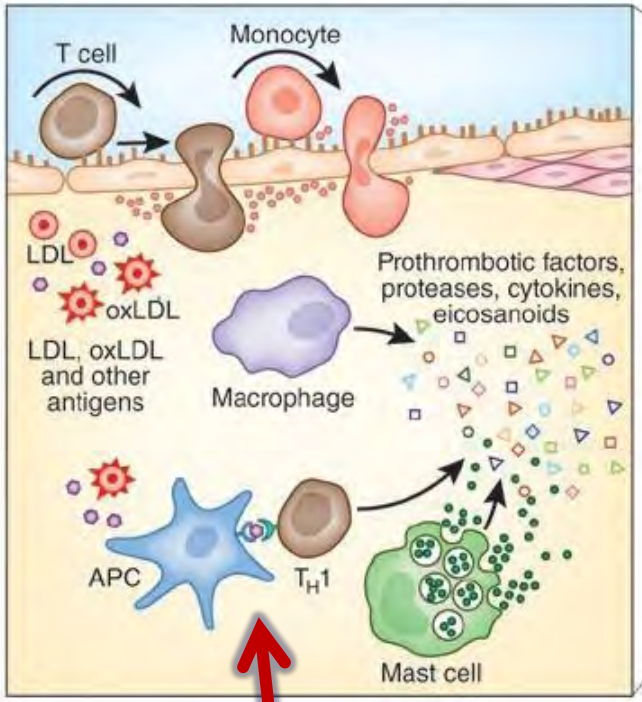
The Leukocyte in Cardiovascular Disease
Geneva, January 2011

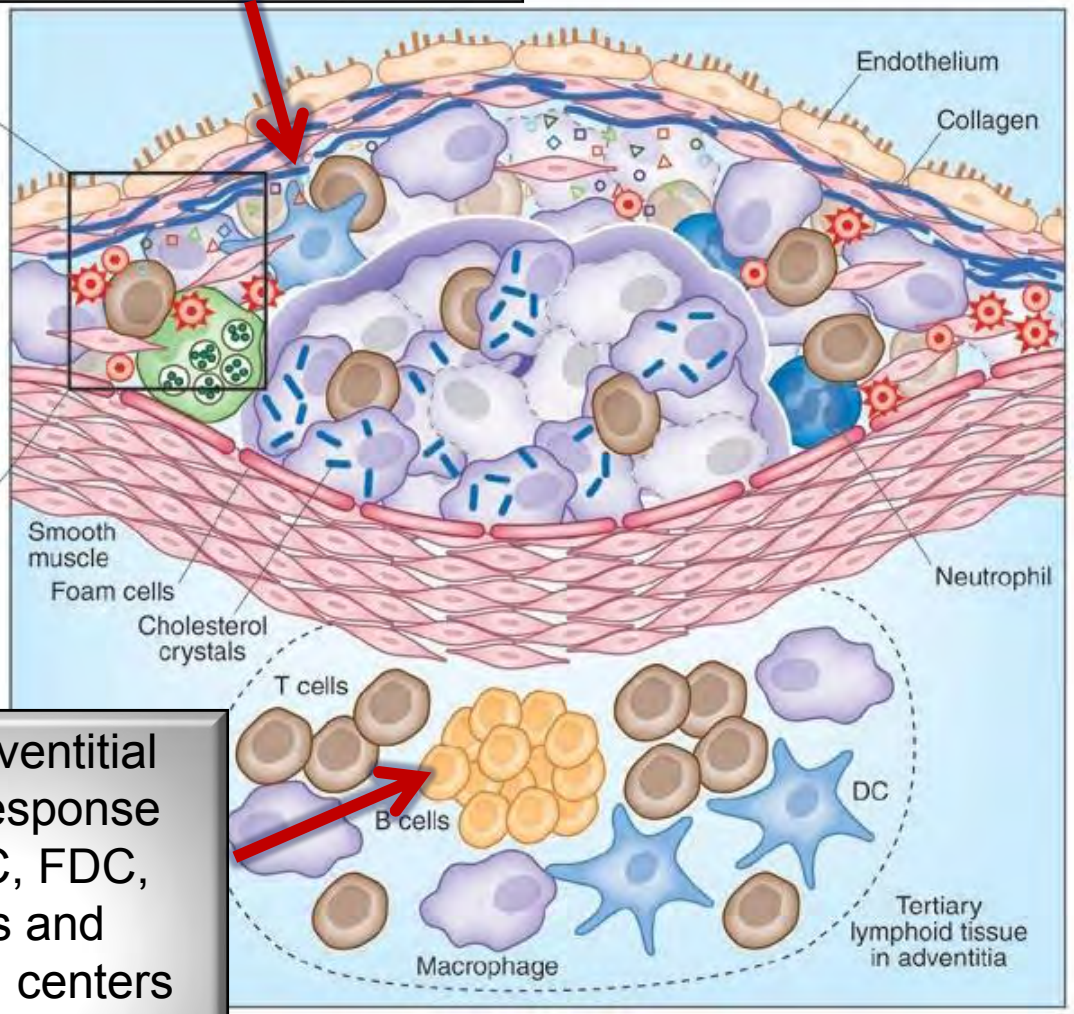
Vaccination against Atherosclerosis

Göran K Hansson
Center for Molecular Medicine and Department of Medicine
Karolinska Institute
Stockholm, Sweden

Immunopathol sclerosis

Inflammatory activation triggers plaque rupture and thrombosis



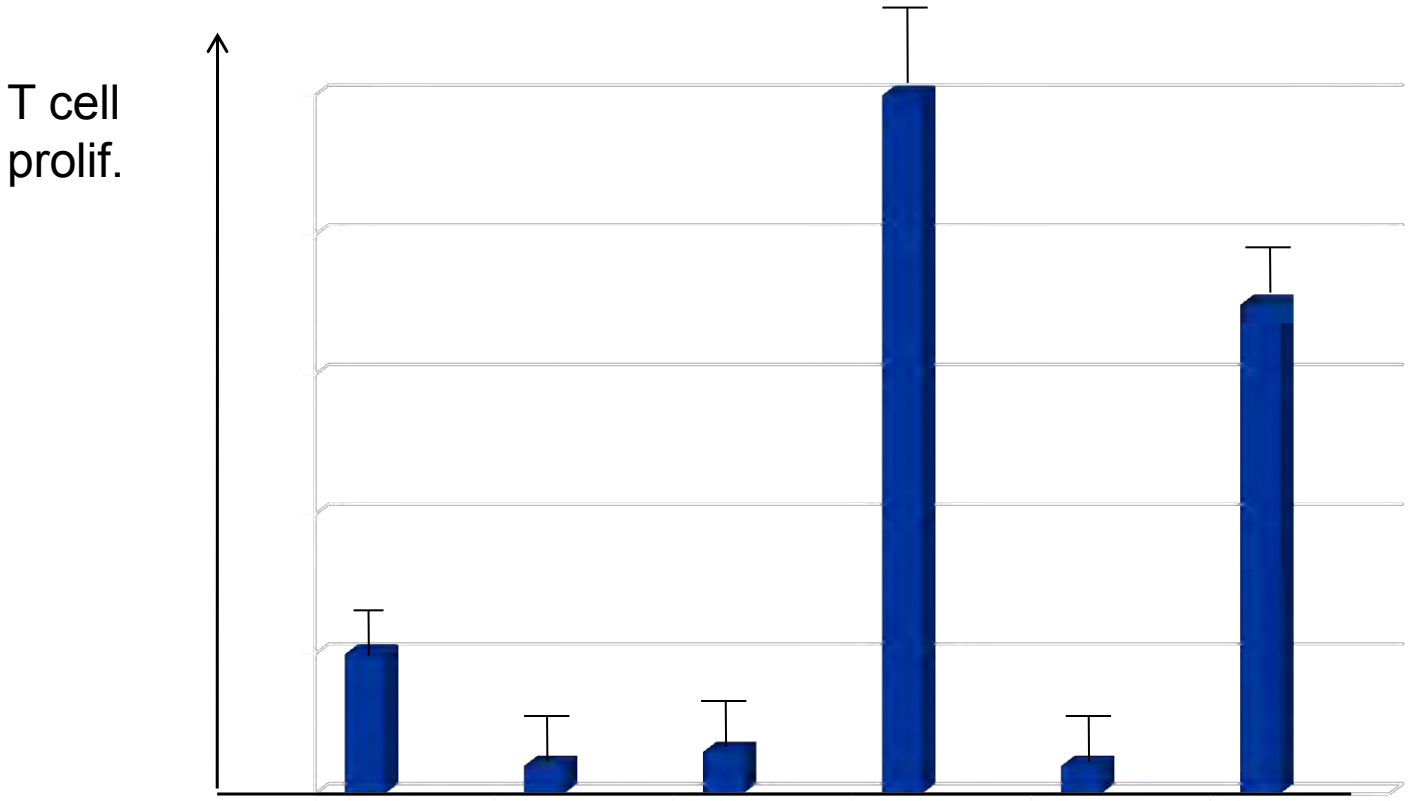


Early macrophage + T cell response in intima and forming atherosclerotic plaque

Late adventitial B cell response with DC, FDC, T cells and germinal centers - adventitial tertiary lymphoid organ -

