IMMUNOLOGICAL ASPECTS OF ATHEROSCLEROSIS

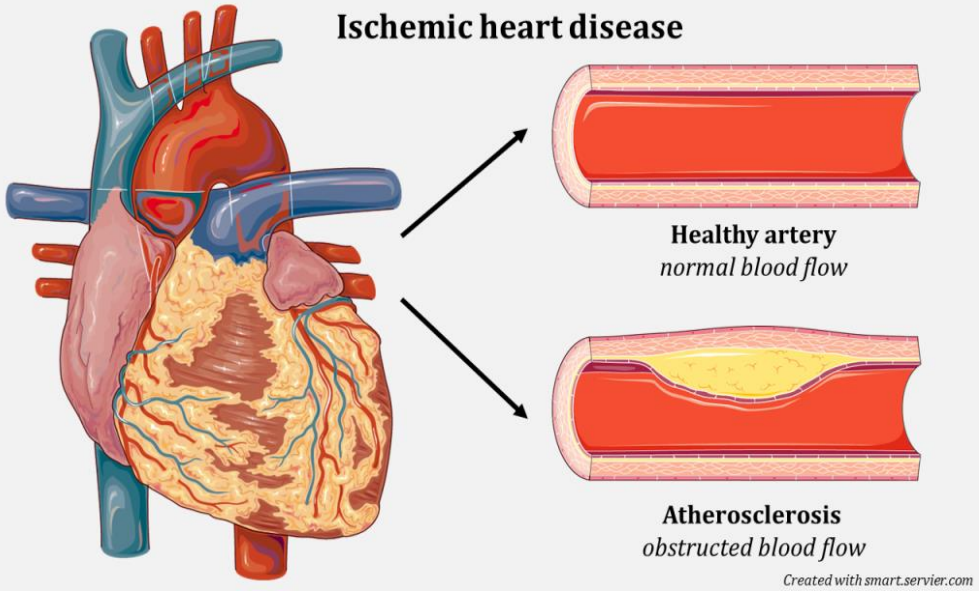
Presenter: Negin Heidari

OUTLINE

- Introduction
- General Characteristics of Atherosclerosis
- Morphology of the Normal Vascular Wall of the Arteries
- The Main Stages in the Development of Atherosclerosis
- Immune Mechanisms of Atherosclerosis Pathogenesis
- Immune checkpoints based-therapy

INTRODUCTION

- Intended for everyone who reaches a certain age
- <u>The main causes of death in developed countries</u>
- A concerning trend of development in young people
- Slowly progress with chronic inflammation



- Build-up of lipid-laden plaques in large and medium-sized arteries and remains unnoticed for the first few decades of its development
- Rupture or erosion of the plaque elicits thrombus formation that leads to ischaemic tissue damage

GENERAL CHARACTERISTICS OF ATHEROSCLEROSIS

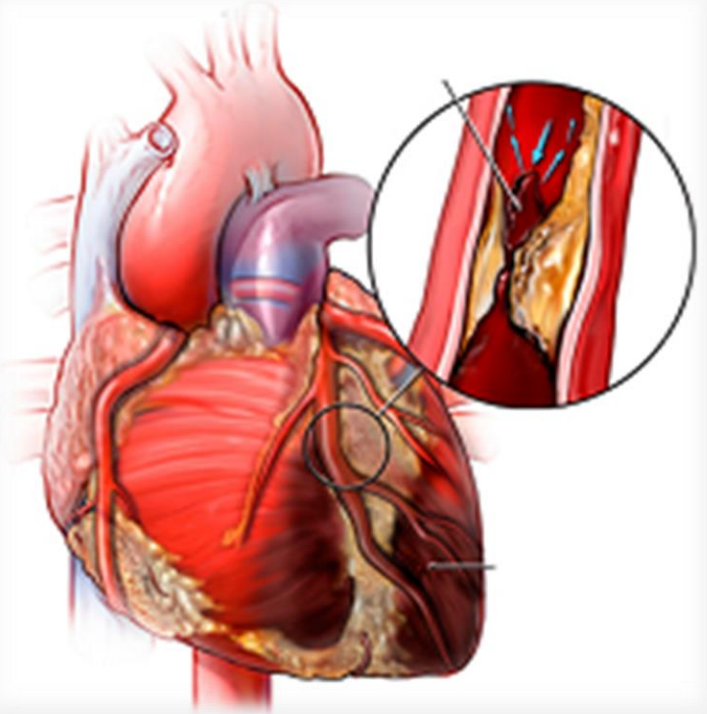
- The main pathogenetic factors of atherosclerosis:

1) Aging
   - Age-related changes in the metabolome, endotheliosis, immune dysfunction, shortening of telomeres, and other systemic changes in homeostasis
   - A vicious circle: dyslipoproteinemia, dyslipidemia, hyperglycemia, and metabolic dysfunctions contribute not only to atherosclerosis but also to cell aging.
2) Lipid metabolism disorders
3) The immune system and inflammation

GENERAL CHARACTERISTICS OF ATHEROSCLEROSIS

- Risk Factors for Atherosclerosis Development:

1) Pro-inflammatory processes and metabolic dysfunctions
2) Congenital risk factors
3) Gender differences
4) Lifestyle risk factors
5) Disorders of intestinal microbiota
6) Infections
7) Anatomical features of the arteries
8) Arterial hypertension



