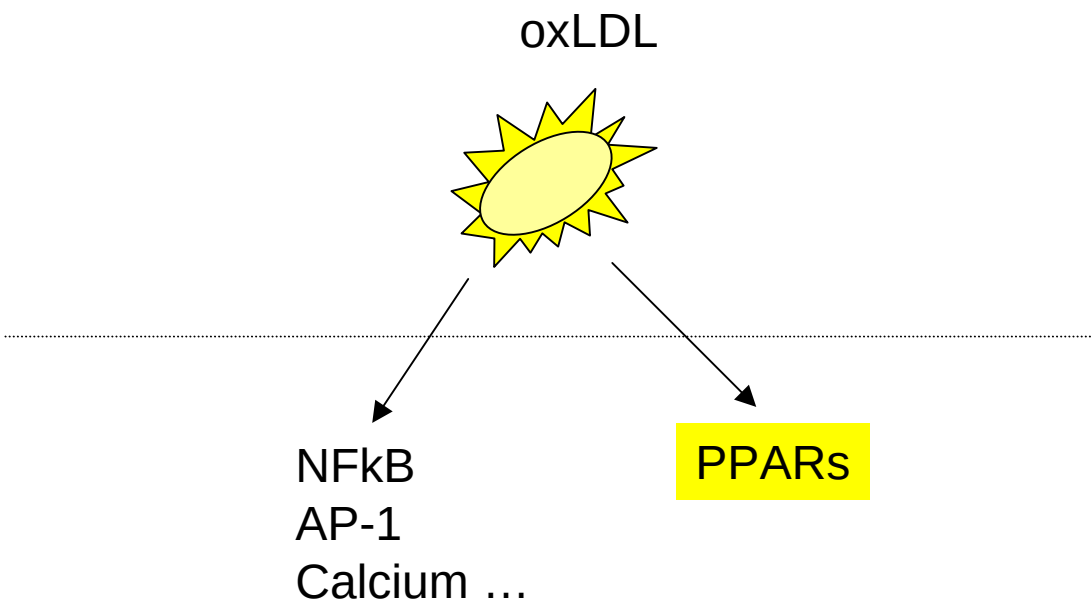
Table 2. Effects of antioxidants in animal models of atherosclerosis

