

Immune modulation for Atherosclerosis Potential Therapeutic option for cardiovascular diseases

Lakshmi Mundkur
Thrombosis Research Institute- Bangalore , India

Cardiovascular Diseases
coronary heart disease, cerebrovascular disease, peripheral arterial disease

17.5 million people die each year from CVDs, an estimated 31% of all deaths worldwide.

80% of all CVD deaths are due to heart attacks and strokes.

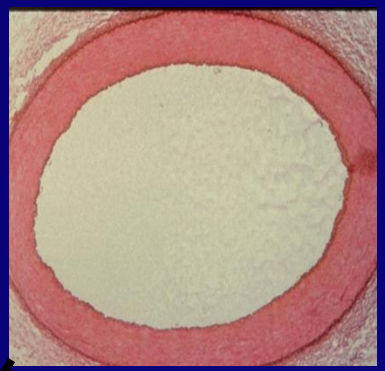
>75% of CVD deaths occur in low-income and middle-income countries.

THE RISK OF DYING EARLY FROM CORONARY ARTERY DISEASE IS TWICE AS HIGH AMONG SOUTH ASIAN COMPARED TO GENERAL POPULATION

WHO –2015 AND NHS –UK

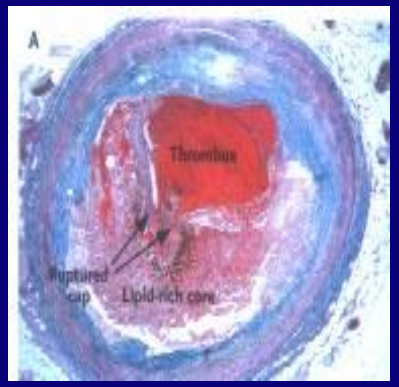
Atherosclerosis

Normal Artery



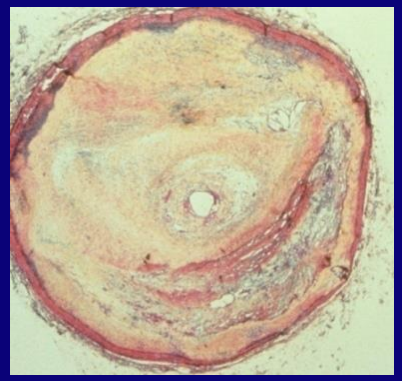
- slow progressive disease
- remains asymptomatic for decades

Sudden Heart attack



Plaque rupture
30-40% occlusion

Angina



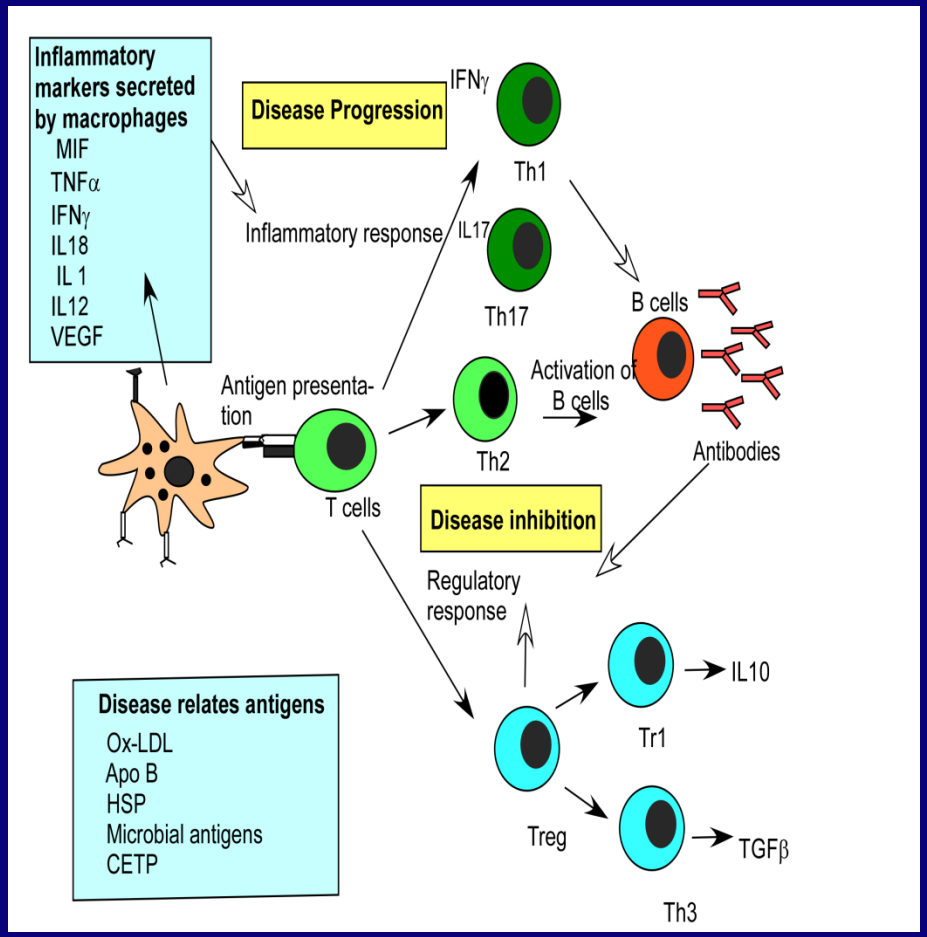
80-90% occlusion

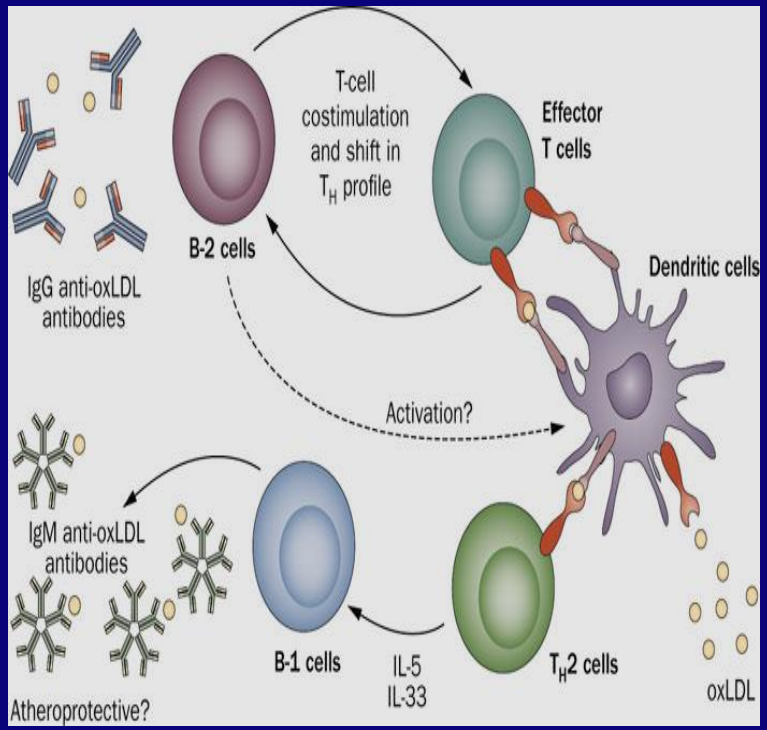
J Autoimmun, 2012, Nat Med 2003, NEJM 1999, NEJM 2012, ATVB 2011