nsson & Hermansson, Nat Imm 2011

T cells of human plaques recognize LDL components as antigen



Stemme et al
PNAS 1995

oxLDL	-	-	+	+	+	+
MC	+	-	-	+	+	+
MAb	-	-	-	-	anti-DR	Ctrl ab

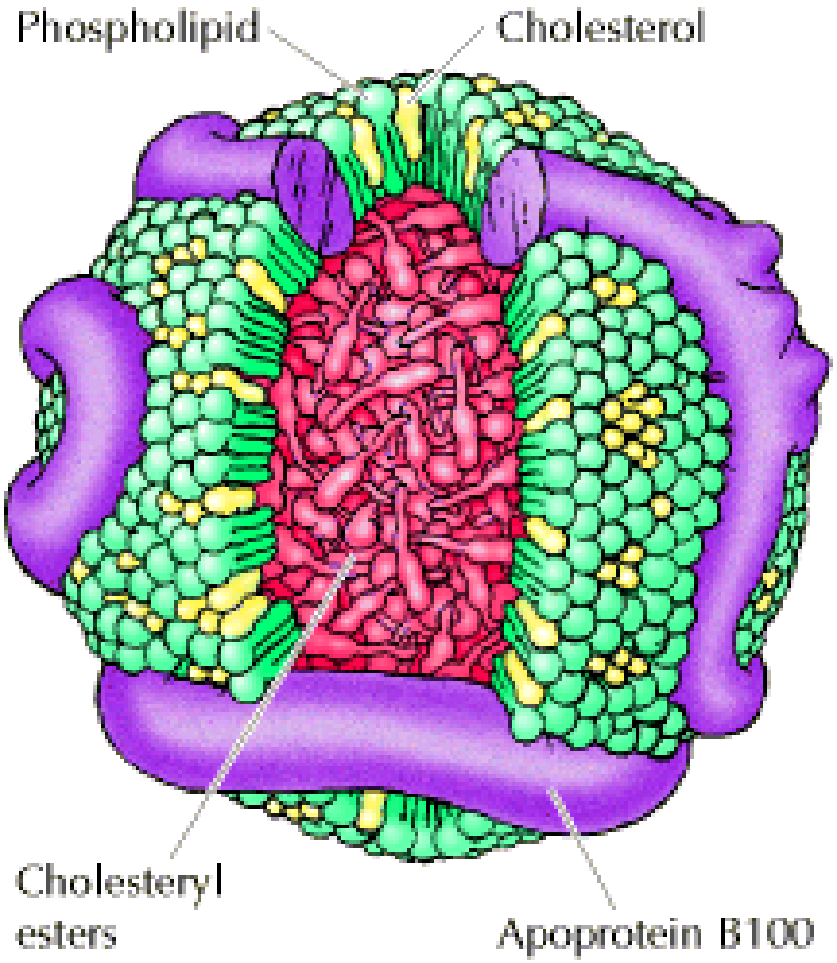
Immunization against LDL components reduces atherosclerosis

- With (ox)LDL
  - *Palinski, PNAS 1995; Ameli ATVB 1996; George 1998; Zhou 2001*
- With native apoB100 sequences
  - *Fredrikson et al, ATVB 2003*
- By mucosal administration
  - Oral tolerance to oxLDL
    - *Van Puijvelde et al, Circ 2006*
  - Nasal immunization with apoB100-CTB fusion protein
    - *Klingenberg et al, ATVB 2010*
- With pneumococci – phosphocholine target of molecular mimickry
  - *Binder et al, Nat Med 2003*

Immunization against LDL components reduces atherosclerosis - But how does it work?

- Complete LDL particles will not be useful for vaccination in humans
  - Expensive, difficult to standardize, potentially dangerous
- Immunodominant epitopes must be identified
- Mechanisms must be clarified
  - Immunomodulation?
  - Antigen (LDL) elimination?

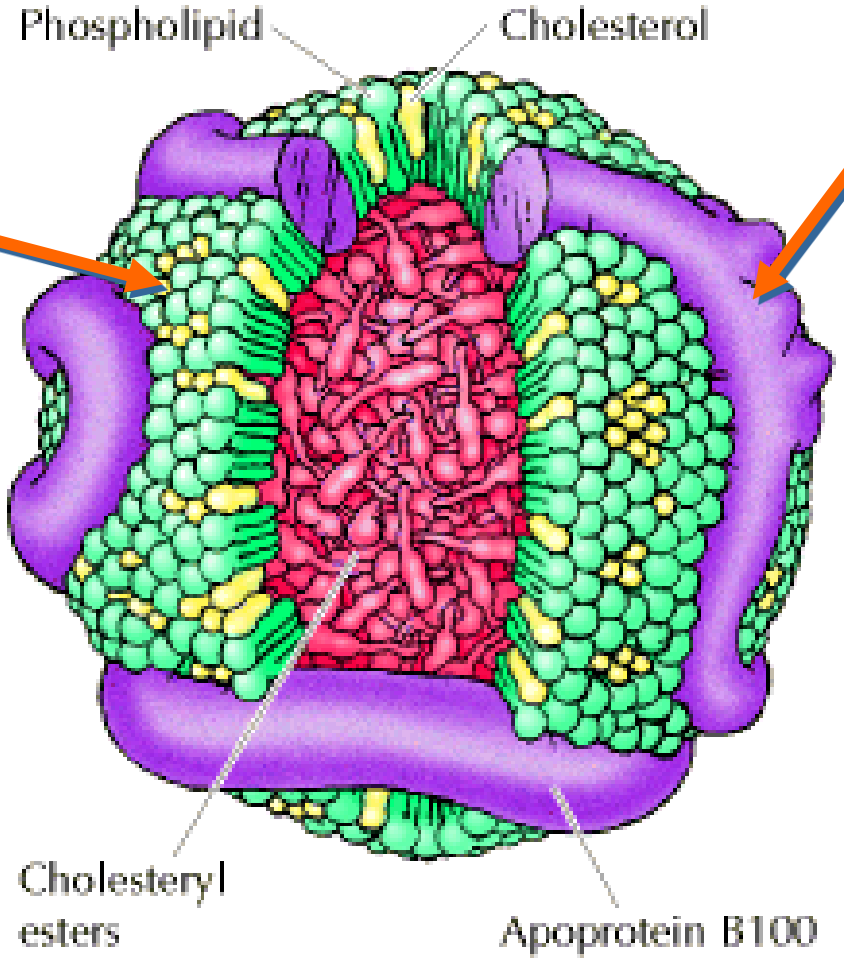
The LDL particle -
at the heart of atherosclerotic disease



G Cooper, The Cell

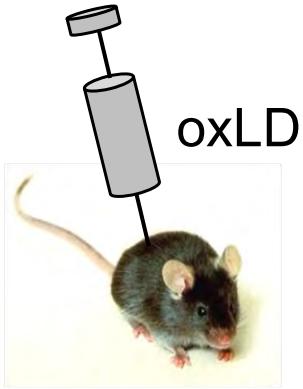
Can oxidative modifications of the LDL particle break immunological tolerance?