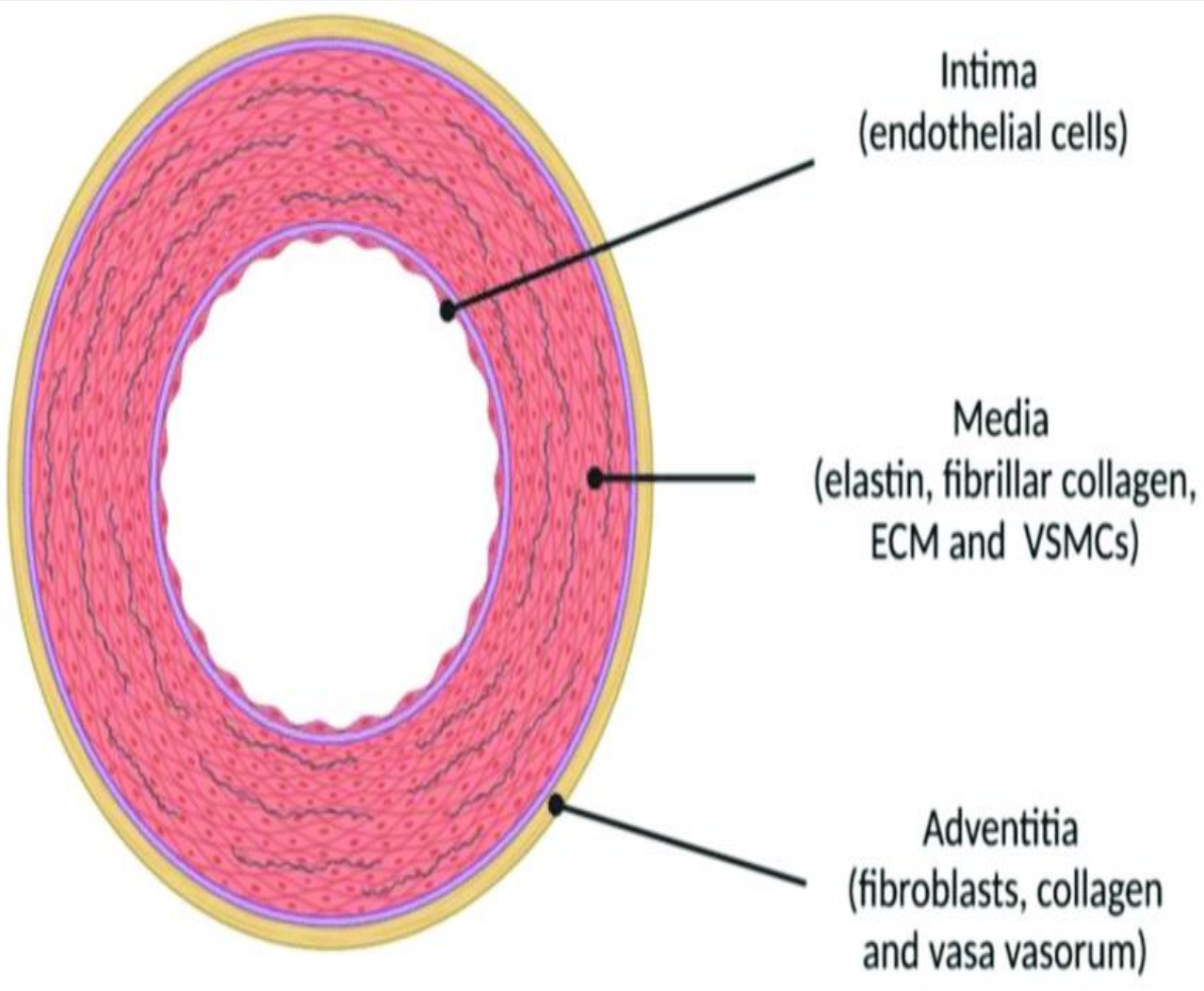
MORPHOLOGY OF THE NORMAL VASCULAR WALL OF THE ARTERIES

(1) intima (endothelial cells)

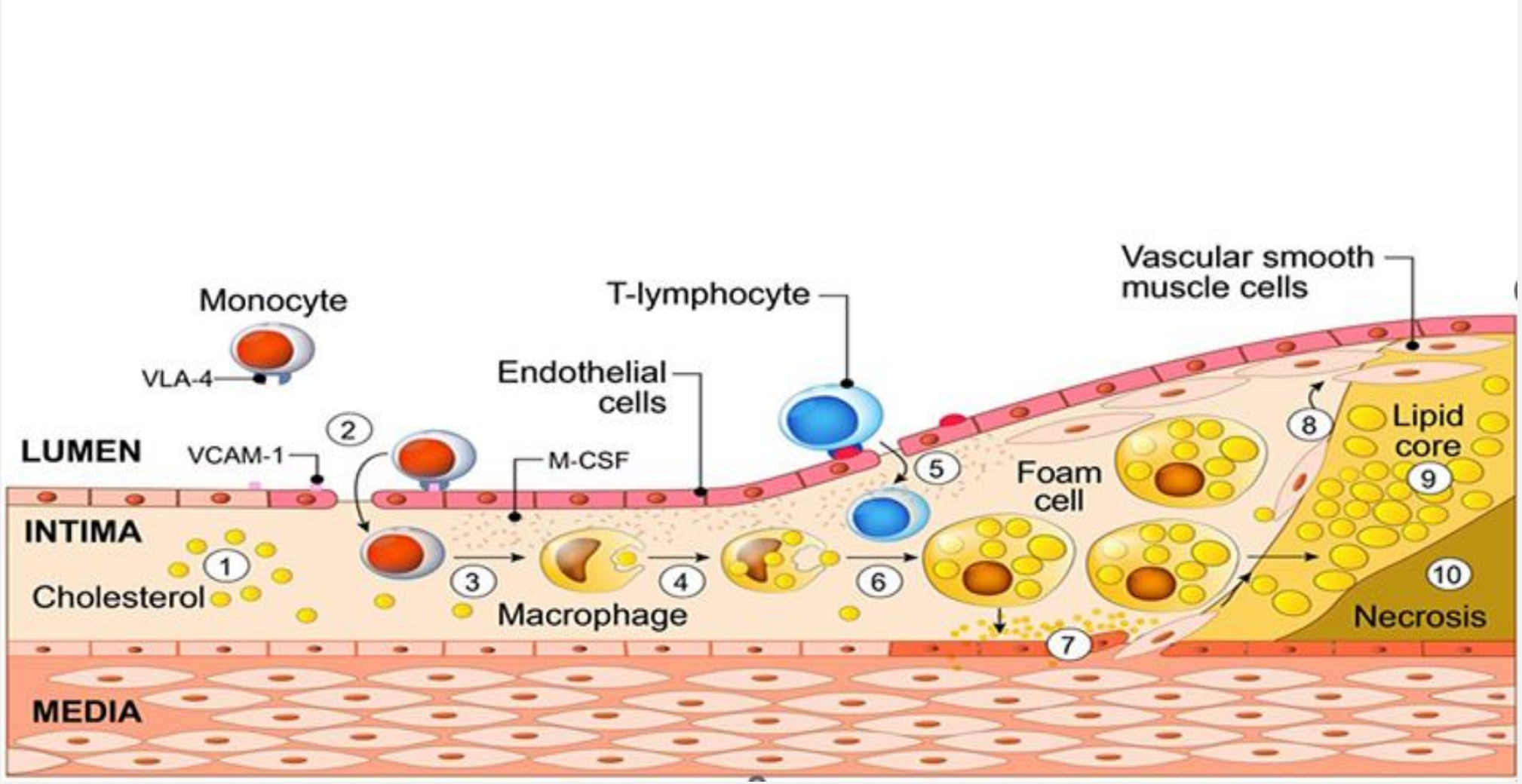
(2) media(concentric layers of elastin, fibrillar collagen and other proteins integrating the extracellular matrix(ECM) and vascular smooth muscle cells (VSMCs))

(3) adventitia (fibroblasts, collagen and, in larger arteries, vasa vasorum).

Atherosclerosis initiates in the intima whereas arteriosclerosis (AS)mostly affects the media.