Atherosclerosis
A Major Paradigm Shift

- 500 publications in major international journals
- Atheroma is a chronic inflammatory disease where lipids and chronic infection play a critical role
- Both innate and adoptive immunity are involved
- Immune modulation have the potential of protecting against atherosclerosis
- New possibilities for significant cost effective reduction of CVD burden

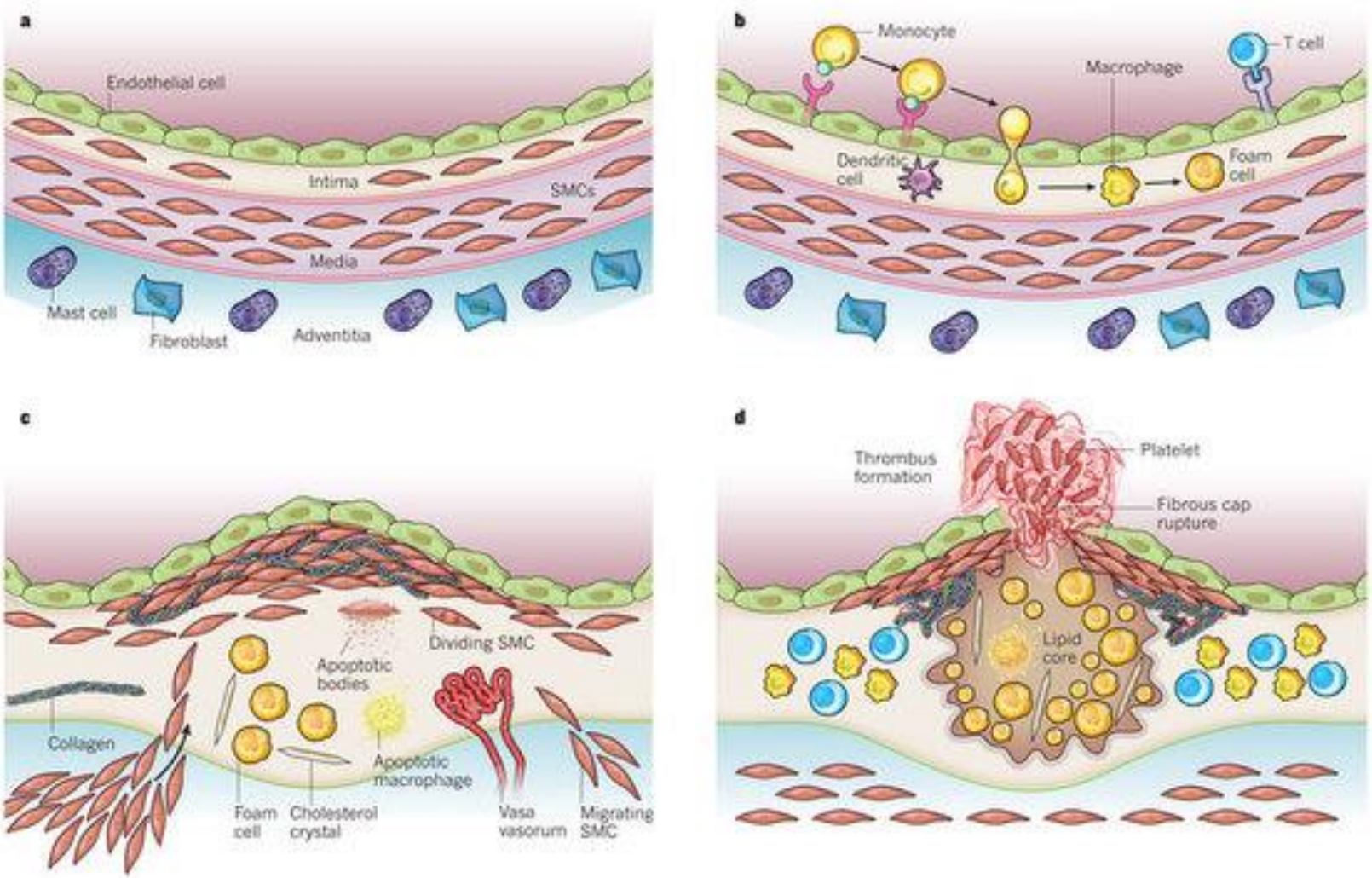
Immune system in Atherosclerosis





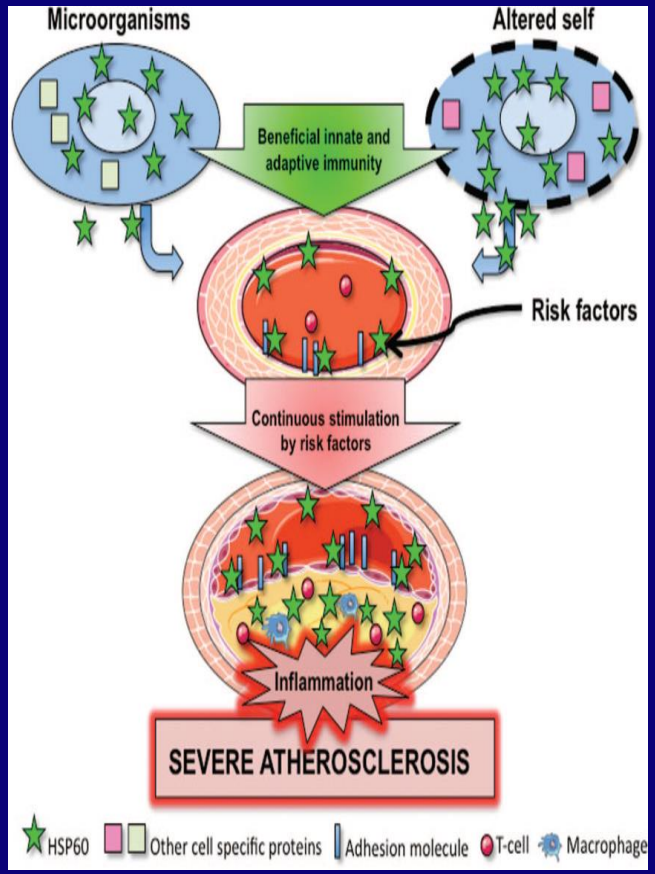
Chyu et al. Discovery medicine 2011, Mundkur et.al CDT-2010