Phospholipid fragments:
Lysophosphatidyl choline
Phosphocholine
PAPC
POVPC



Aldehyde adducts on apoB protein:
Malondialdehyde
Hydroxynonenal

Strategy to identify T cell epitope in oxLDL



oxLDL + CFA

hu*APOB100*^{tg} mice

T cell clones

Thymoma

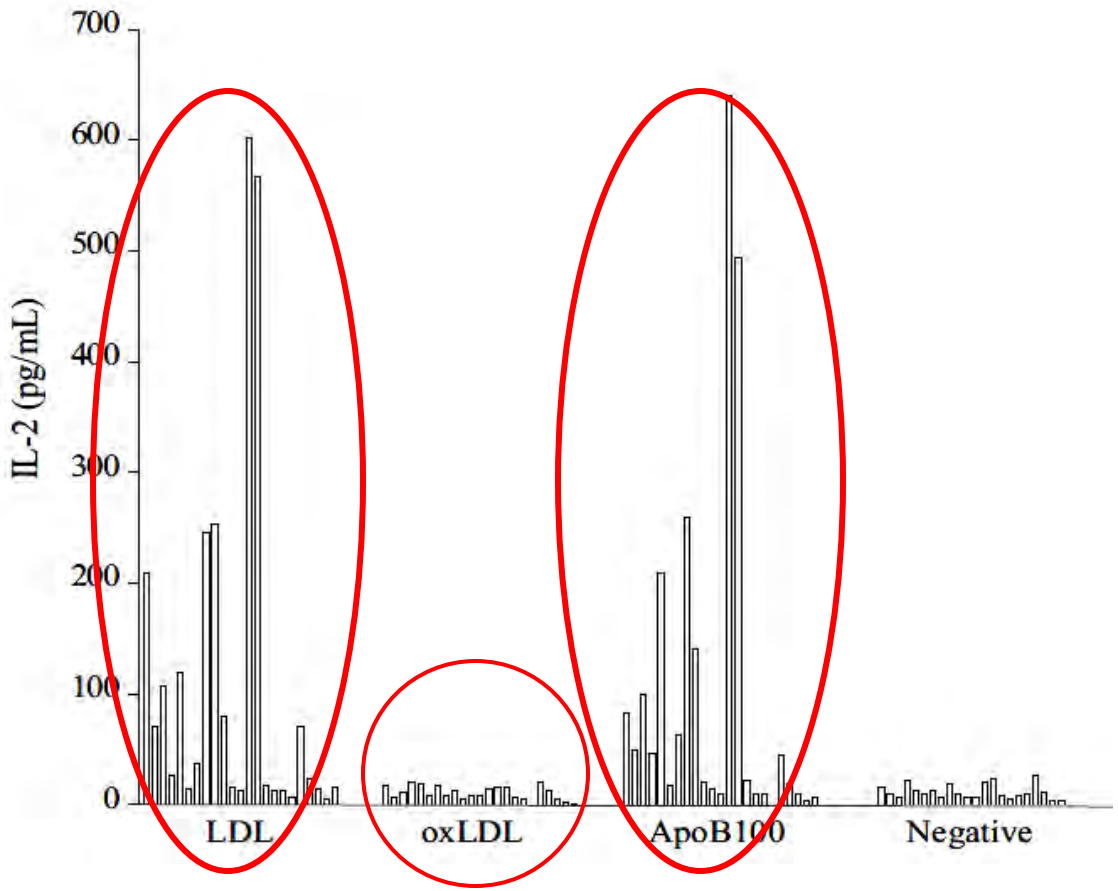
T cell hybridomas

+ APC
(H-2^{b})

+ Antigen
oxLDL
nLDL
apoB100

Activation
IL-2 secretion

Specificity of LDL reactive T cell hybridomas

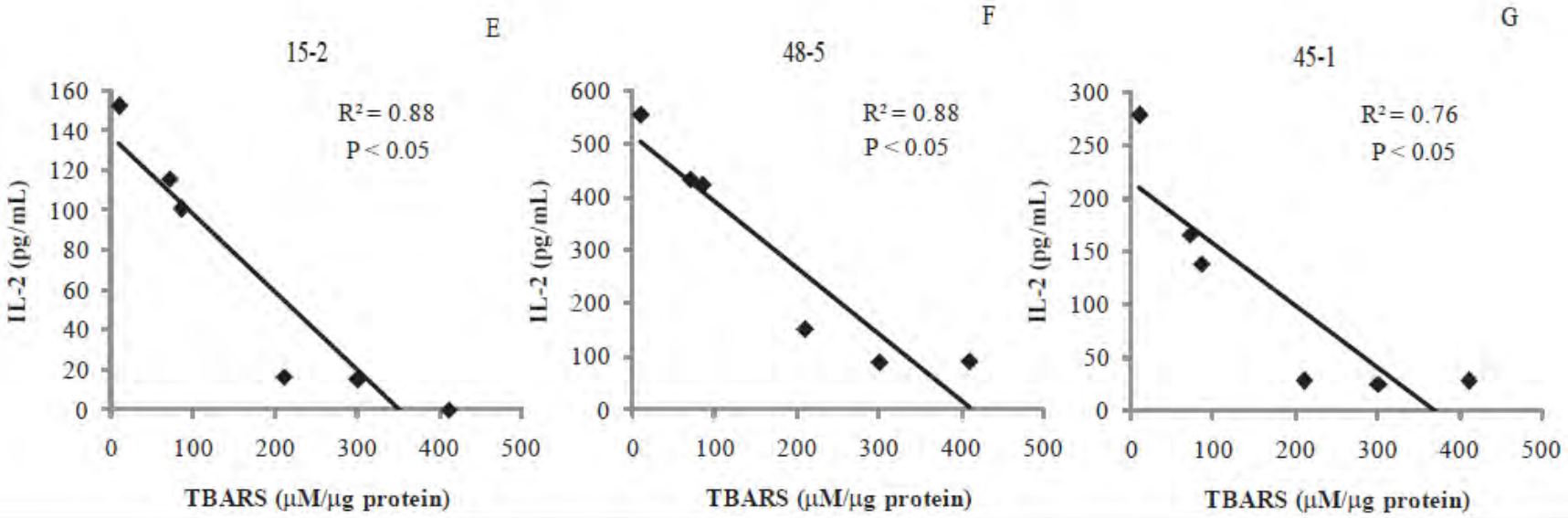


Hermansson, Ketelhuth et al J Exp Med 2010

T cells from oxLDL immunized mice recognize apoB100 and native LDL, not oxLDL!

Oxidation inhibits T cell response!

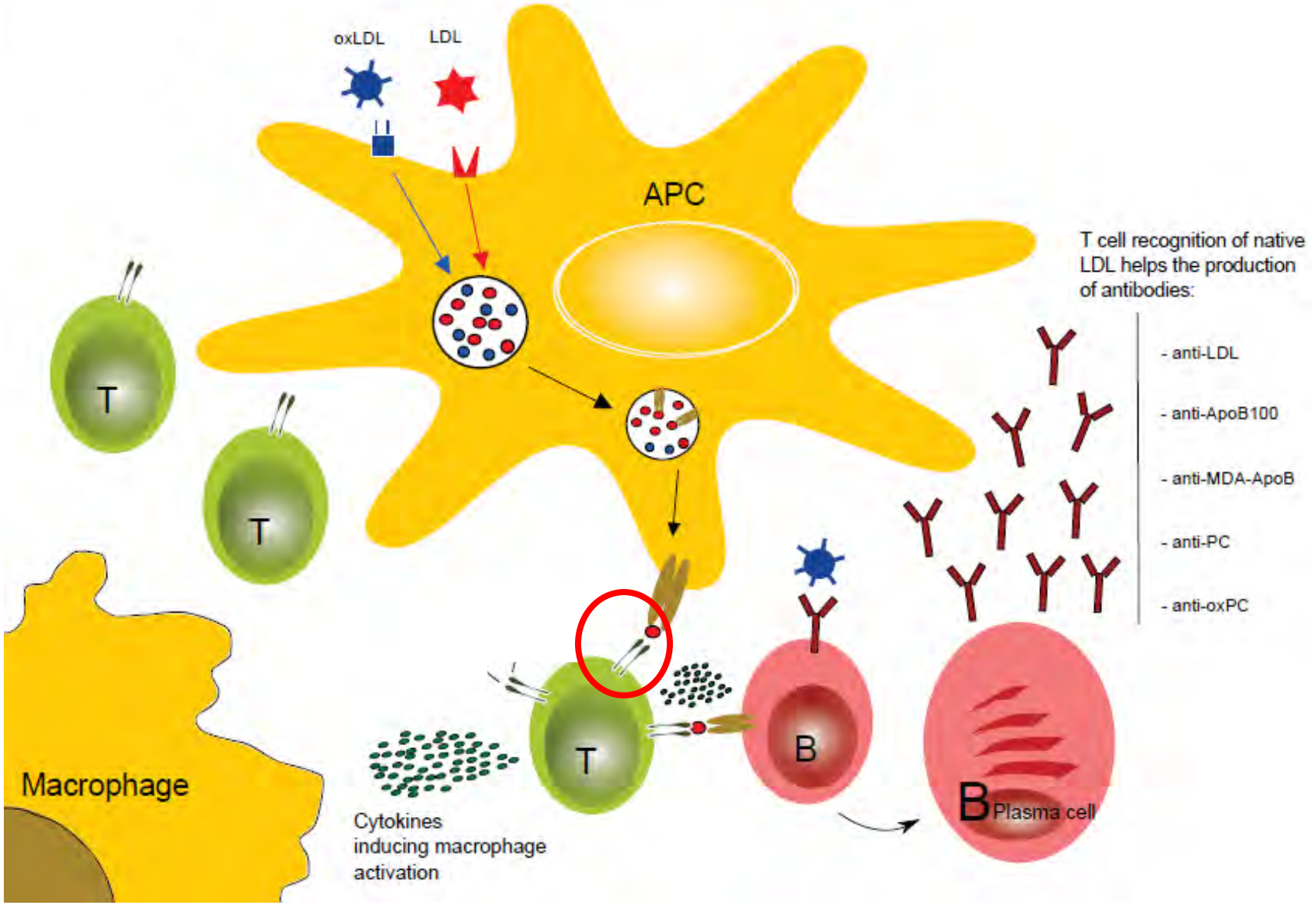
What happens to LDL immunogenicity when we oxidize?