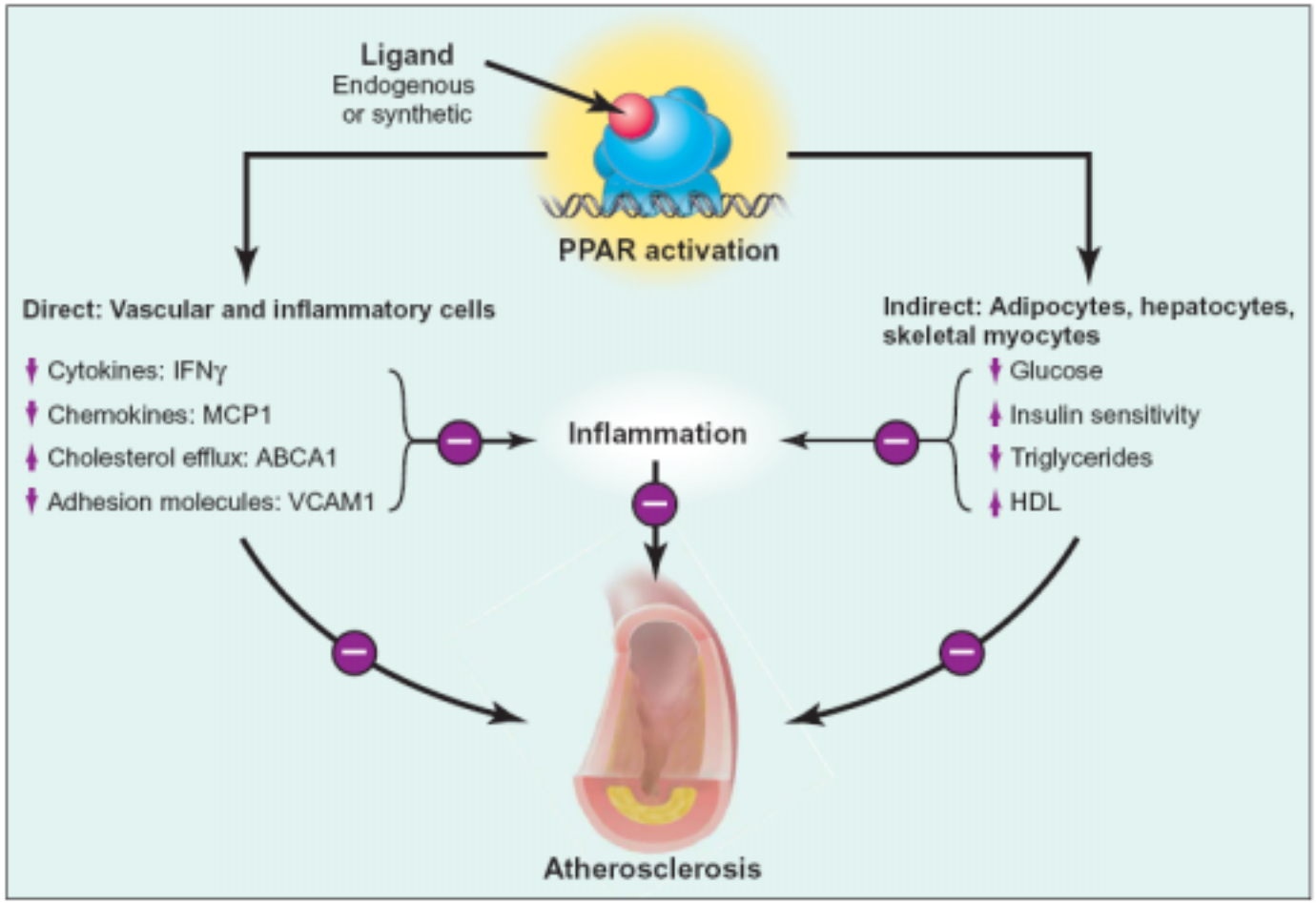
Type of study	*Reference*	*Result*
Probucol in $LDLR^{-/-}$ rabbits	Carew et al. (1987)	+
	Kita et al. (1987)	+
	Mao et al. (1991)	+
	Daugherty et al. (1991)	±
	Fruebis et al. (1994)	+
	Witting et al. (1999a)	+
Probucol analogs in $LDLR^{-/-}$ rabbits	Mao et al. (1991)	+
	Fruebis et al. (1994)	−
	Witting et al. (1999a)	−
Probucol in cholesterol-fed rabbits	Stein et al. (1989)	−
	Daugherty et al. (1989)	+
	Prasad et al. (1994)	+
	Shaish et al. (1995)	+
Other antioxidants in rabbits		
DPPD	Sparrow et al. (1992)	+
BHT	Bjorkhem et al. (1991)	+
Vitamin E	Mantha et al. (1993)	+
	Morel et al. (1994)	−
	Kleinveld et al. (1995)	−
	Shaish et al. (1995)	−
	Fruebis et al. (1996)	−
Antioxidants in rodents		
Probucol in hamsters	Parker et al. (1995)	+
Vitamin E in hamsters	Parker et al. (1995)	+
DPPD in $apoE^{-/-}$ mice	Tangirala et al. (1995)	+
Probucol in $apoE^{-/-}$ mice	Zhang et al. (1997)	−*
Probucol in $LDLR^{-/-}$ mice	Bird et al. (1998)	−*
Probucol in $LDLR^{-/-}$ mice	Cynshi et al. (1998)	−*
Probucol in $apoE^{-/-}$ mice	Witting et al. (2000)	+/−*
Probucol analog in $LDLR^{-/-}$	Cynshi et al. (1998)	+
Probucol metabolite in $LDLR^{-/-}$/$apoE^{-/-}$	Witting et al. (1999)	+
Vitamin E in $apoE^{-/-}$	Praticò et al. (1998)	+
Dietary antioxidants in $LDLR^{-/-}$	Crawford et al. (1998)	+
Antioxidants in nonhuman primates		
Probucol	Sasahara et al. (1994)	+
Vitamin E	Verlangieri and Bush (1992)	±

Modified from Steinberg and Witztum (1999). + = positive study (atherosclerosis decreased); − = negative study (atherosclerosis unchanged); ± = atherosclerosis equivocal; −* = atherosclerosis enhanced.

Look at the other side of coin.