Atherosclerosis



Libby and Hansson Nature 2011

Autoimmune concept of Atherosclerosis



❖ Atherogenesis begins as a qualitative change to intact endothelial cells– (oxidative ,hemodynamic, risk factors)

❖Changes their permeability to promote the entry and retention of monocytes and LDL

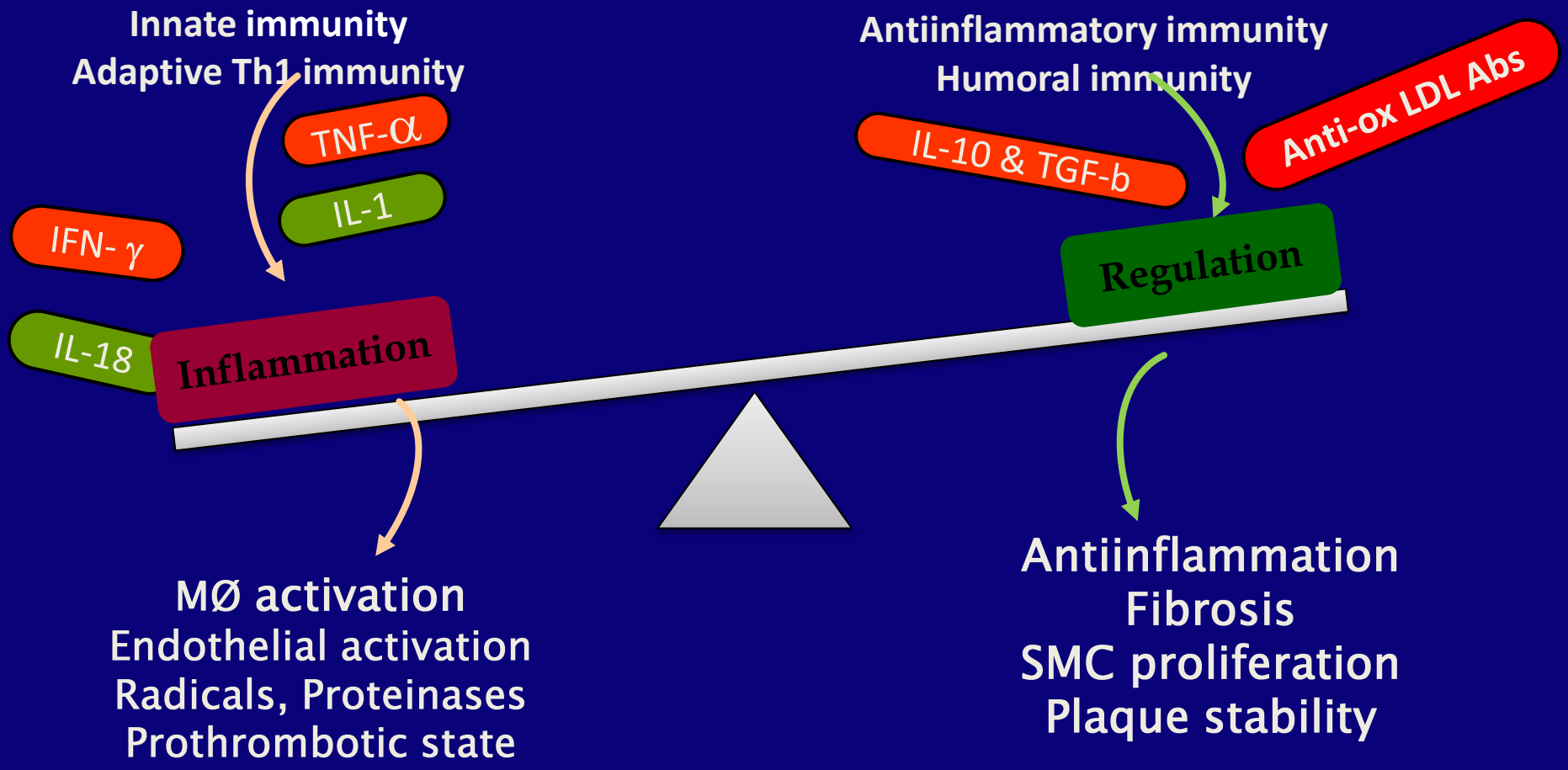
❖Oxidation of LDL exposes neo epitopes in ApoB100 and LDL

❖ Classical risk factors / Infection modify the expression of HSP60 on the surface of arterial endothelial cells (ECs)

❖T cells reactive to Oxidized lipoproteins and HSP are seen the early plaques

Nabel et. Al NEJM 2012, Gruntman et al ATVB 2011

Immune modulation for atherosclerosis



Restoring tolerance to auto antigens and reduce inflammation

Why an Immune based therapy ?

- Current treatments and prevention include
  - Lifestyle changes
  - Reduction in risk factors

- More than 90% of sudden heart attacks are due to
  - Rupture of atherosclerotic plaque

- No treatments available for
  - preventing plaque rupture
  - stabilizing a vulnerable plaque

Target the cause of the disease rather than risk factors to get additional effects

The Global Economic Burden of Noncommunicable Diseases
(World Economic Forum, Geneva, 2011.

TRI- Approach

➢Hypothesis

- ❖ Multiple antigens would confer better protection compared to single antigens.
- ❖ Infection and molecular mimicry plays a very important role in the development of atherosclerosis

➢Approach

- ❖ Use peptides as antigen
- ❖ Express multiple peptides in a single protein scaffold
- ❖ Oral dosing to induce tolerance

Sutcliffe MJ, .Nat Struct Biol. 1994,Lu X,. J Biol Chem. 1996.

Selection Of Epitopes

T cell epitopes promiscuous MHC binding

Self-antigens

ApoB 100* (AA 688-707)

Human HSP60,- (AA 153-163) molecular mimicry with CMV early proteins

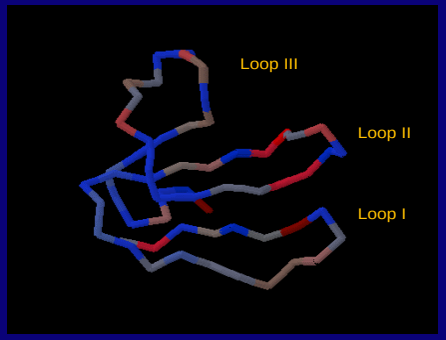
Proteins from pathogens

Chlamydia pneumonia ,CMV, P gingivalis (molecular mimicry with HSP60 and ApoB)