T cell clones were exposed to LDL preparations that had been Cu oxidized to varying extent

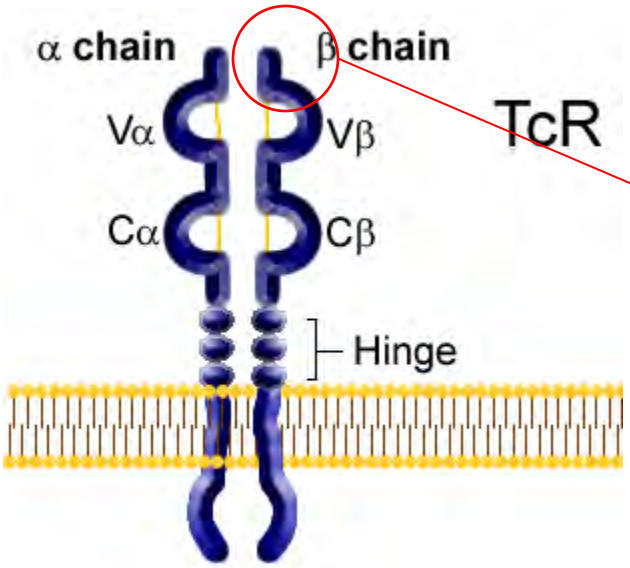
Hermansson, Ketelhuth et al
J Exp Med 2010

T cells recognize apoB100 motifs and help B cells make anti-oxLDL antibodies

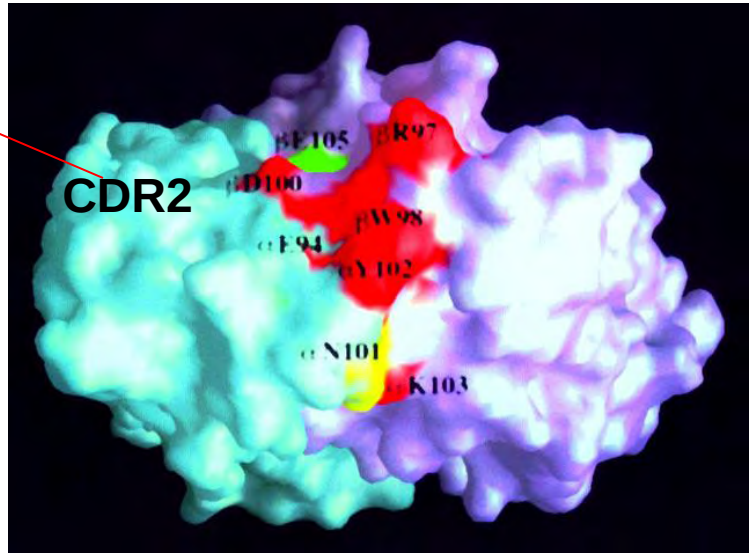


T cells recognizing LDL express TCR with TRBV31-type β chains

Clones recognizing LDL/ApoB100			*Clones not recognizing LDL/ApoB100*		
Clone	*Vα gene*	*Vβ gene*	*Clone*	*Vα gene*	*Vβ gene*
15-2	TRAV14 and 3	TRBV31	**96-7**	TRAV13	TRBV19
15-3	TRAV14 and 3	TRBV31	**97-3**	TRAV13	TRBV19
15-4	TRAV14 and 3	TRBV31	**32-1**	TRAV20	TRBV19
15-5	TRAV14 and 3	TRBV31	**32-4**	TRAV20	TRBV19
15-6	TRAV14 and 3	TRBV31	**117-1**	TRAV5 and 3	TRBV3
45-1	TRAV4	TRBV31	**117-3**	TRAV5 and 3	TRBV3
45-2	TRAV4	TRBV31	**117-5**	TRAV5 and 3	TRBV3
45-4	TRAV4	TRBV31	**117-7**	TRAV5 and 3	TRBV3
48-5	TRAV12 and 13	TRBV31	**50-0**	TRAV20	TRBV29
48-8	TRAV12 and 13	TRBV31	**50-2**	TRAV20	TRBV29
			50-3	TRAV20	TRBV29
			50-4	TRAV20	TRBV29
			50-5	TRAV20	TRBV29



TRBV31

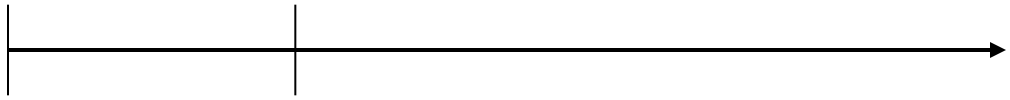


hApoB100tg x *Ldlr* $^{-/-}$

TRBV31-KLH or KLH

1st shot
100 ug Ag+CFA

2nd shot
100 ug Ag+IFA



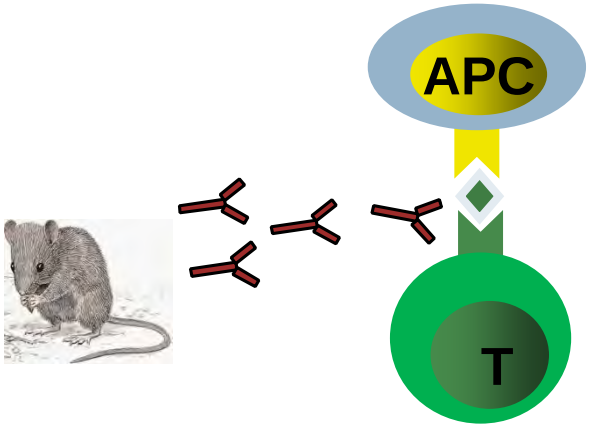
Weeks of age

11

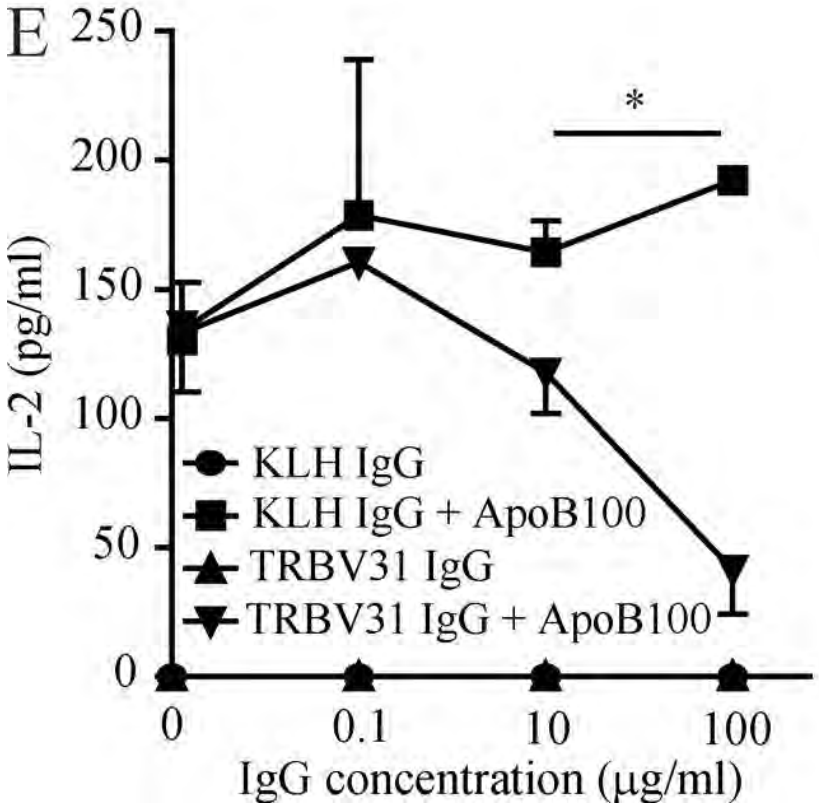
15

21

Immunization with TRBV31 peptide induces TCR blocking antibodies



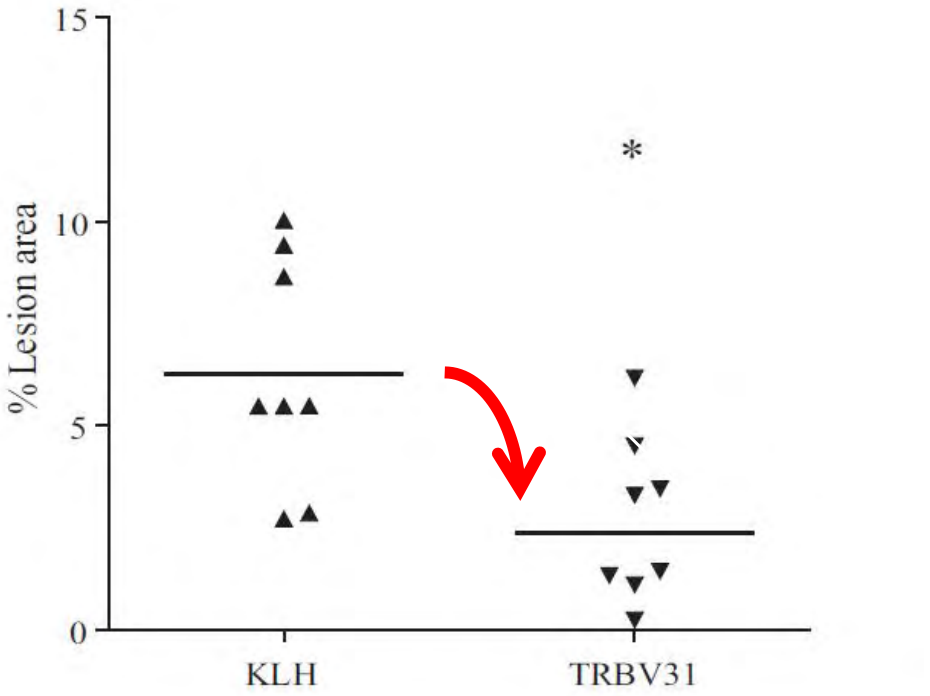
Anti-TRBV31 impairs hybridoma response to ApoB100