THE MAIN STAGES IN THE DEVELOPMENT OF ATHEROSCLEROSIS



IMMUNE MECHANISMS OF ATHEROSCLEROSIS PATHOGENESIS

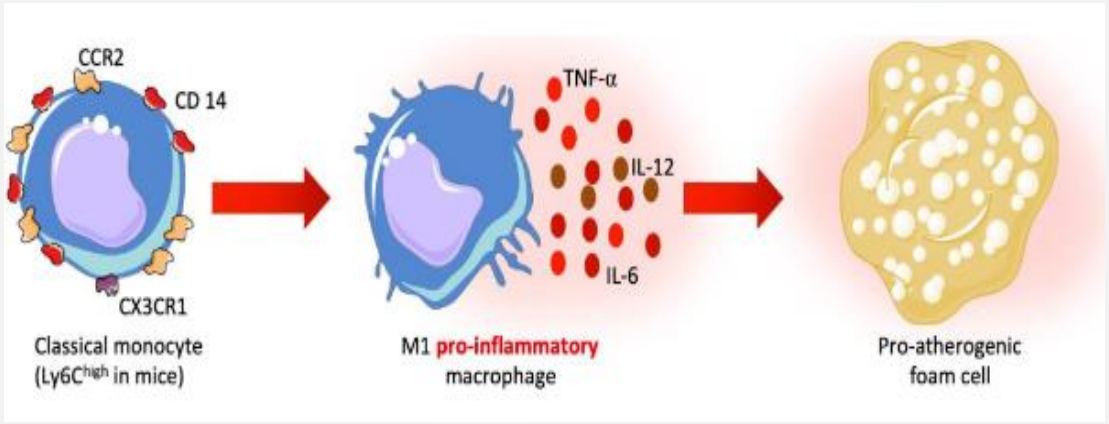
Macrophages

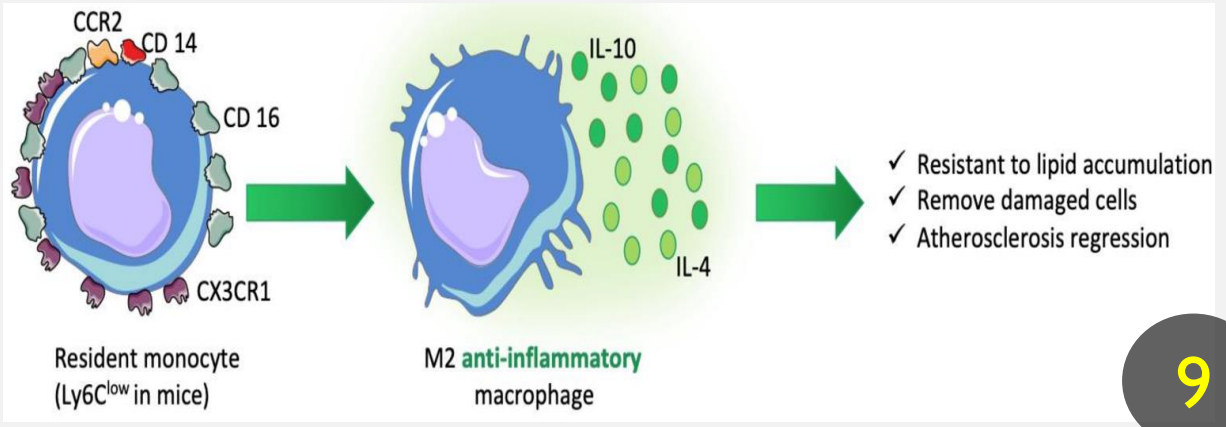
- embryonic origin / MQ originating from bone marrow monocytes.
- primarily in mucous membranes and lymphoid organs, as well as in the heart and arteries
- MQ of monocytic origin: greater immune and pro-inflammatory activity
- embryonic cells: more homeostatic activity.
- The number of stromal MQ of bone marrow origin increases with age, including in arteries and adipose tissue.

IMMUNE MECHANISMS OF ATHEROSCLEROSIS PATHOGENESIS

❑ Macrophages

- oxLDL uptake by MQ ➡ protective mechanism (remove cytotoxic elements from the intima)
- The increased migration of monocytes ➡ formation of an atherosclerotic plaque, which can become unstable and lead to severe hemodynamic disorders.





IMMUNE MECHANISMS OF ATHEROSCLEROSIS PATHOGENESIS

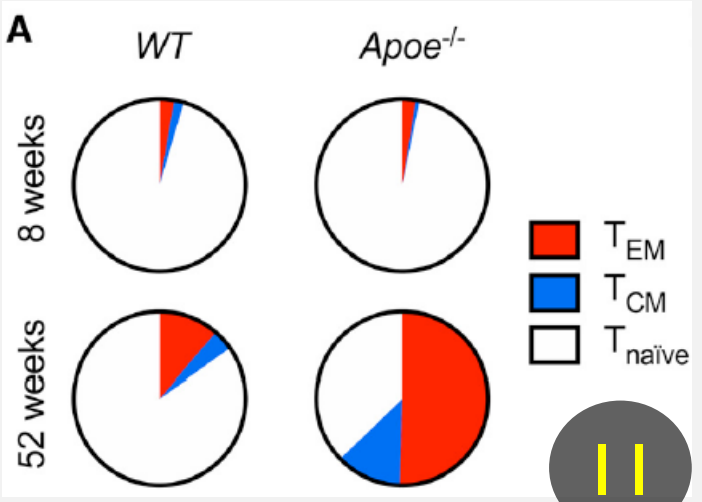
❑ Macrophages

- cholesterol efflux is activated by HDL :
- plaque MQ ➡ M2-like phenotype, releasing anti-inflammatory cytokines such as IL-10 and TGF-β, thereby promoting tissue repair or fibrosis.
- fibroblast like VSMCs ➡ producing collagen and other extracellular matrix proteins contribute to fibrosis and stable plaque formation.
- Dominance of M1 MQ, macrophage-like, and osteoblast-like VSMCs is a prerequisite for ➡ unstable plaque formation

IMMUNE MECHANISMS OF ATHEROSCLEROSIS PATHOGENESIS

❑ Evidence for an Autoimmune Response in Atherosclerosis

- Hypothesis that atherosclerosis includes an autoimmune response: the presence of T and B cells in the plaque.
- An increased rate of APC-CD4+ T-helper cell interactions in the plaque specifically in hypercholesterolemia that resulted in proinflammatory cytokine secretion
- T-helper cells show an increasing maturation into antigen-experienced TEM and TCM in the lymph nodes



WHY APOE-/- MOUSE?

- **LDLR:** plays a crucial role in the receptor-mediated pathway of lipoprotein metabolism.
- **ApoB:** the major protein of LDL and VLDL, is essential for the receptor-mediated uptake of LDL.
- **ApoE:** metabolism of cholesterol and triglycerides by binding to hepatic ApoE receptor or LDLR in the liver
- Differences in the lipid homeostasis between human and murine organisms
- gene manipulation (disturbances in plasma lipoprotein metabolism) ➡ atherosclerosis in mice.

➢ the atherosclerotic lesions in Apoe−/− mice resemble human lesions.

IMMUNE MECHANISMS OF ATHEROSCLEROSIS PATHOGENESIS

LDL: An Autoantigen Within the Plaque

- LDL as (auto) antigen was first suggested by Gero et al in 1959: immunization with LDL protected against atherosclerosis in rabbits, suggesting that autoimmune response against LDL can be atheroprotective.
  - Many CD4+ T cells in human plaques recognize oxLDL by binding to MHC-presented peptide epitopes from Apo B100.
  - atherosclerosis is accompanied by IgG antibodies against LDL, oxLDL, and ApoB.

these findings strongly suggest LDL as a relevant self-antigen in the atherosclerotic plaque

IMMUNE MECHANISMS OF ATHEROSCLEROSIS PATHOGENESIS

❑T-Helper Cell–Dependent Immunity in Atherosclerosis

- ≈25% to 38% of all leukocytes in mouse aortic and human atherosclerotic plaques are CD3+ T cells
- CD3+CD4+ T-helper cells accounting for ≈10%.
- T cells predominantly populate atherosclerotic lesions in the fibrous cap
- Also found in the adventitia of older lesions.
- Their recruitment to the plaque: via chemokine receptors CCR5, CXCR6.