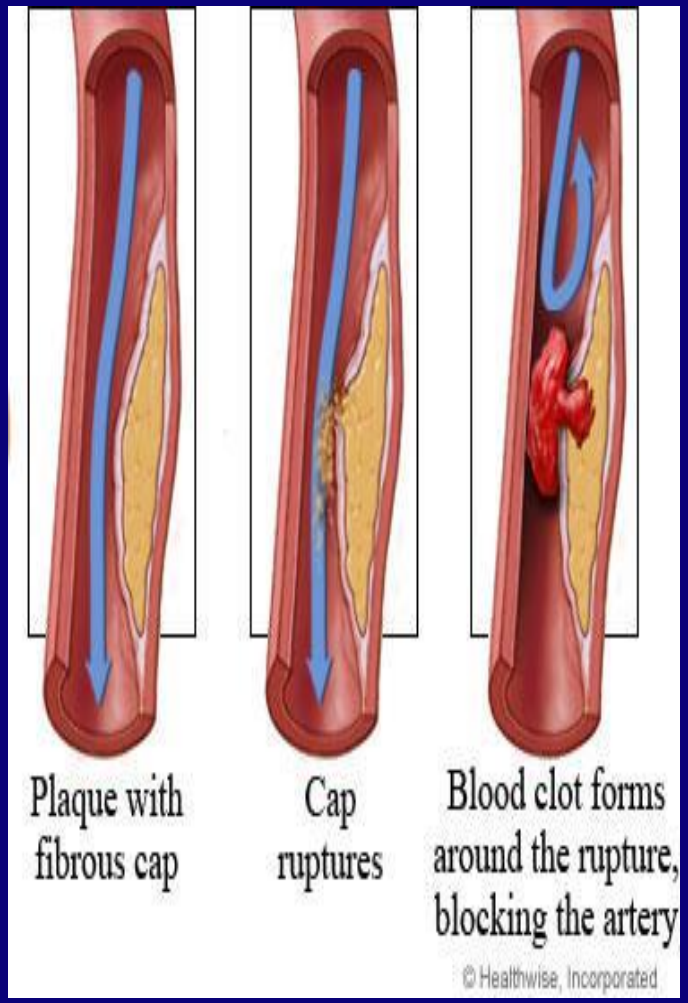
Protein scaffold Dendroaspin

- Short-chain neurotoxin homolog from Elapidae snakes, with no neurotoxicity

- Novel arrangement of loops-
  Can be modified to incorporate further functional amino acid sequences



Focus of Immune Therapy



Focus of Vaccine Development

Development

Progression

Stabilization

Plaque Rupture

- Unmet Clinical need
- Major cause of ACS, Stroke

Experimental Design

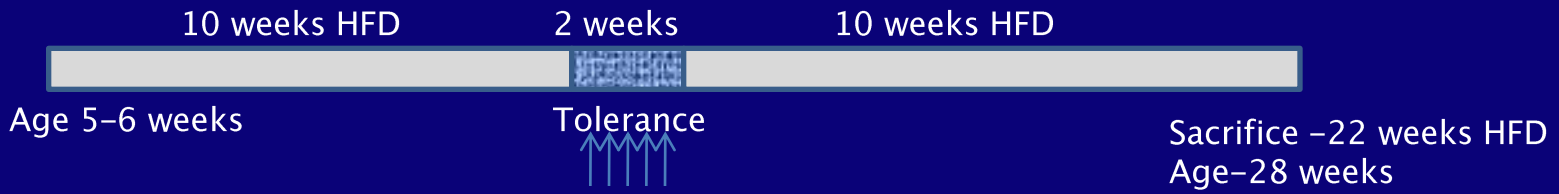
Animal models

- **Mice-Gene knock out C57/BL6**
  - ApoE-/-, LDL r-/-, ApoB48 LDLr-/-)
- Rabbits
  - New Zealand White rabbits