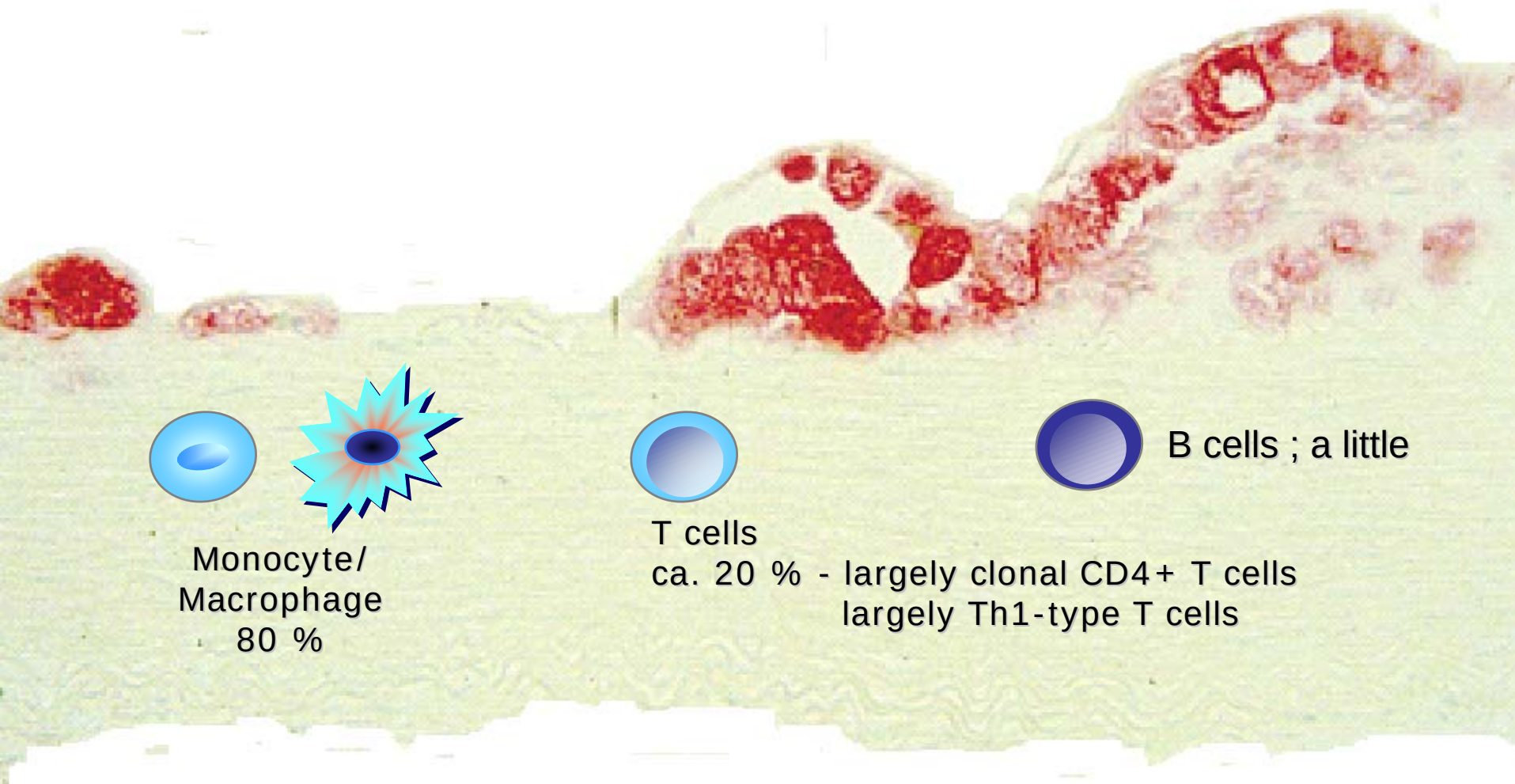

Oxidative
stress
Cellular response


oxLDL
NFkB
AP-1
Calcium …
PPARs



Direct and indirect effects of PPAR nuclear receptors. PPAR activation by synthetic ligands induces metabolic changes that may limit inflammation and atherosclerosis indirectly. Alternatively, the expression of PPARs in most major vascular and inflammatory cells and PPAR regulation of relevant target genes in those cells raises the possibility that PPARs have a direct effect on inflammation and atherosclerosis.