Hermansson, Ketelhuth et al
J Exp Med 2010

Immunization against TRBV31 reduces atherosclerosis in hypercholesterolemic mice

HuB100tg x Ldlr- mice
immunized w/
TRBV31 peptide-KLH
Western diet 10 wk



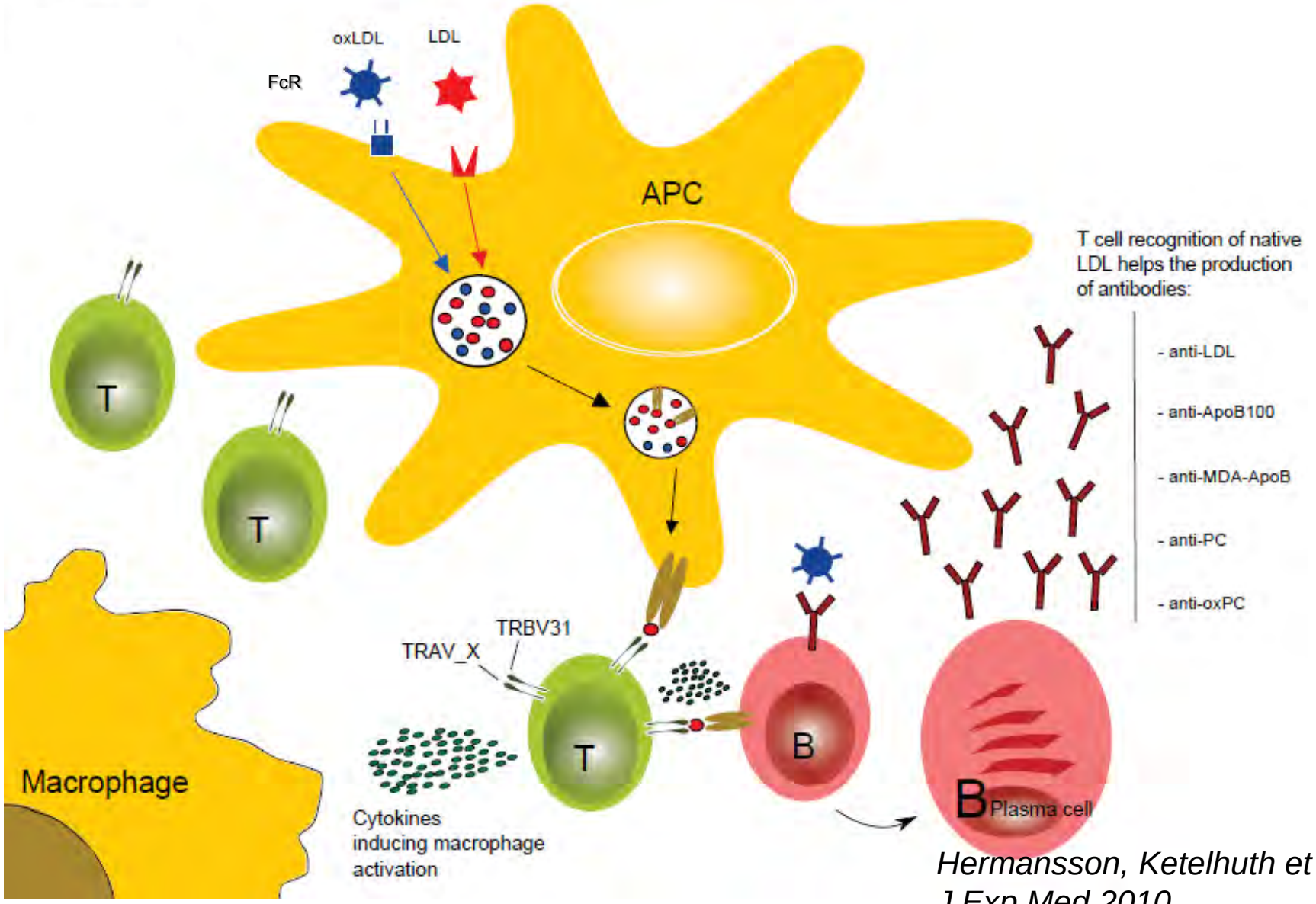
57% lesion reduction in aortic arch

65% lesion reduction in aortic root

Hermansson, Ketelhuth et al
J Exp Med 2010

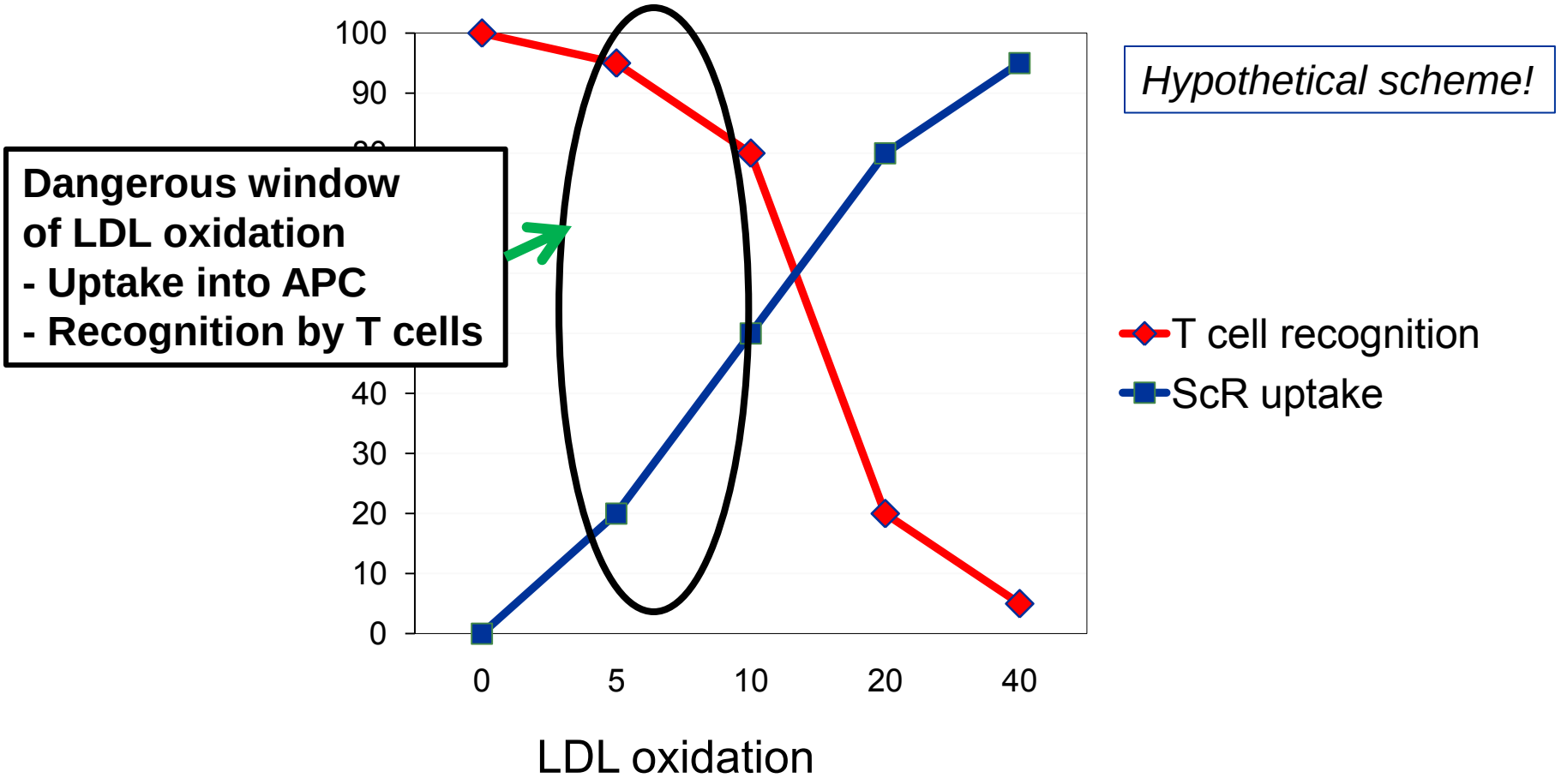
- ApoB100 reactive T cells
  - Express TRBV31+ TCR
  - Recognize native apoB100 epitopes
  - Help B cells make antibodies to oxLDL epitopes
  - Promote atherosclerosis
  - Increase lesion inflammation

T cells recognizing apoB100 motifs trigger plaque inflammation and anti-oxLDL antibodies