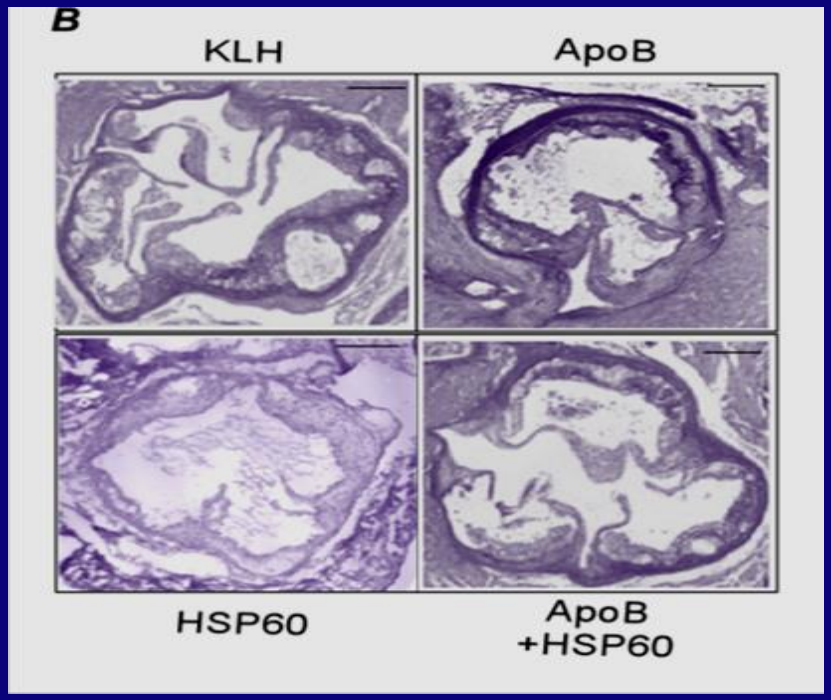
Disease Development

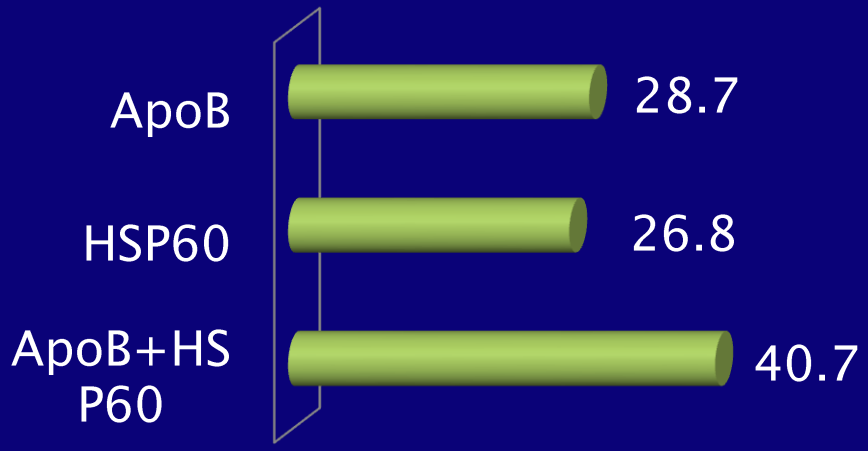


Disease Progression



Combination of peptides –enhanced protection





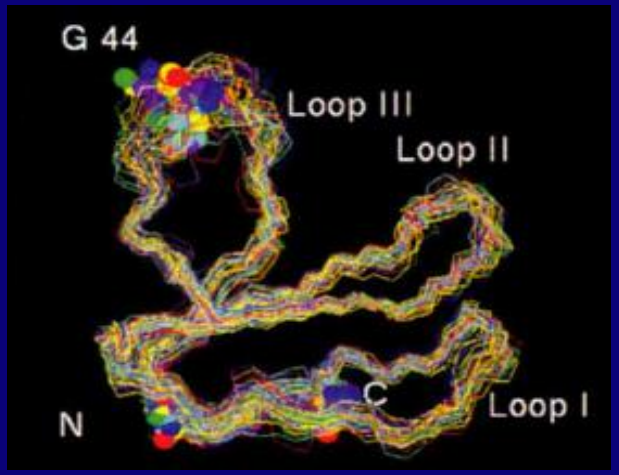
- ApoB : ↓ lipid and macrophage
- HSP60 : reduction in CD4 and SMC

ApoB + HSP60

- Macrophage infiltration (77%) and TNF-α (64%)
- ↑ Treg cells in and TGF-β in plaque
- No difference in lipids levels

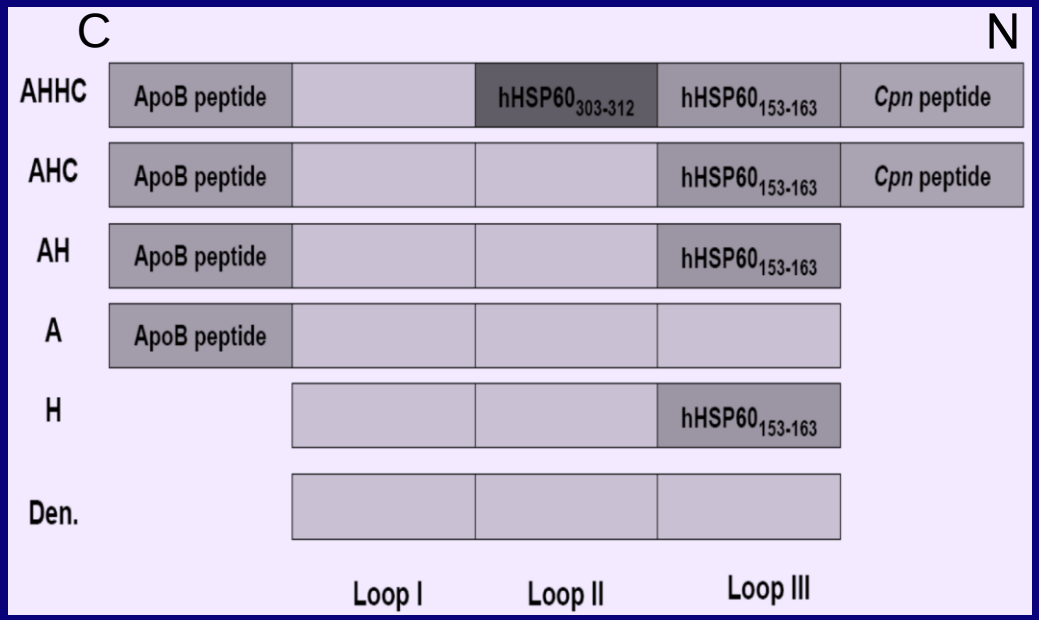
Xinjie et al. Atherosclerosis 2010, *Mundkur et al. J .vaccine 2013, , PLOS one 2013*