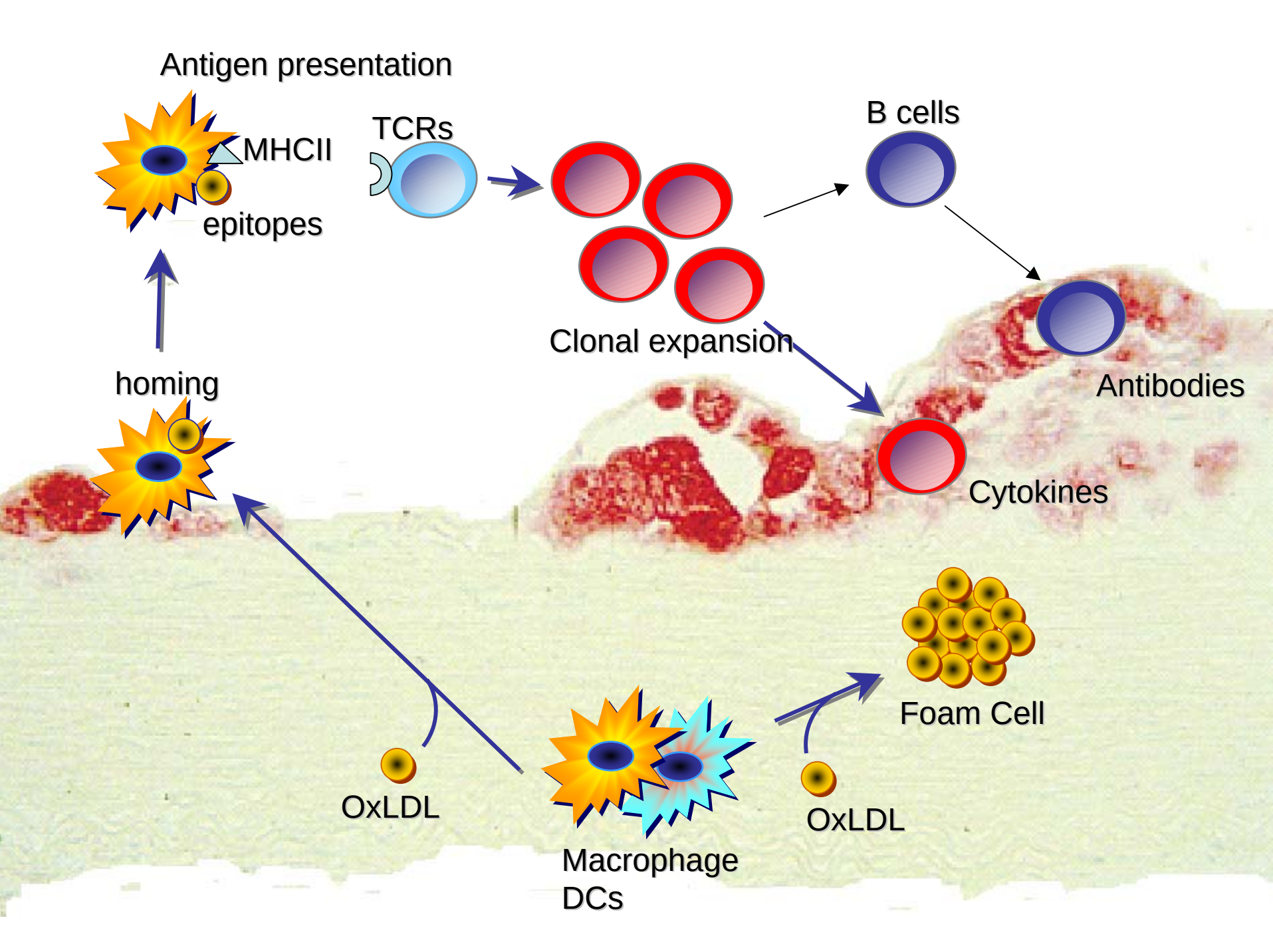
Inflammatory Cells in Atheroma



Monocyte/
Macrophage
80 %

T cells
ca. 20 % - largely clonal CD4+ T cells
largely Th1-type T cells

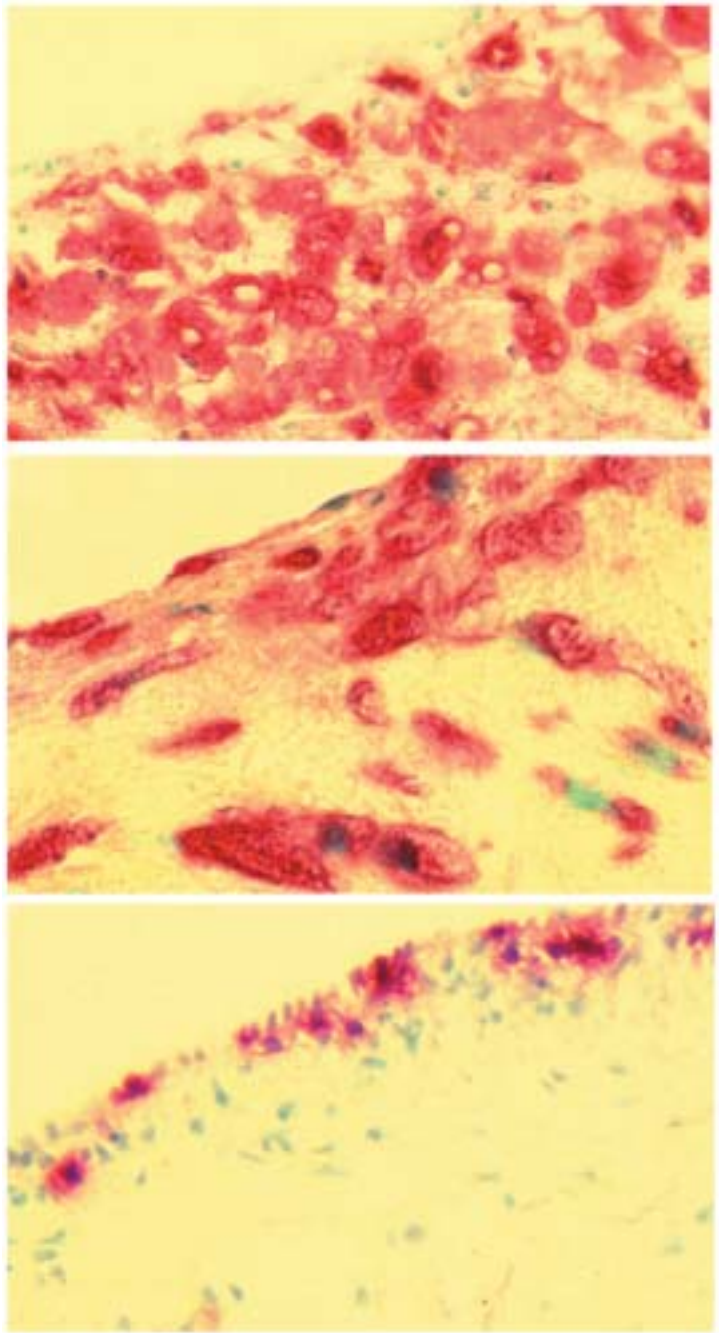
B cells ; a little


Antigen presentation
MHCII
epitopes
TCRs
B cells
Clonal expansion
homing
Antibodies
Cytokines
Foam Cell
OxLDL
Macrophage
DCs
OxLDL