IMMUNE MECHANISMS OF ATHEROSCLEROSIS PATHOGENESIS

T-Helper Cell–Dependent Immunity in Atherosclerosis

- the function of T-helper cells in atherosclerosis is multi-faceted
- TH1: + Atherosclerosis is a known TH1 disease. Many CD4+ T cells in the plaque express the proinflammatory, TH1-associated cytokines IFN-γ, IL-2, IL-3, TNF and LT (lymphotoxin).
- Tregs: - FoxP3 / the high-affinity IL-2 receptor CD25 / theroprotective properties by secreting IL-10, TGF-β, and by suppressing the proliferation of proinflammatory T-effector cells.
- TH2: ? IL-4 antagonizes TH1 responses and diminished lesion formation in 1 study, although depletion of IL-4 has also been reported to be atheroprotective.

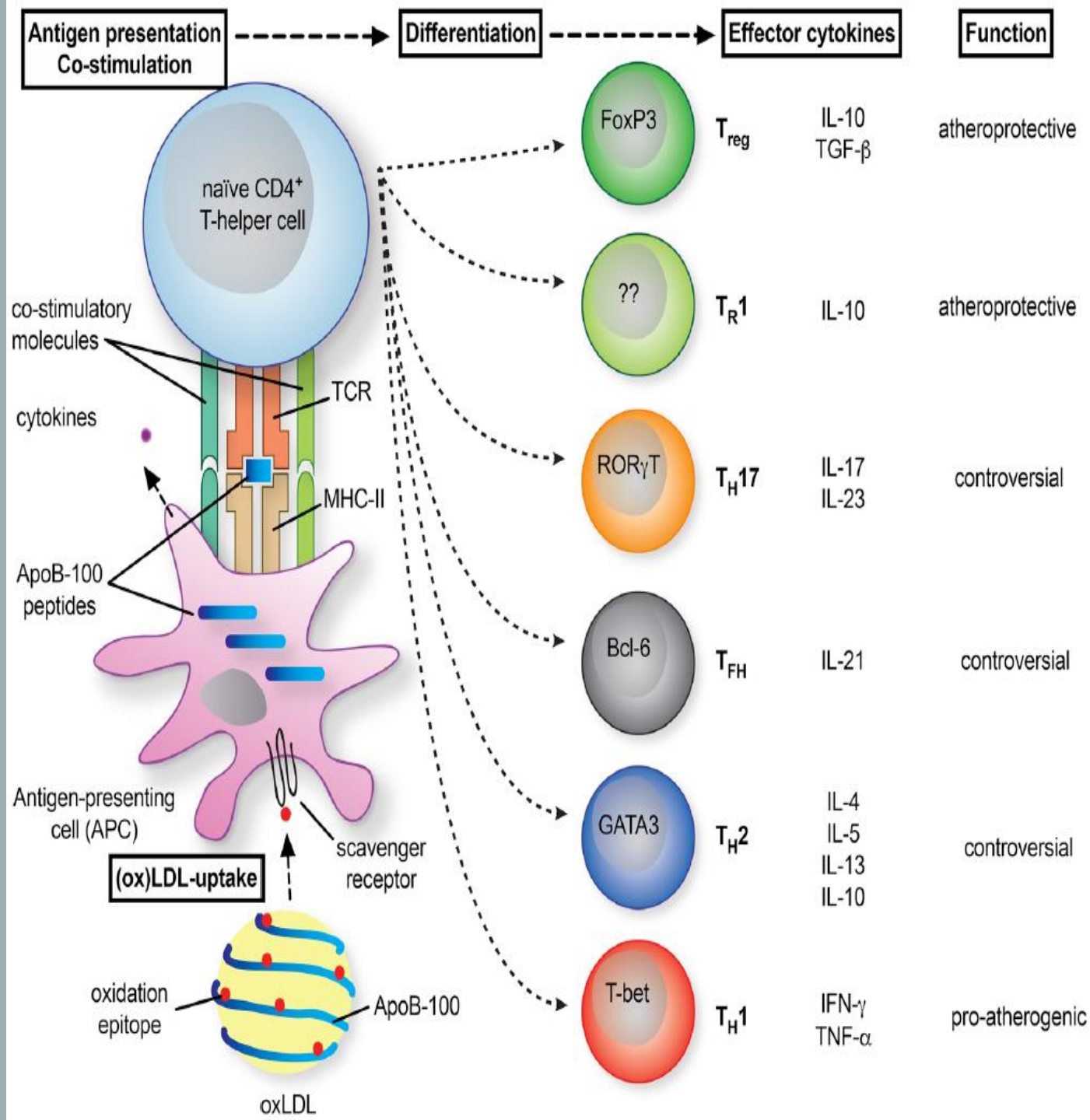
T-CELL POLARIZATION IN ATHEROSCLEROSIS

TR1:

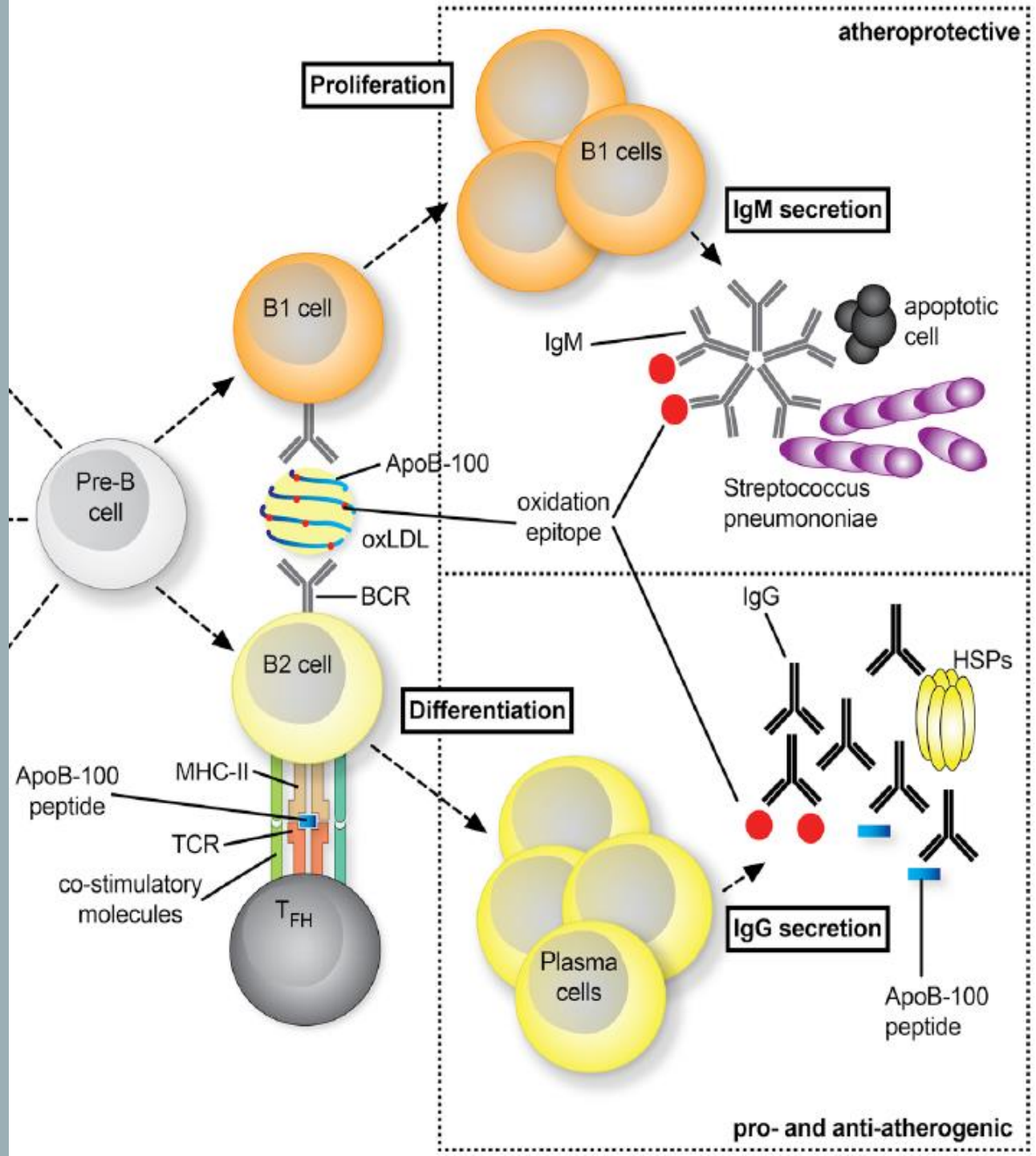
lack FoxP3 expression

express CD49b and Lag-3

secrete IL-10

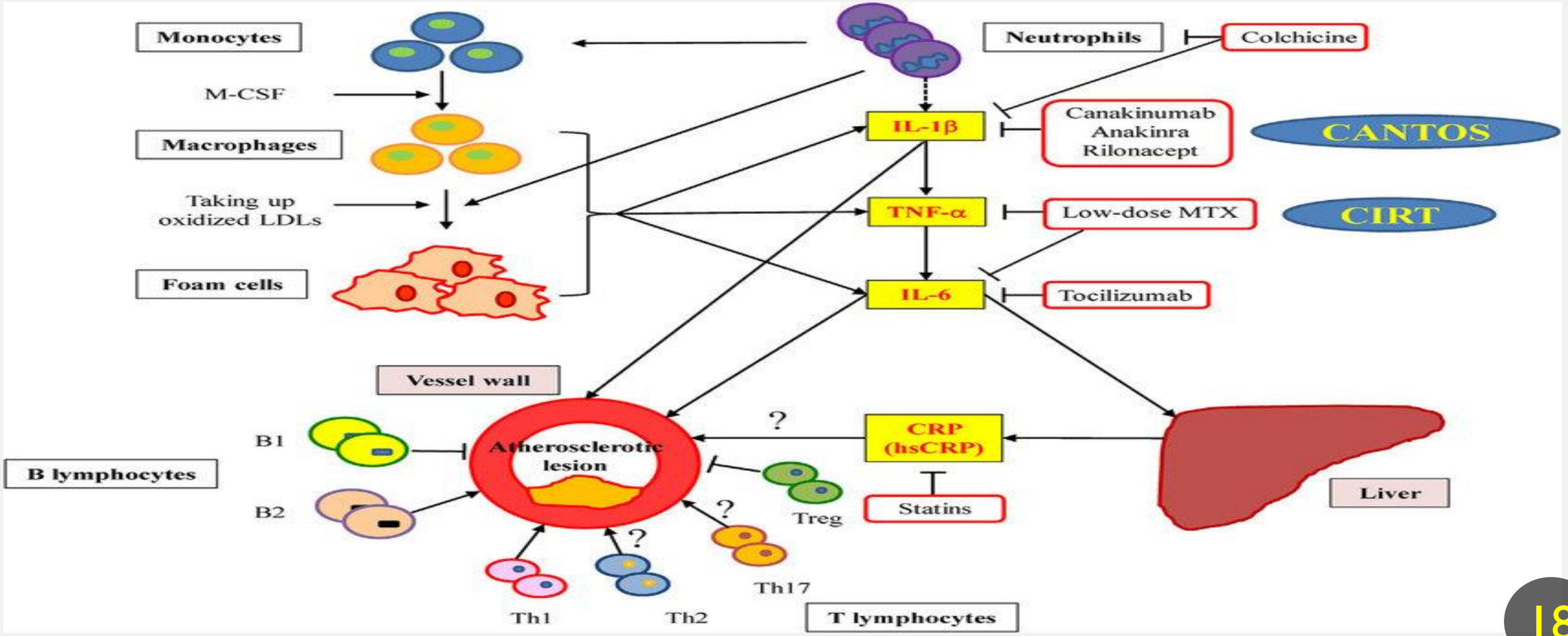


DISTINCT ROLE OF B CELLS IN ATHEROSCLEROSIS



CURRENT IMMUNOTHERAPY FOR ASCVD

- Targeting innate immune system:



CURRENT IMMUNOTHERAPY FOR ASCVD

- CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study)
- IL-1 inhibitors:
  - anakinra (an IL-1 receptor antagonist)
  - rilonacept (an IL-1 trap)
  - canakinumab (an anti-IL-1β antibody)