Improve efficacy by introducing the peptides into Dendroaspin



Peptides linked to the N –terminal, loop2,3 and C terminal end of dendroaspin

Recombinant construct was expressed in Escherichia coli and purified



AHHC – 42.81 %

AHC – 51.82 %

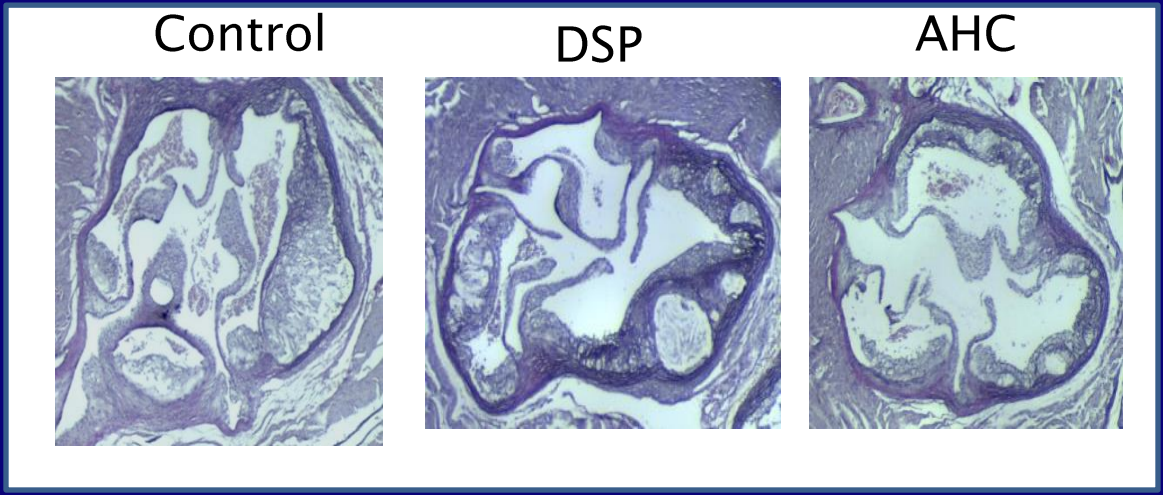
AH – 40.3 %

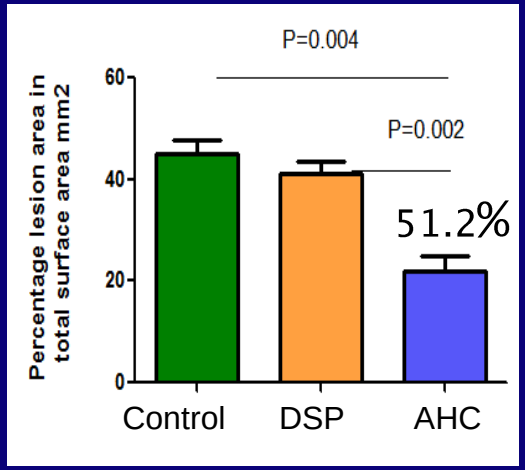
A – 22.8 %

H – 20.8 %

Lu et al Atherosclerosis 2012,

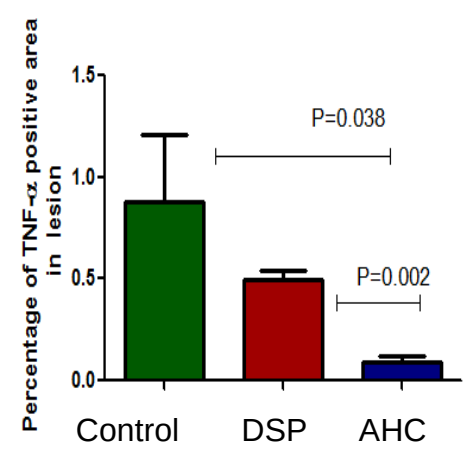
Oral tolerance to AHC



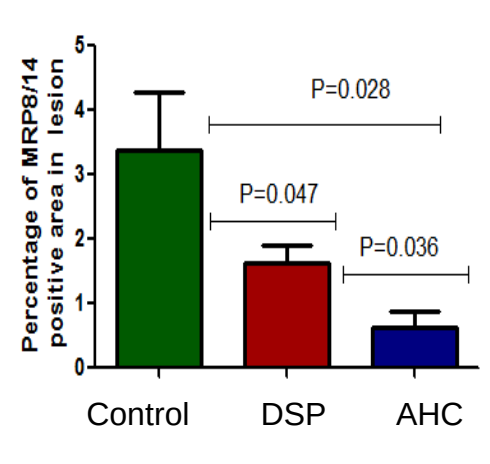


Reduction in inflammatory markers

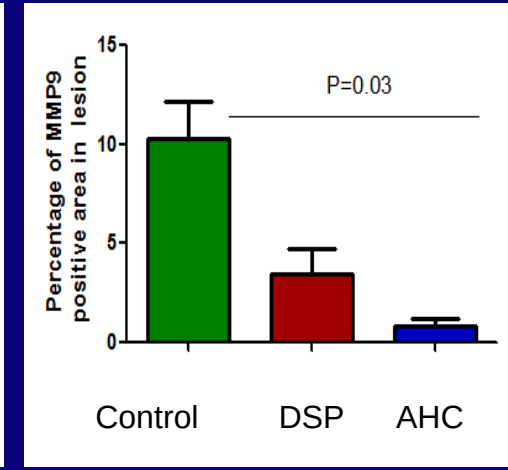
TNF-α – 83.6%



MMP9 - 91.7%



MRP8/14 - 61.2 %

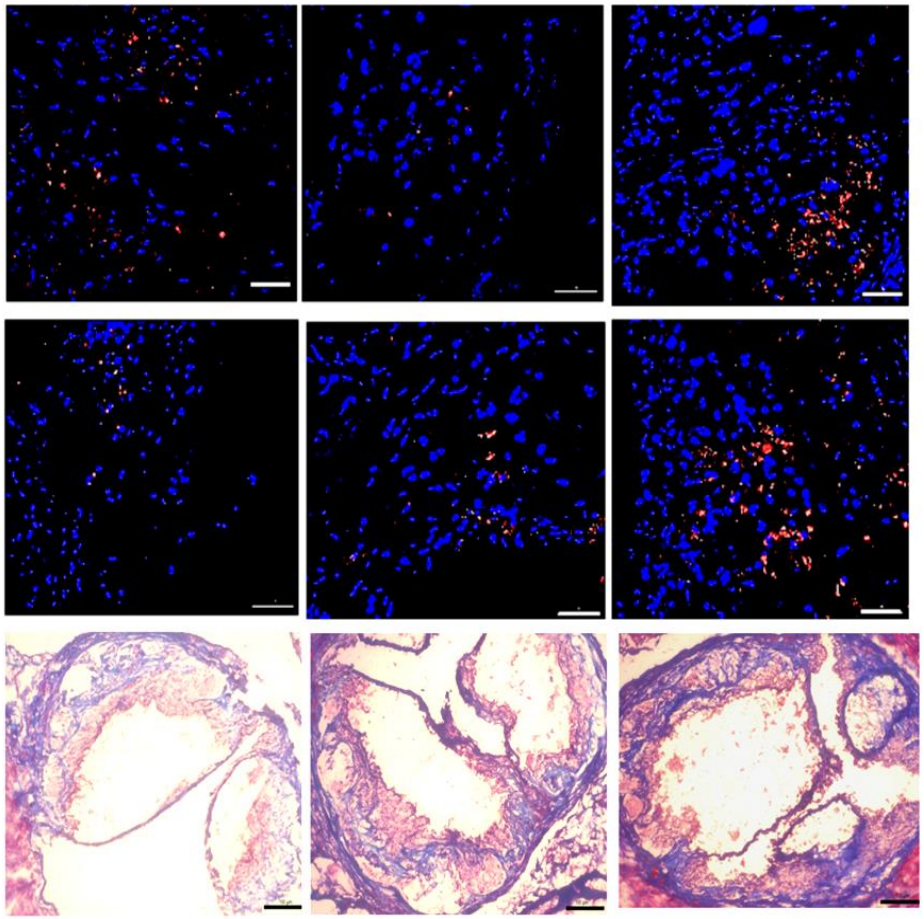


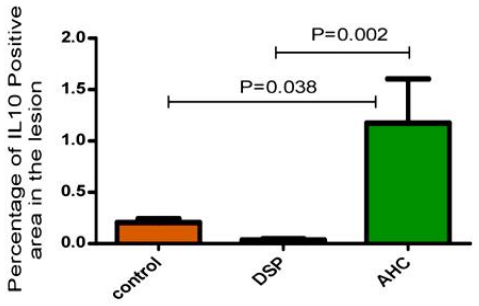
Increase in Expression of regulatory markers in aorta