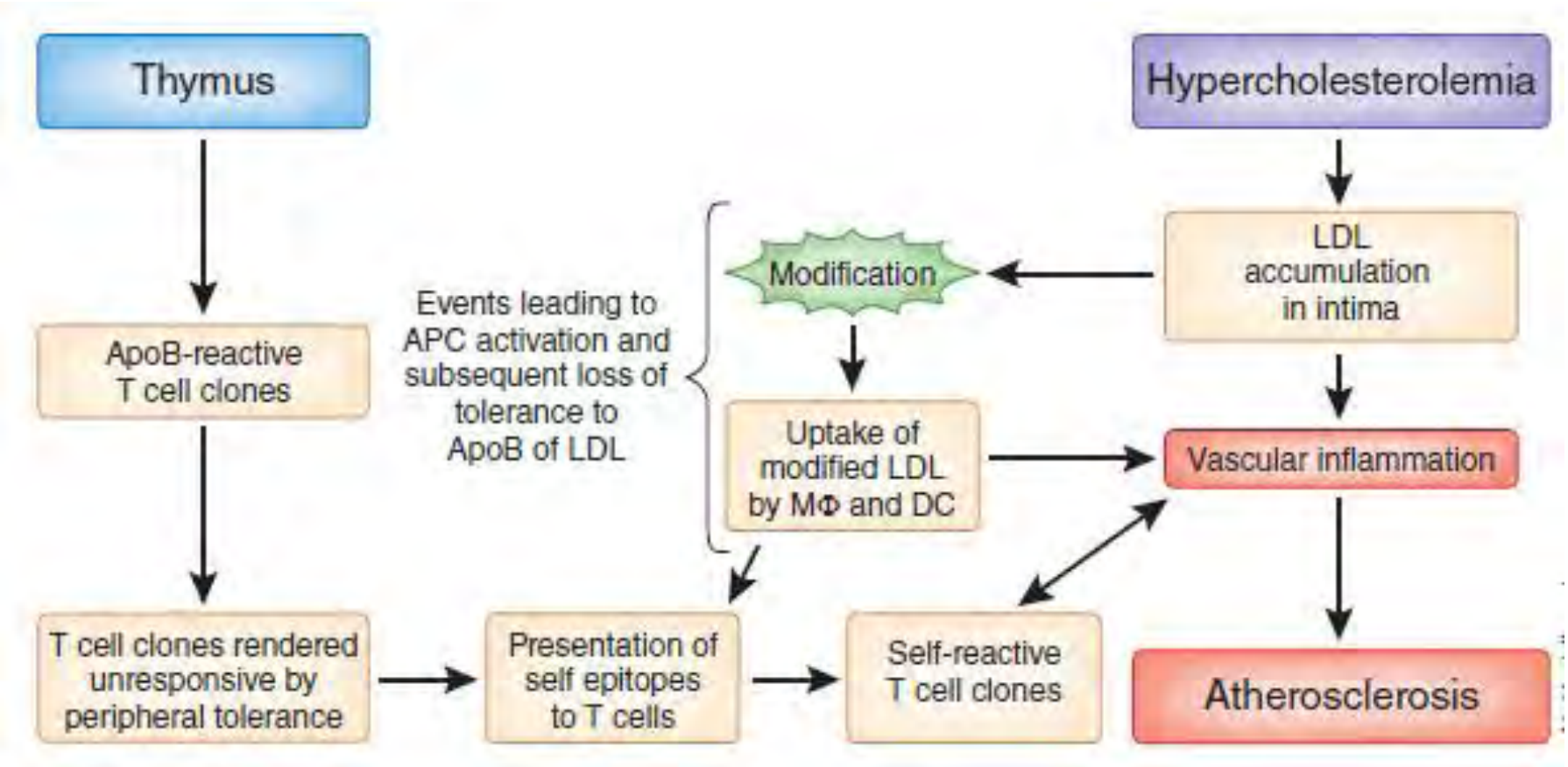
Hermansson, Ketelhuth et al J Exp Med 2010

Inverse relationship between oxLDL uptake into APC and T cell recognition of LDL epitopes



Hansson & Hermansson, Nat Imm 2011

Vascular inflammation breaks peripheral tolerance to LDL



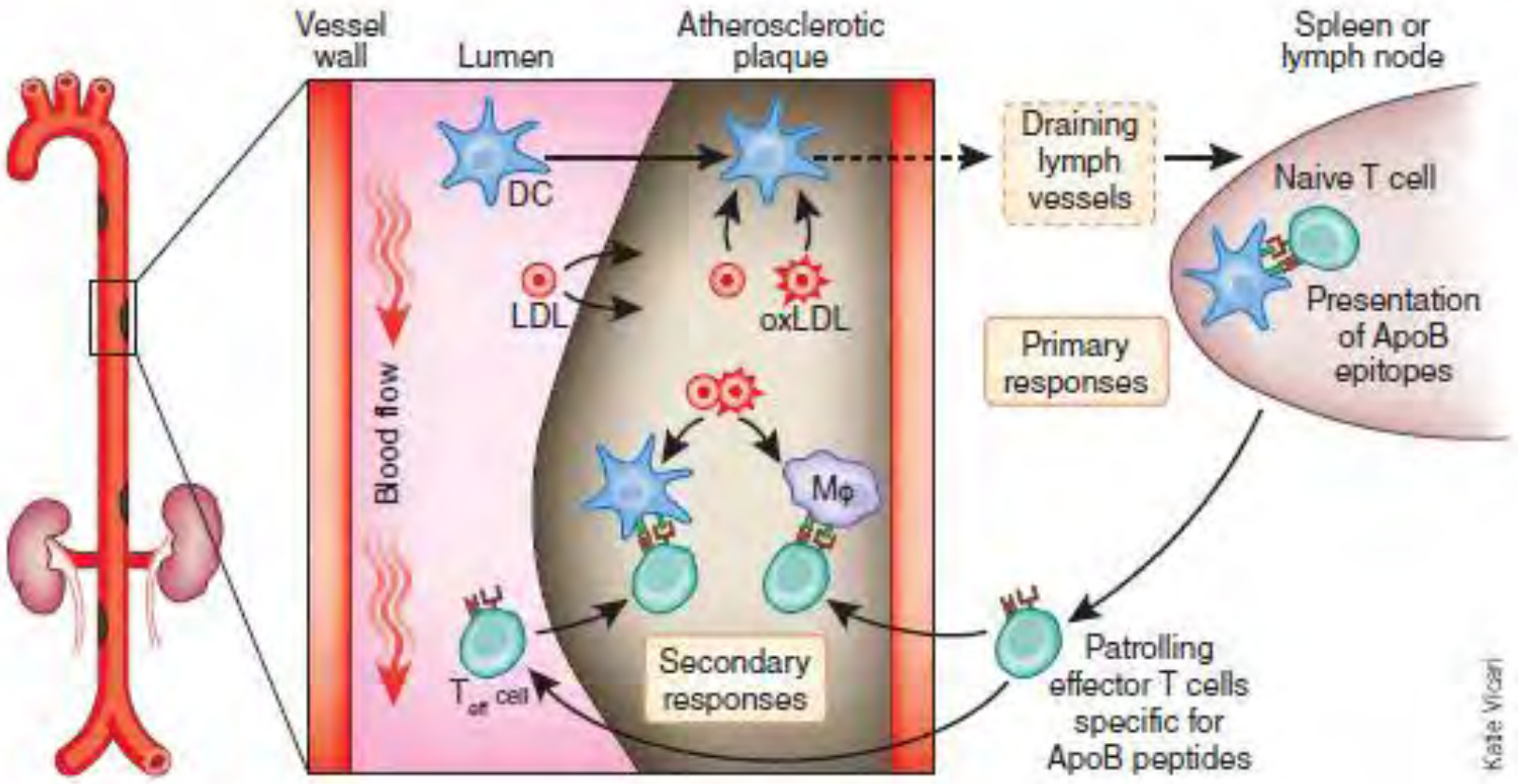
Hansson & Hermansson, Nat Imm 2011

Dendritic cells and atherosclerosis

- Professional antigen-presenting cells
- Present in atherosclerotic lesions (Bobryshev YV et al, *Histol Histopathol. 2001; Niessner et al, Circ 2006*)
- Patrol artery wall to bring antigen to lymph nodes

  (Angeli et al, Immunity 2004)
- Plaque DCs are able to present antigen to T cells in vitro (Choi JH et al, *J Exp Med, 2009*)