Macrophages

oxidized
epitope

T cells



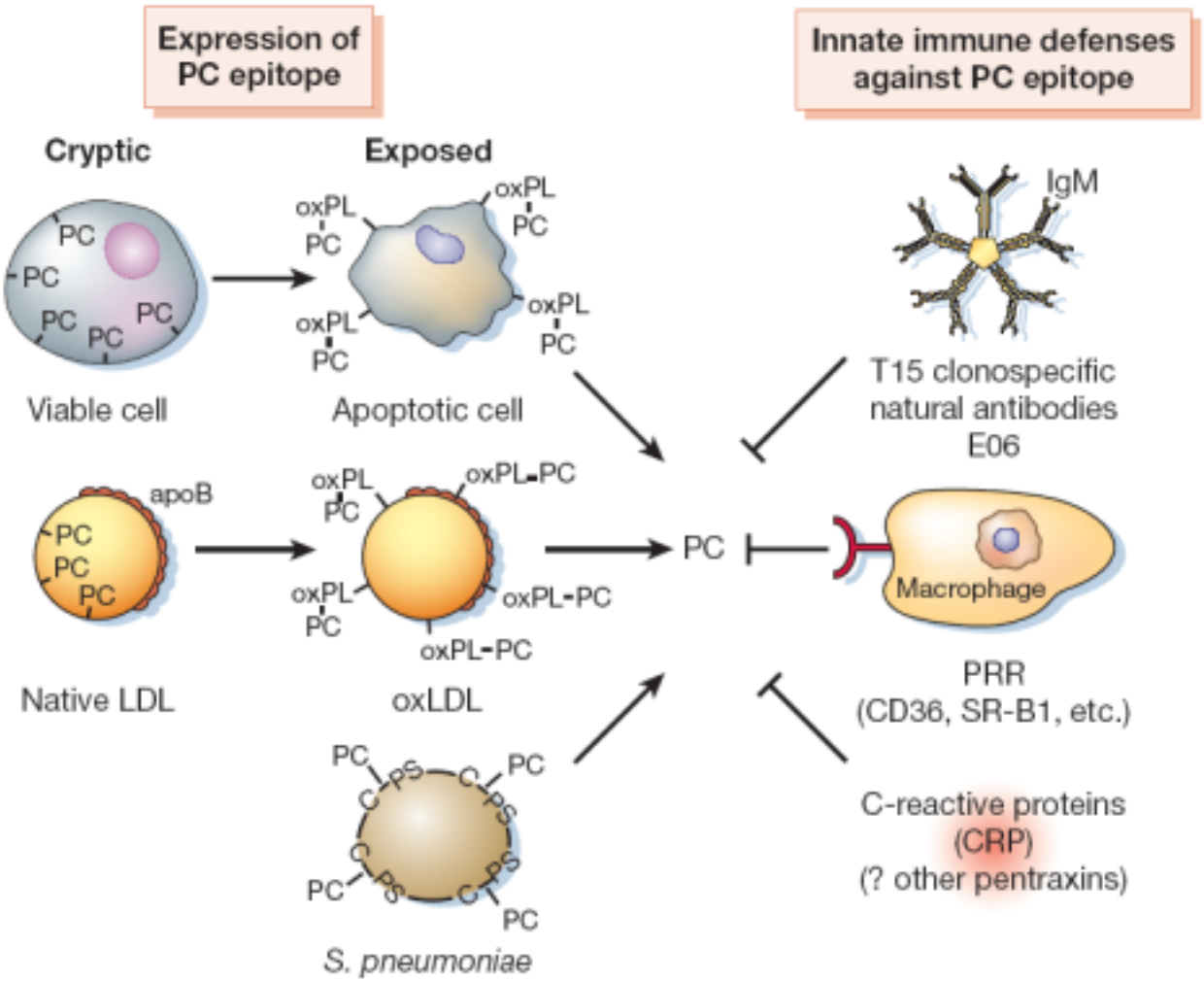
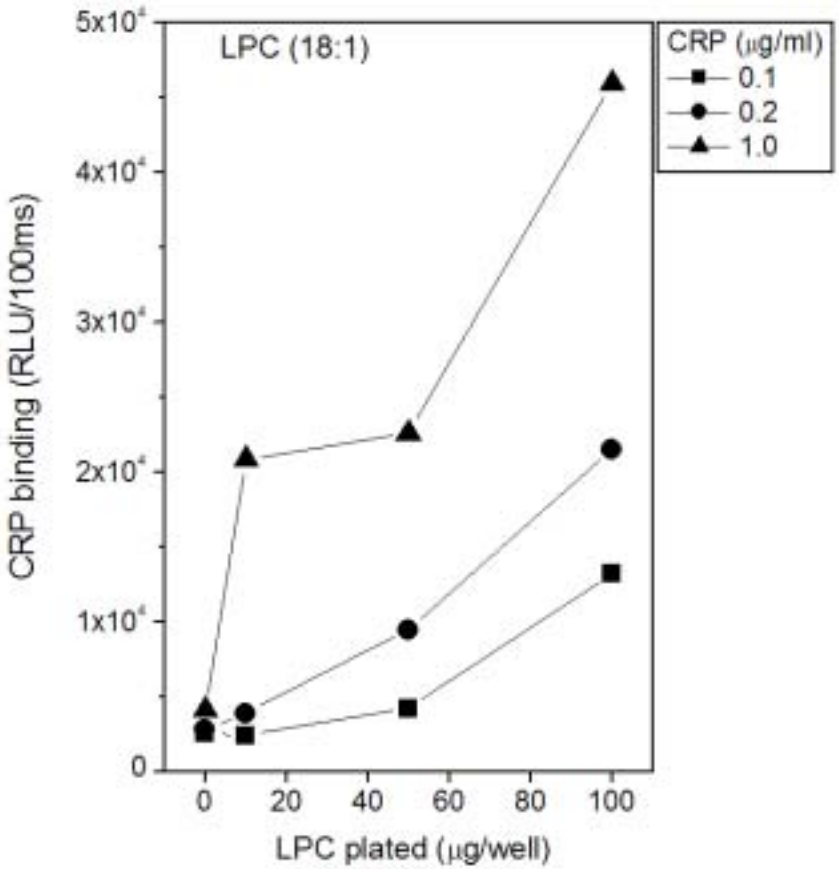


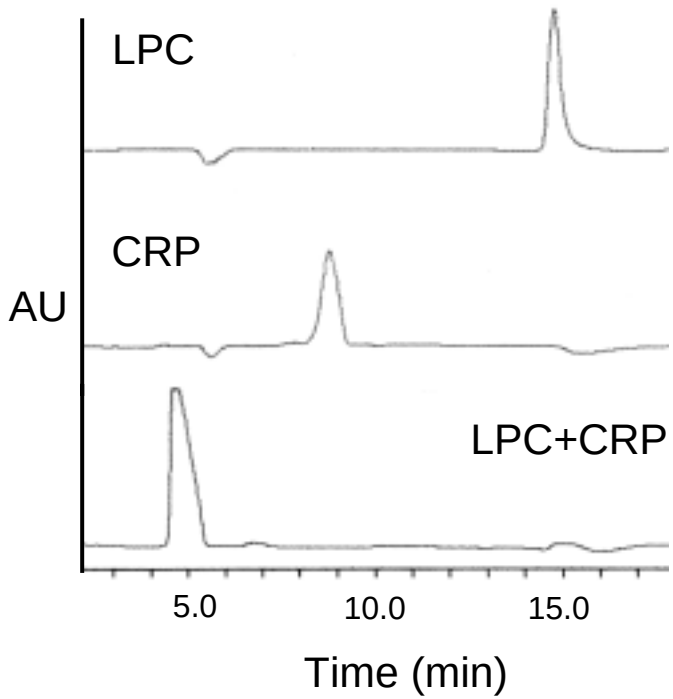
Fig. 3 Molecular mimicry between epitopes of oxLDL, apoptotic cells and the PC of the C-PS of pathogens. For native LDL and viable cells, the PC-containing phospholipids need to be oxidized (oxPL) to have the PC moiety exposed for recognition by innate immune defenses, represented by natural antibodies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