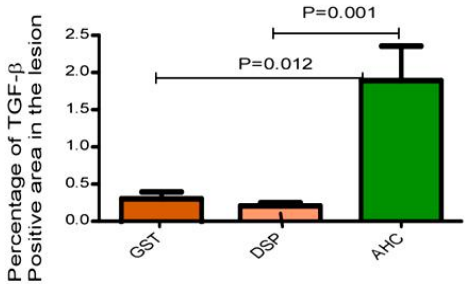
IL10

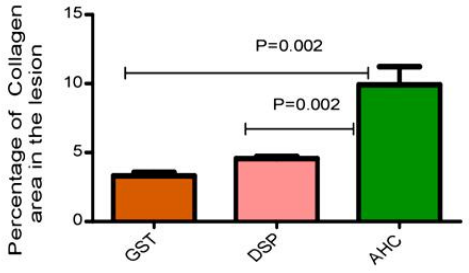
TGF-β

Collagen









Mechanism of Protection

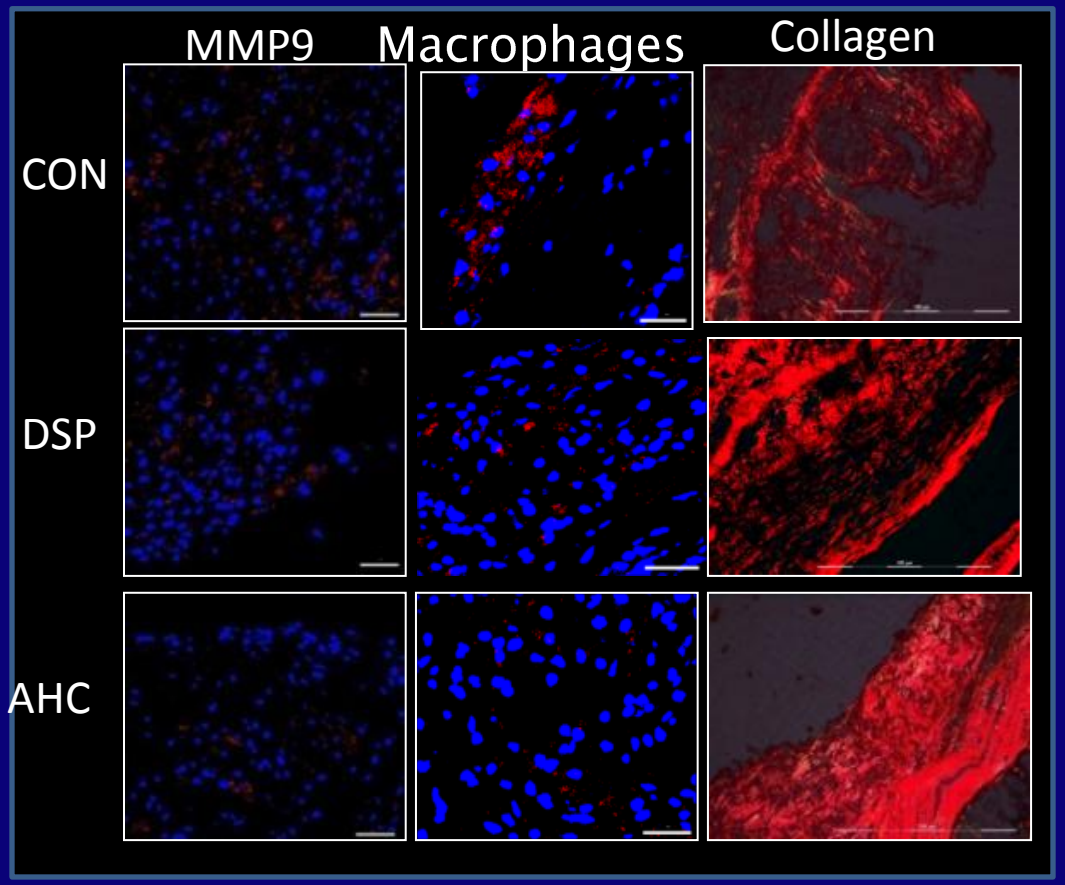
Antigen specific Tolerance to the peptides

- Increase in Foxp3 +ve and CTLA4+ve cells in Lymphoid organs
- Increase in $CD11c^{+}$ $11b^{+}$ 103^{+} tolerogenic Dendritic cells
- Antigen specific regulatory cell function
- Reduction in proliferation of peptide specific T cells by 31.02% for ApoB, 27.6% for HSp60 and 25% for Cpn peptides
- Increase in regulatory cells in aorta
- Reduction in Th17 and Th1 cells in aorta,spleen and peripheral circulation

Mundkur et al Int journal of cardiology 2014

Mundkur et al Int journal of cardiology 2014

Rabbit model of Atherosclerosis



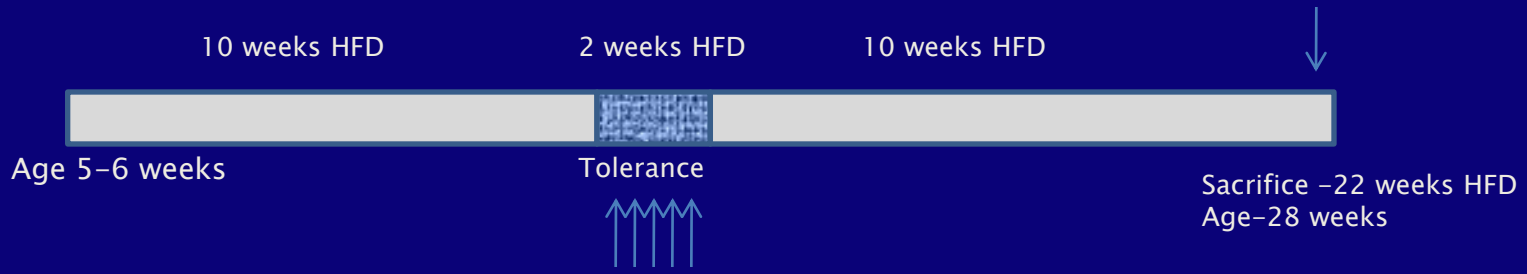
48.6% reduction in lesion
Reduction in

- ❖Macrophage infiltration
- ❖Plaque MMP9
- ❖48.6% in lesion
- ❖Increase in collagen
- ❖Increased Treg activity

Reduction in plaque inflammation and increase in regulatory response

Sheena and Mundkur Canadian journal of cardiology 2015

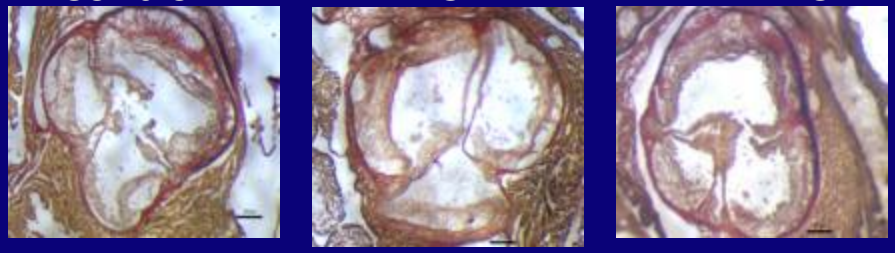
Effect of immune modulation in Disease Progression