A human monoclonal antibody that selectively neutralizes IL-1β but not IL-1α

no effect on LDL or HDL

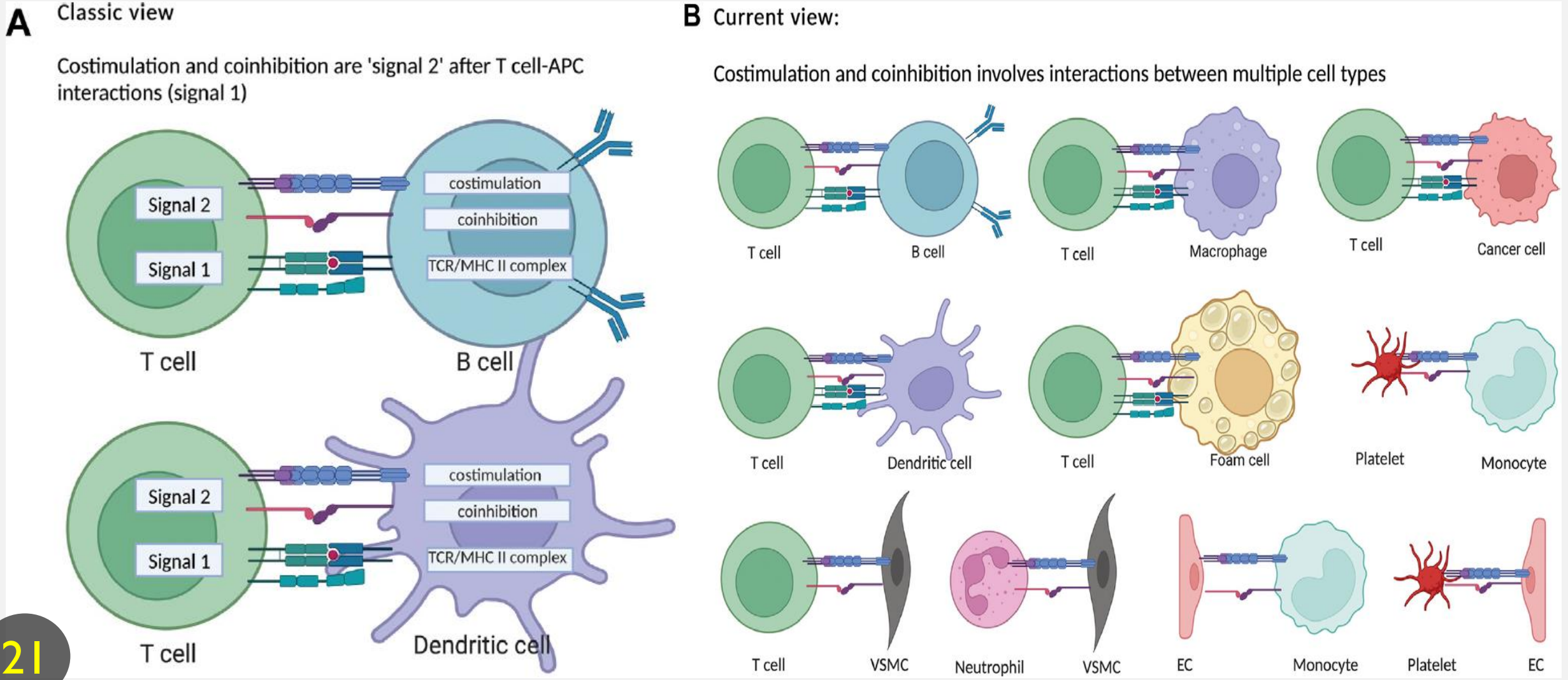
→ Canakinumab, special candidate to test the inflammatory hypothesis

- The CANTOS trial is a randomized, double-blind, placebo-controlled trial, enrolled over 10,000 patients with previous myocardial infarction, levels of hsCRP, defined as 2 mg or more per liter.

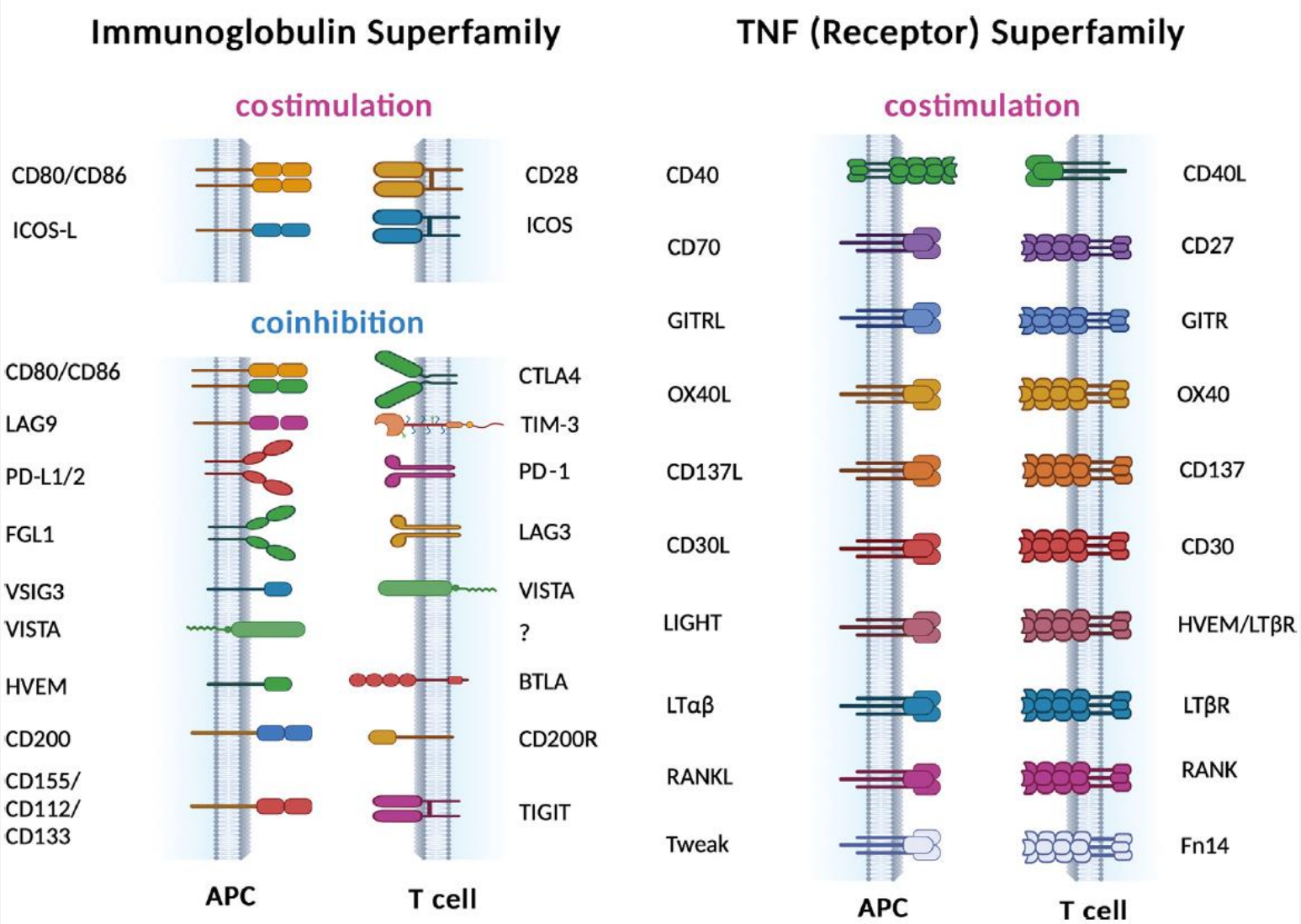
CURRENT IMMUNOTHERAPY FOR ASCVD

- Targeting adaptive immune system:
- RITA-MI (Rituximab in Patients With Acute ST-Elevation Myocardial Infarction)
  - anti-CD20 antibody rituximab
  - mature B cells were depleted in patients with acute ST-segment elevation myocardial infarction
  - Safe and effective
- LILACS (Low Dose IL-2 in Patients With Stable Ischemic Heart Disease and Acute Coronary Syndromes)
  - low-dose IL-2 is given to patients with acute coronary syndromes
  - significant expansion of regulatory T (Treg) cells without major adverse events in patients with ischemic heart disease