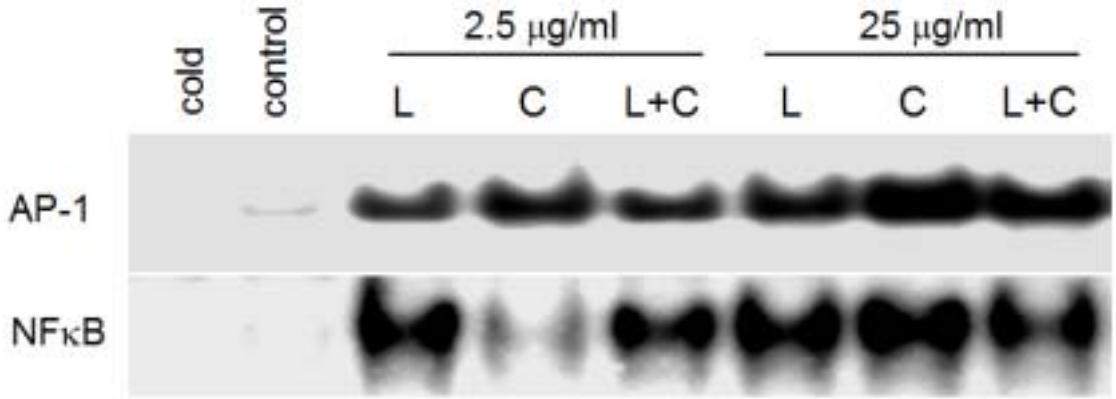
Table 1. Immune modulation of atherogenic murine models

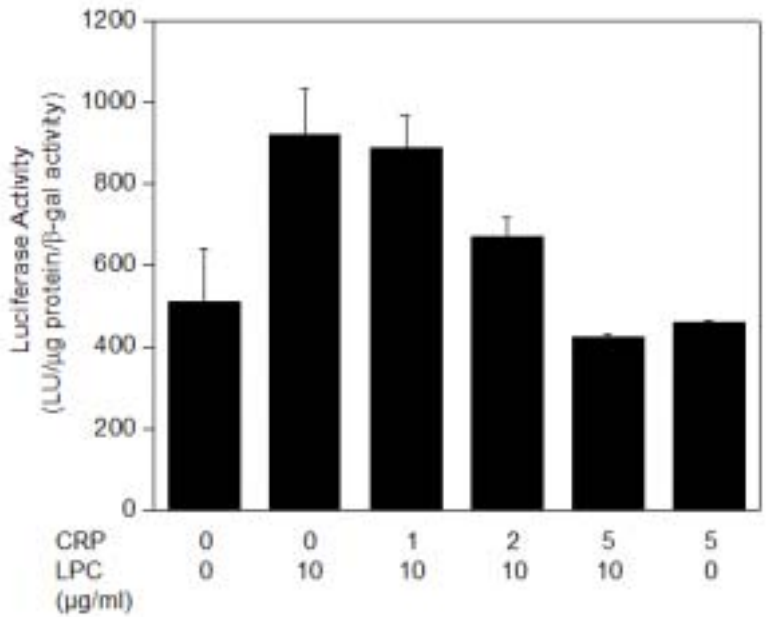
Defect or treatment	Immunological effect	Effect on atherosclerosis	Mouse model	Diet	Ref.
Rag1$^{-/-}$*Rag2*$^{-/-}$	T- and B-cell defect	↓	*Apoe*$^{-/-}$	Chow diet	21,24
Rag1$^{-/-}$*Rag2*$^{-/-}$	T- and B-cell defect	No effect	*Apoe*$^{-/-}$	High-fat diet	21,22
Rag1$^{-/-}$	T- and B-cell defect	↓ (early)	*Ldlr*$^{-/-}$	High-fat diet	23
Splenectomy		↑	*Apoe*$^{-/-}$	High-fat diet	72
plus B-cell transfer from old *Apoe*$^{-/-}$		Rescue	Normal/splenectomized *Apoe*$^{-/-}$	High-fat diet	72
plus T-cell transfer from old *Apoe*$^{-/-}$		Rescue	Splenectomized *Apoe*$^{-/-}$	High-fat diet	72
CD4^{+} cell transfer from old *Apoe–/–*		↑	*Apoe*$^{-/-}$ × SCID	Chow diet	25
Anti-CD40L	No CD40 signaling	↓	*Ldlr*$^{-/-}$	High-fat diet	56–58
CD40L$^{-/-}$	No CD40 signaling	↓	*Apoe*$^{-/-}$	Chow diet	59
IFN-γR$^{-/-}$	No IFN-γ effects	↓	*Apoe*$^{-/-}$	High-fat diet	62
IFN-γ treatment	More IFN-γ effects	↑	*Apoe*$^{-/-}$	Chow diet	63
IL-10 transgenic	More IL-10 by T cells	↓	*Ldlr*$^{-/-}$	High-fat diet	66
IL-12 treatment	More IL-12 effects	↑	*Apoe*$^{-/-}$	High-fat diet	64
IL-18 treatment	More IFN-γ effects	↑	*Apoe*$^{-/-}$/*Apoe*$^{-/-}$ × IFN-γ$^{-/-}$	Chow diet	65
Anti-TGF-β1,2,3	No TGF-β signaling	↑	*Apoe*$^{-/-}$	Chow diet	70,71
Polyspecific IgG	Immunosuppression	↓	*Apoe*$^{-/-}$	High-fat diet	31
Hsp65	Vaccination	↑	*Ldlr*$^{-/-}$	Chow diet	80
oxLDL	Vaccination	↓	*Ldlr*$^{-/-}$	High-fat diet	28
oxLDL	Vaccination	↓	*Apoe*$^{-/-}$	High-fat diet	29,30