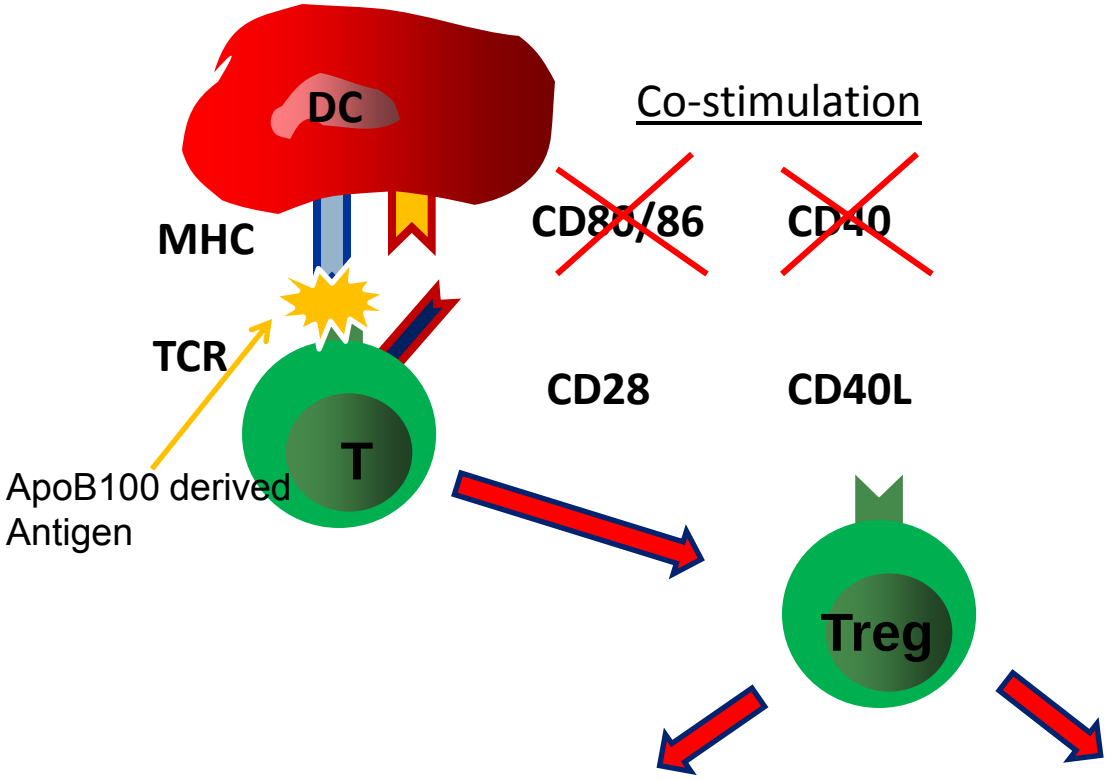
Dendritic cells patrol artery, move to lymph nodes, and activate naïve T cells



Hansson & Hermansson, Nat Imm 2011

Induction of tolerogenic dendritic cells

IL-10 treatment



- Activation of effector T cells
- DC secretion of pro-inflammatory cytokines:
  IL-12, IL-6, TNFalpha, MCP-1

Increase atherosclerosis

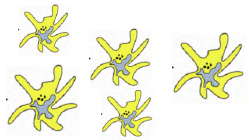
Tolerance to the ApoB100 derived antigen

Decrease atherosclerosis?

DC therapy

Bone marrow from mice

Culture with GM-CSF and IL-4

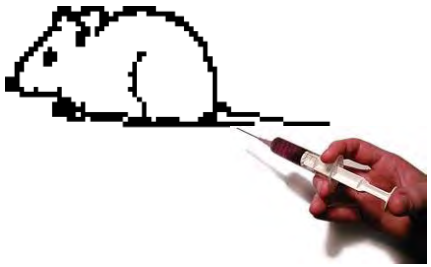


Enrich DC with CD11c MACS separation column

Pulse DC with ApoB100 and IL-10 together with LPS

Exp. Groups

- DC (no antigen)
- DC + ApoB100
- DC + ApoB100 + IL-10
- DC + IL-10 (no antigen)



Inject pulsed DCs into huB100tg x LDLr-/- mice (I.V)

Hermansson et al Circ 2011

Tolerogenic DC induce Treg and inhibit T effector responses

Figure 2

A

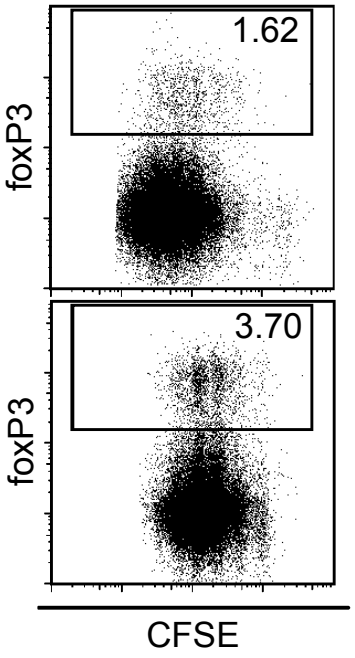
DC IL-10 Anti-CD3

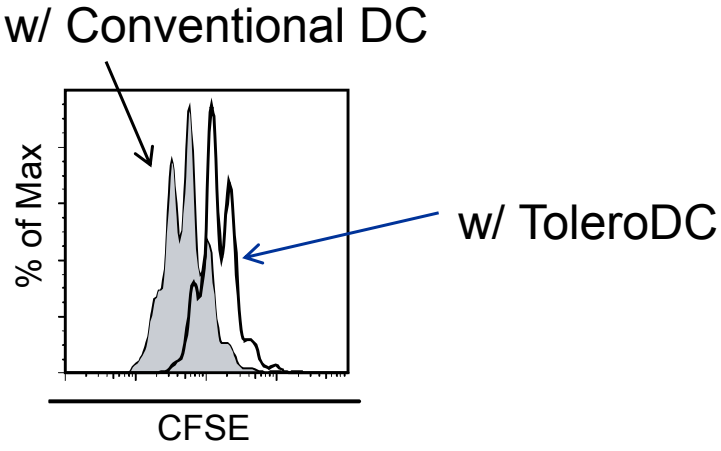
+ - +

+ + +

1.56

IFNγ



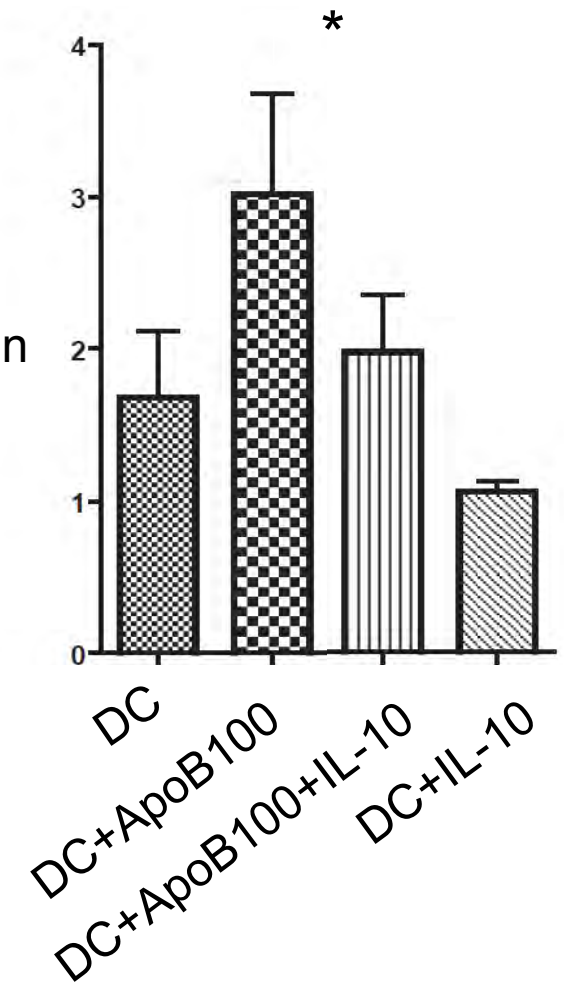


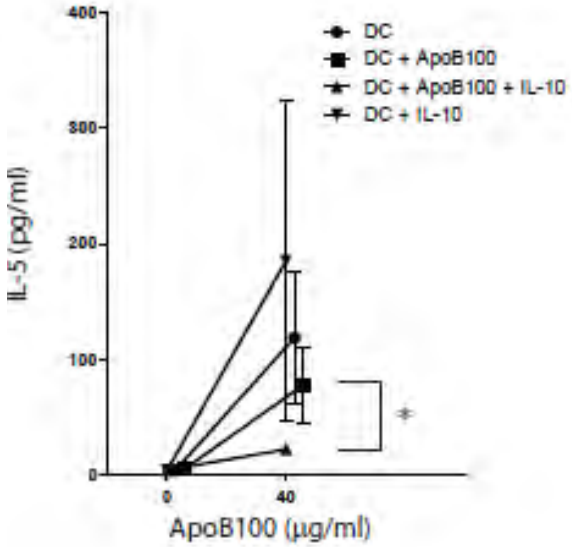
D

Hermansson et al, Circ 2011

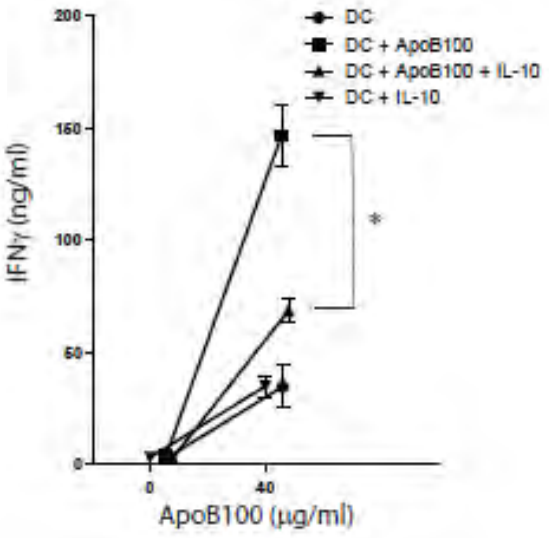
The T cell response to ApoB100 is modulated in mice receiving DC