IMMUNE CHECKPOINTS



IMMUNE CHECKPOINTS

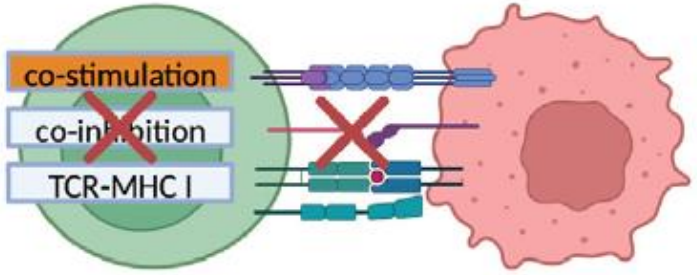


IMMUNE CHECKPOINT–BASED THERAPIES IN ONCOLOGY AND AUTOIMMUNE DISEASES

Cancer

Therapeutic action:

Enhance immune mediated killing of tumor cells by inhibition of coinhibition and/or enhancing costimulation



T cell Cancer cell

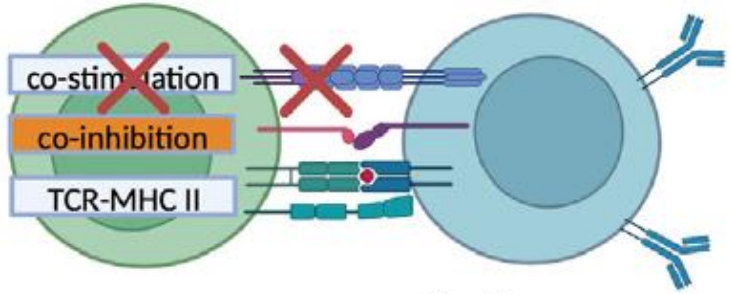
Drugs approved/under development

Anti-PD1, Anti-CTLA4
CD40, GITR, OX40 agonists

Auto-immune disease

Therapeutic action:

Reduce inflammation by inhibiting costimulation or enhancing coinhibition



T cell B cell

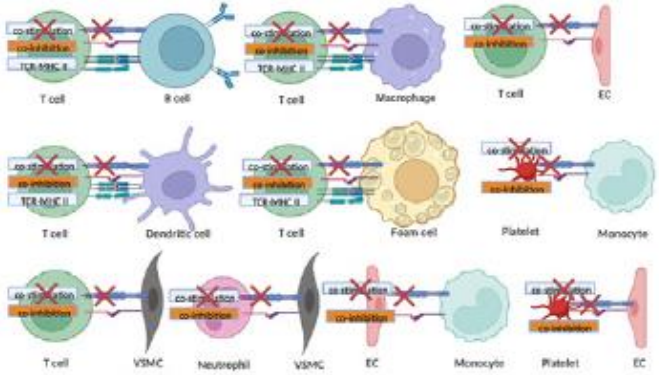
Drugs approved/under development

CTLA4-fusion protein, anti-CD80/86, anti-CD40(L), anti-CD137, anti-ICOS
PD1, BTLA agonists

Cardiovascular disease

Therapeutic action:

Reduce inflammation by inhibiting costimulation or enhancing coinhibition



Multiple cell-types

Drugs in pre-clinical development

Anti-CD80/CD86, Anti-CD40(L),
CD40-TRAF6 SMI
PD1 agonists

EC = endothelial cell; SMI = small-molecule inhibitor; TCR = T cell receptor; VSMC = vascular smooth muscle cell.

IMMUNE CHECKPOINT–BASED THERAPIES IN ONCOLOGY

- Inhibition of Coinhibitory immune checkpoints, activation of Costimulatory checkpoints, or a combination of both efficiently.
- Dr James Allison, received the Nobel Prize in 2018:
  - blocking the Coinhibitory immune checkpoint CTLA4 can regress tumors in mice.
  - blocking the Coinhibitory immune checkpoint PD-1 or PD-L1 had similar effects.

❖ ipilimumab, CTLA4 antibodies, was approved for metastatic melanoma in 2011

❖ pembrolizumab and nivolumab, anti-PD-1 therapy, was approved in 2014.

- Since then, the use of immune checkpoint–based immunotherapies has been approved for >50 indications and is now being used in different types of primary and metastasized cancers.

EXPERIMENTAL ATHEROSCLEROSIS STUDIES ON COSTIMULATORY AND COINHIBITORY IMMUNE CHECKPOINT

Immune Checkpoint Dyad	Atherosclerosis Model (Genetic Model/ICI/SMI)	Atherosclerotic Plaque Phenotype	Intraplaque Immune Cell Phenotype	First Author (Year)
Costimulation				
ICOS-ICOSL	Icos$^{-/-}$ Ldlr$^{-/-}$ BM chimera (8-wk atherogenic diet)	AR: ↑ lesion size, ↑ collagen, ↑ αSMA	↑ macrophages, ↑ CD4^{+} T cells	Gotsman et al (2006)[131]
CD28-CD80/CD86	B7-1/B7-2$^{-/-}$ (CD80/CD86) Ldlr$^{-/-}$ (8- and 20-wk atherogenic diet)	AR: ↑ lesion size, ↓ αSMA, ↓ collagen	↓ macrophages (8 wk)	Buono et al (2004)[52]
	Cd80/Cd86$^{-/-}$ Ldlr$^{-/-}$ BM and Cd28$^{-/-}$ Ldlr$^{-/-}$ BM chimera (20-wk atherogenic diet)	AR: ↑ lesion size	ND	Ait-Oufella et al (2006)[53]
Coinhibition				
CTLA4-CD80/CD86	Abatacept (CTLA4 ligand fusion protein) in ApoE3*Leiden; cuffed FA	FA: ↓ intimal thickening, ↓ lumen stenosis, ↑ αSMA	↓ macrophages and CD3^{+} T cells	Ewing et al (2013)[61]
	Anti-CTLA4, ApoE3*Leiden; cuffed FA	FA: ↑ intimal thickening, ↑ lumen stenosis	↑ CD3^{+} T cells	Ewing et al (2013)[61]
	Ctla4Tg ApoE$^{-/-}$ (16-wk NCD)	AR: ↓ lesion size	↓ macrophages, ↓ CD4^{+} T cells	Matsumoto et al (2016)[54]
	Anti-CTLA4 (9D9) in Ldlr$^{-/-}$ (6-wk atherogenic diet)	AR: no effect on lesion size, ↑ PIT and FCA, ↑ necrotic core	↑ CD4^{+} T cells	Poels et al (2020)[56]
PD-1-PD-L1/2	Pd-l1/l2$^{-/-}$ Ldlr$^{-/-}$ (6-wk and 16-wk atherogenic diet)	AR: ↑ lesion size, ↑ collagen, ↑ αSMA	↑ macrophages, ↑ CD4^{+}/CD8^{+} T cells	Gotsman et al (2007)[102]
	Pd-1$^{-/-}$Ldlr$^{-/-}$ (5-wk and 10-wk atherogenic diet)	AR: ↑ lesion size (10 wk)	↑ macrophages, ↑ CD4^{+}/CD8^{+} T cells	Bu et al (2011)[103]
	Anti-PD-1 in Ldlr$^{-/-}$ (5-wk atherogenic diet)	No effect on lesion size	↑ CD4^{+}/CD8^{+} T cells	Bu et al (2011)[103]
	Pd-1$^{-/-}$Ldlr$^{-/-}$ (9-wk atherogenic diet)	AR: ↑ lesion size	↑ macrophages, ↑ CD4^{+}/CD8^{+} T cells, ↑ T_{reg} cells	Cochain et al (2014)[132]
	Anti-PD-1 (BE0146) and anti-CTLA4 (BE0164) in Ldlr$^{-/-}$ (6-wk atherogenic diet)	AA/AR: no effect on lesion size, ↓ IX, ↑ PIT, ↑ necrotic core	↓ macrophages, ↑ CD3^{+} T cells	Poels et al (2020)[104]
	PD-1 agonist (PIM-2) in Ldlr$^{-/-}$ (6-wk atherogenic diet)	AR: ↓ lesion size	↓ CD4^{+} T cells	Grievink et al (2021)[105]