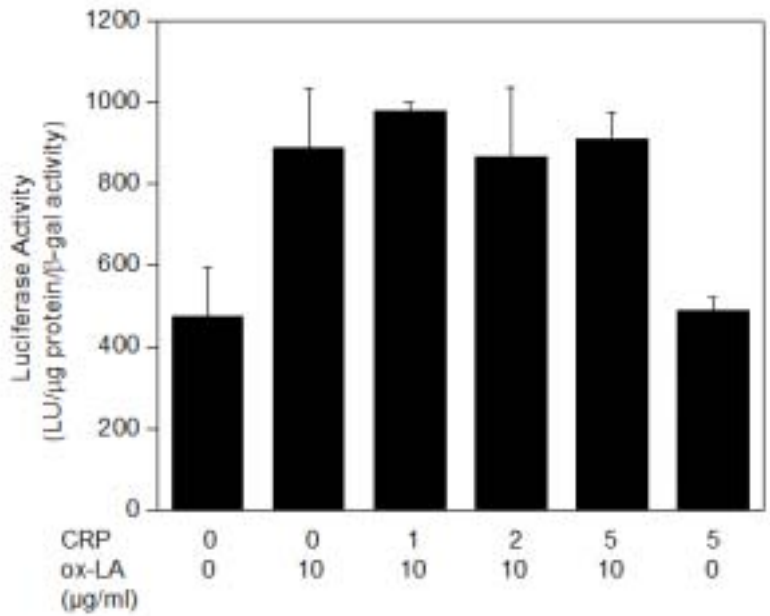
SCID, severe combined immunodeficiency; Anti-CD40L, antibody against CD40L;IFN-γR, IFN-γ receptor; Anti-TGF-β1,2,3, antibody against TGF-β1,2,3.


LPC (18:1)
CRP (μg/ml)
0.1
0.2
1.0
CRP binding (RLU/100ms)
LPC plated (μg/well)
0
20
40
60
80
100


LPC
CRP
LPC+CRP
AU
5.0
10.0
15.0
Time (min)


cold
control
2.5 μg/ml
25 μg/ml
L
C
L+C
L
C
L+C
AP-1
NFκB


Luciferase Activity
(LU/μg protein/β-gal activity)
1200
1000
800
600
400
200
0
CRP 0 0 1 2 5 5
LPC 0 10 10 10 10 0
(μg/ml)


Luciferase Activity
(LU/μg protein/β-gal activity)
1200
1000
800
600
400
200
0
CRP 0 0 1 2 5 5
ox-LA 0 10 10 10 10 0
(μg/ml)

Future prospects

- Oxidative or inflammatory stress itself may not totally determine cellular response *in vivo*.
- The characteristics of cell condition may be more important to decide final output.
- Cellular signaling to determine the cellular response may be more precise target to control the process of atherogenesis.