Proliferation Index



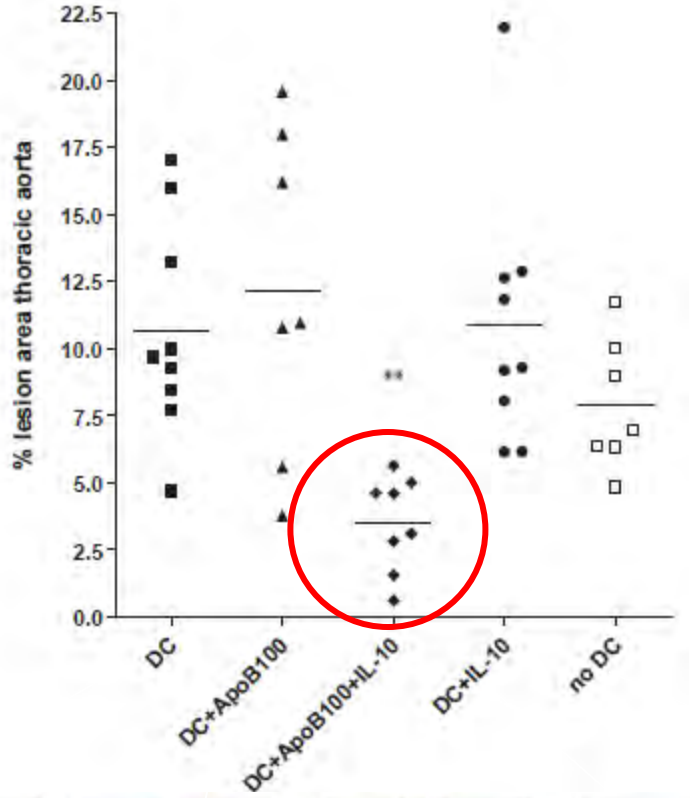


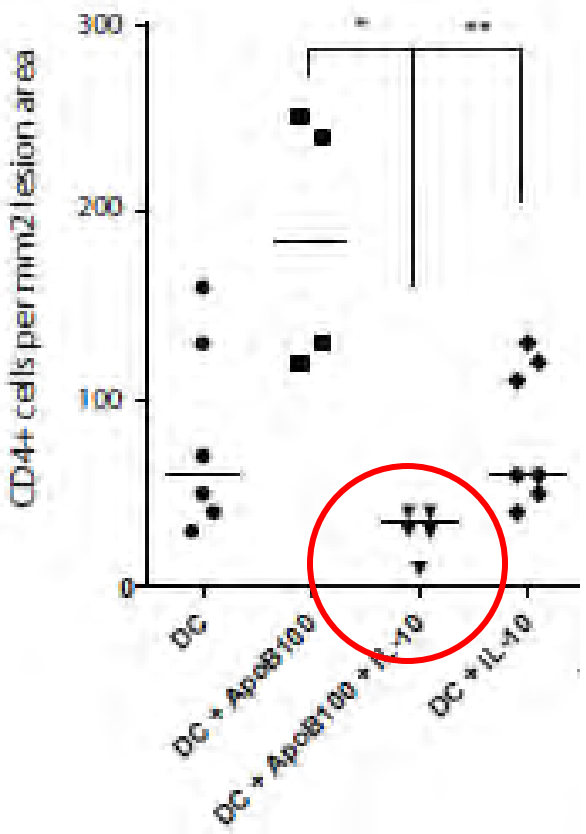
Th2



Th1

IL-10 treated DC reduce atherosclerosis and CD4+ T cell infiltration in lesions





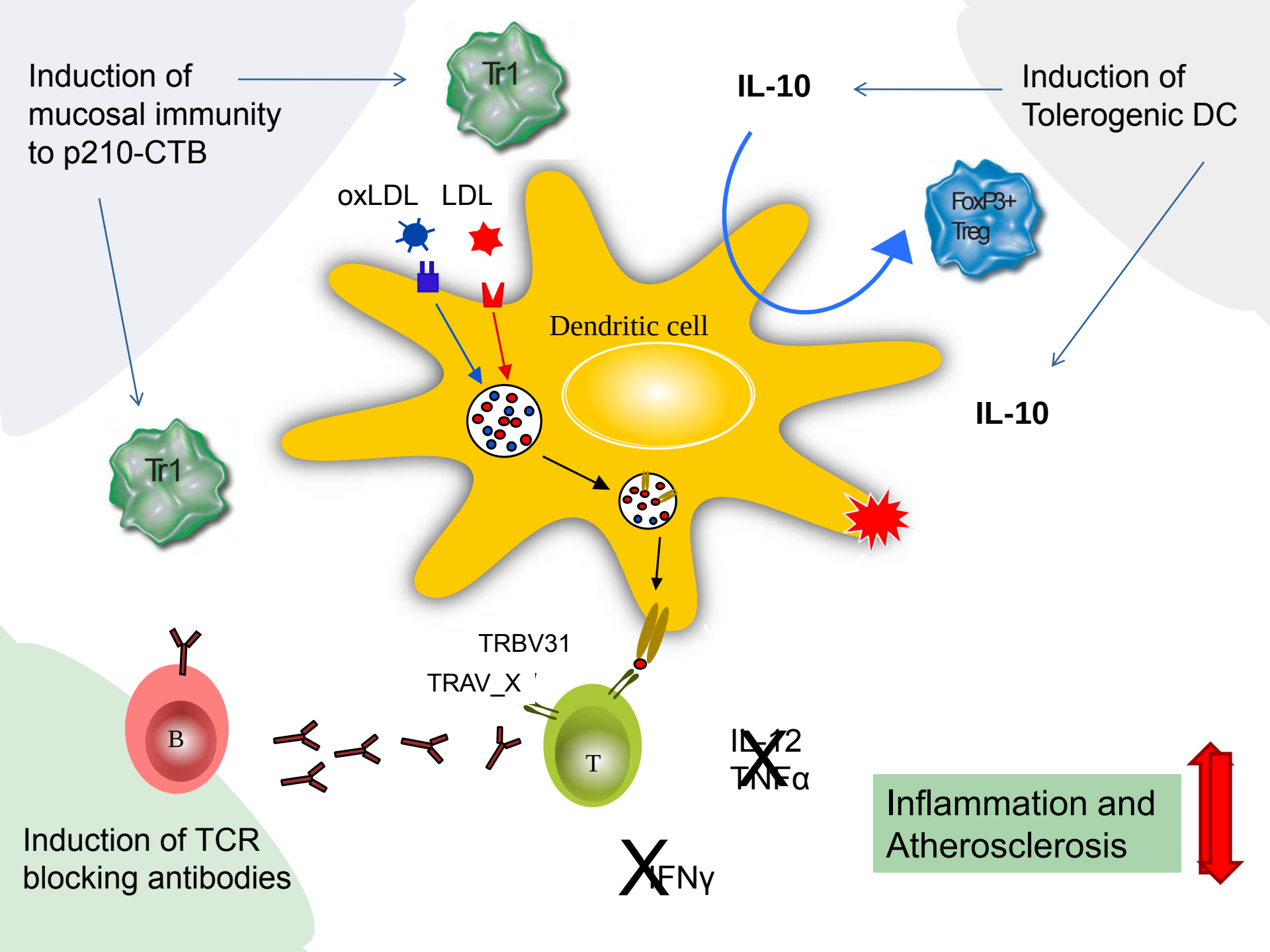
Hermansson et al
Circ 2011

Immunization with ApoB100 loaded tolerogenic DC reduces atherosclerosis

- Mice receiving IL-10-treated DC presenting ApoB100 show significantly reduced atherosclerosis.

- In vivo transfer of IL-10-treated ApoB100-pulsed DC dampens the autoreactive cellular immune response to ApoB100 and reduces pro-inflammatory cytokine responses.

- DC pulsed with ApoB100 and treated with IL-10 can suppress the specific T cell response to ApoB100 and induce foxP3+ regulatory T cells.


Induction of
mucosal immunity
to p210-CTB
Tr1
IL-10
Induction of
Tolerogenic DC
oxLDL
LDL
FoxP3+
Treg
Dendritic cell
Tr1
IL-10
TRBV31
TRAV_X
B
T
IL-12
TNFα
IFNγ
Induction of TCR
blocking antibodies
Inflammation and
Atherosclerosis

Cardiovascular Research Laboratory

Center for Molecular Medicine, Karolinska Institutet

Collaborators:
Antonino Nicoletti
Jan Nilsson
Jan Holmgren

Fund
Swedish Research Council
SSF
Vinnova Foundation
Heart-Lung Foundation
European Union
Leducq Foundation

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Center for Molecular Medicine, Karolinska Institutet

Thank you!