EXPERIMENTAL ATHEROSCLEROSIS STUDIES ON COSTIMULATORY AND COINHIBITORY IMMUNE CHECKPOINT

Immune Checkpoint Dyad	Atherosclerosis Model (Genetic Model/ICI/SMI)	Atherosclerotic Plaques Phenotype	Atherosclerotic Plaques Phenotype	Intraplaque Immune Cell Phenotype	First Author (Year)
CD40-CD40L	CD40L	Anti-CD40L in $Ldlr^{-/-}$ (12-wk atherogenic diet)	AA: ↓ wall area, ↓ wall thickness, ↓ lipid content	↓ macrophages and $CD3^+$ T cells, ↓ V-CAM	Mach et al (1998)[82]
		$Cd40l^{-/-}ApoE^{-/-}$ (23-wk NCD)	AA: ↓ lesion size, ↑ collagen	↓ macrophages and $CD3^+$ T cells	Lutgens et al (1999)[79]
		Anti-CD40L in $ApoE^{-/-}$: 5 wk old (early stage), and 17 wk old (late stage) (12-wk NCD)	AA: no effect on lesion size, ↑ collagen, ↑ αSMA (early and late); ↑ fibrous cap, ↓ lipid content (late)	↓ $CD3^+$ T cells (early); ↓ macrophages, ↓ $CD3^+$ T cells (late)	Lutgens et al (2000)[80]
		Anti-CD40L in $Ldlr^{-/-}$ (13-wk atherogenic diet)	AA: ↓ wall area and thickness, ↓ collagen, ↑ αSMA, ↓ lipid content	↓ macrophages	Schonbeck et al (2000)[81]
		$Cd40l^{-/-}Ldlr^{-/-}$ (16-wk atherogenic diet)	AA/AR: ↓ lesion size, ↑ collagen, ↑ αSMA, ↓ lipid content	↓ macrophages	Bavendiek et al (2005)[137]
		$Cd40l^{-/-}Ldlr^{-/-}$ BM chimera (16-wk atherogenic diet)	AA/AR: no effect on lesion size	No effect	Bavendiek et al (2005)[137]
		$Cd40l^{-/-}Ldlr^{-/-}$ BM chimera (20-wk atherogenic diet)	AR: no effect on lesion size	No effect	Smook et al (2005)[138]
		Adoptive transfer of thrombin-activated $Cd40l^{-/-}$ platelets in $ApoE^{-/-}$ (29-wk NCD) and in collar-induced model (6-wk atherogenic diet)	AA/AR: ↓ lesion size, ↓ collagen (collar induced) CA: ↓ lesion size, ↓ αSMA	Collar induced: ↓ macrophages	Lievens et al (2010)[72]
		cM7 peptide in $Ldlr^{-/-}$ (20-wk atherogenic diet)	AR: ↓ lesion size, ↑ collagen, ↓ lipid content	↓ macrophages	Wolf et al (2011)[98]
		Cd4-CreTg$Cd40lf/fl$ $ApoE^{-/-}$ (28-wk NCD)	AR: ↓ lesion size, ↓PIT, ↓ FCA, ↑ IX, ↑ fibrous cap, ↑ αSMA, ↓ necrotic core	↑ macrophages, ↓ $CD4^+$ T cells	Lacy et al (2021)[83]
		Pf4-CreTg $Cd40lf/fl$ ApoE-/; WI model (28-wk NCD)	Only with WI: ↓ lesion size (↓ atherothrombosis)	ND	Lacy et al (2021)[83]
	CD40	$Cd40^{-/-}Ldlr^{-/-}$ (16-wk atherogenic diet)	AA: no effect on lesion size, ↑ lipid content	↑ macrophages	Zirlik et al (2007)[139]
		$Cd40^{-/-}ApoE^{-/-}$ (26-wk NCD)	AA: ↓ lesion size, ↑ collagen, ↑ αSMA	↓ macrophages, ↓ T cells	Lutgens et al (2010)[73]
		Adoptive transfer of thrombin-activated $Cd40^{-/-}$ platelets in $ApoE^{-/-}$ (29-wk NCD)	AA: ↓ lesion size, ↓ collagen, ↑ FCA and PIT, ↓ lipid content	↓ macrophages	Gerdes et al (2016)[140]
		TRAF-6/SMI 6877002 and 6860766 in $ApoE^{-/-}$ (18-wk [early] and 28-wk [advanced] NCD)	AA: ↓ lesion size, ↓ FCA, ↑ IX (early); ↓ lesion size, ↑ collagen, ↓ necrotic core (advanced)	↓ macrophages, ↓ $CD3^+$ cells (early); ↓ macrophages (advanced)	Seijkens et al (2018)[96]
		TRAF-6i-HDL with SMI6877002 in $ApoE^{-/-}$ (12-wk atherogenic diet)	ND	↓ macrophages and monocyte in aorta	Lameijer et al (2018)[97]
		Bmx-CreERT2/$CD40fl/fl$ $ApoE^{-/-}$ (25-wk NCD)	AR: no effect on lesion size, ↓ lipid content, ↑ collagen, ↑ αSMA	↓ macrophages	Gissler et al (2021)[84]
		Cd11c-CreTg/$Cd40fl/fl$ $ApoE^{-/-}$ (28-wk NCD)	AR: ↓ lesion size, ↑ αSMA	↓ $CD4^+$ T cells	Lacy et al (2021)[83]
		LysM-CreTg$Cd40fl/fl$-$ApoE^{-/-}$ (14-wk atherogenic diet)	AA: ↓ lesion size, ↓ necrotic core AR: ↓ lipid content	↓ macrophages	Bosmans et al (2023)[74]
		AdipoQ-CreTg$Cd40fl/fl$-$ApoE^{-/-}$ (11-wk atherogenic diet)	AR: ↓ lesion size, ↑ necrotic core	↓ $CD3^+$ T cells	Reiche et al (2023)[85]

IMMUNE CHECKPOINTS IN ATHEROSCLEROSIS

- in ASCVD is still in a preclinical stage
  - CD28/CTLA4-CD80/86.
  - CD40L-CD40.
  - PD-1-PD-L1.
  - LAG3.
- Costimulatory and Coinhibitory immune checkpoints translation into the cardiovascular disease arena lags behind:
- The diversity and complexity of the immune response in atherosclerosis
- cellular diversity, and alternative signaling pathways of Costimulatory and Coinhibitory immune checkpoints

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THANK YOU
FOR YOUR ATTENTION