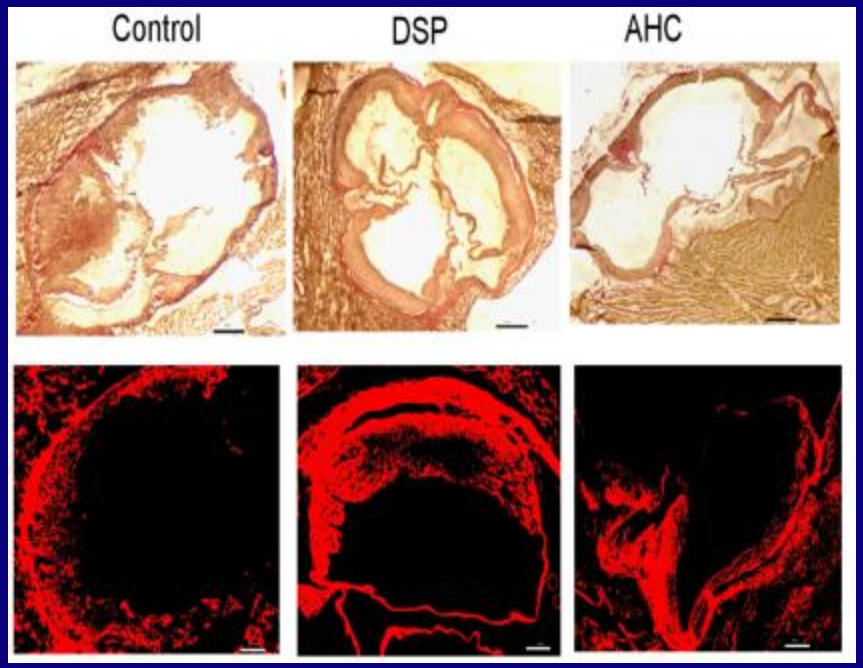
Plaque stabilization

Mice

Control DSP AHC



Rabbits



- Reduction in necrotic core in the lesion (p=0.001)
- Reduction in MMP9 (p=0.006)
- reduction in Apoptosis as observed by TUNEL, (P=0.037)
- Reduction in Tissue factor expression (P<0.001)
- increase in collagen content

AHC treatment showed markers of stabilization in mice

Immune Therapy for Atherosclerosis

Summary

- Reduces development of Atherosclerosis
  - Mice Apob48 /LDLr-/- (51%)
  - Rabbits (48.6%)
- Controls plaque progression
- Induces markers of plaque stabilization in mice and Rabbits
- Reduction in inflammation
- Increase in immune regulatory cells and cytokines in plaque

Preliminary Toxicity studies

Acute toxicity test

- 20×, 100X and 200x administered as a single dose
- Clinical signs of toxicity and mortality were not observed during 15 days

Sub chronic toxicity

- 1X 5 X and 10 X – 5 doses
- No clinical signs of toxicity observed 15 and 30 days

Chronic toxicity

- 1X 5 X for 28 day daily dosing
- No clinical signs of toxicity observed

Immune Therapy for Atherosclerosis

Challenges ahead

- Long term effect of immune therapy?
- Potentiating of immune tolerance?
- Translation in human?
- Delivery methods
- Clinical Development of the molecule

Acknowledgements

Prof V V Kakkar – Scientific Chairman Thrombosis Research Intitute

Prof Xinjie Lu- Collaborator, TRI- London

Scientific Staff- TRI- Bangalore

Department of Biotechnology- Govt .of India- Program Grant

Tata social welfare trust

THANK YOU



Clinical studies

Summary

- Patients with coronary artery disease
- Higher Th17 cells
- Higher IL17 and IL6 levels
- Lower Treg cells
- Imbalance in Th17/Treg ratio
- Th17/Treg ratio showed an association with MI with an odds ratio of 2.92 (95% CI: 1.73-4.92), $P<0.001$
- PBMC showed reactivity to Ox-LDL and HSP60 with an expansion of Th17 cells

Mechanism

?





Immune Tolerance

Regulatory Immune response Treg

High fat diet

Reduced inflammation and Atherosclerosis

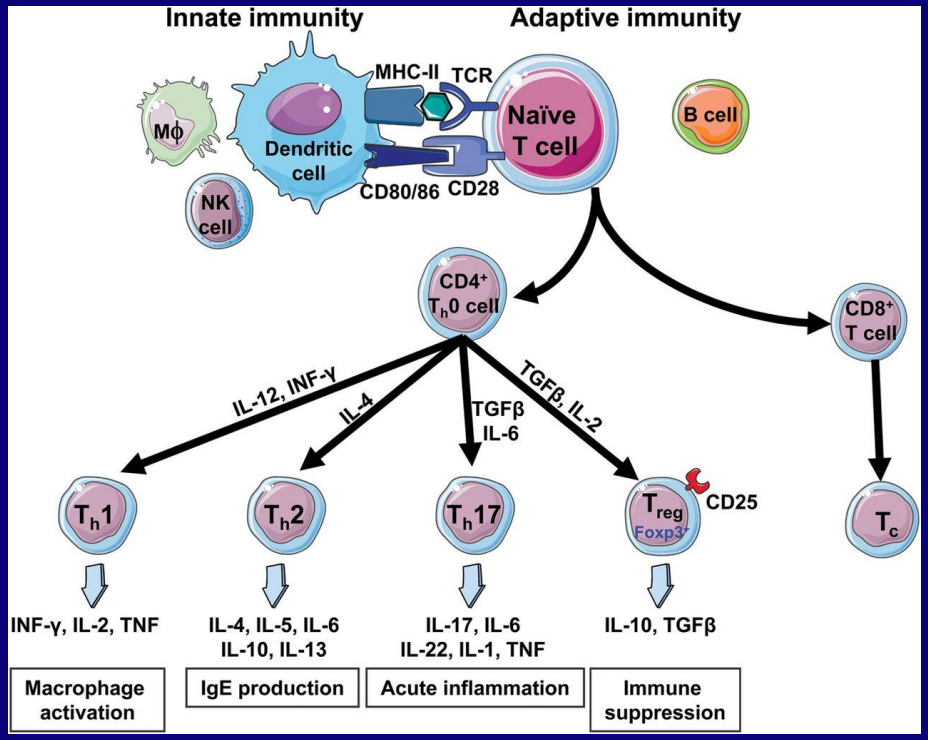
Adaptive immune response to self antigens

Autoimmunity

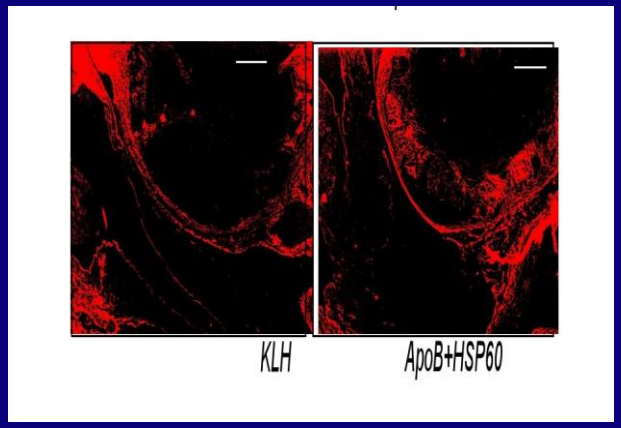
Restoring tolerance

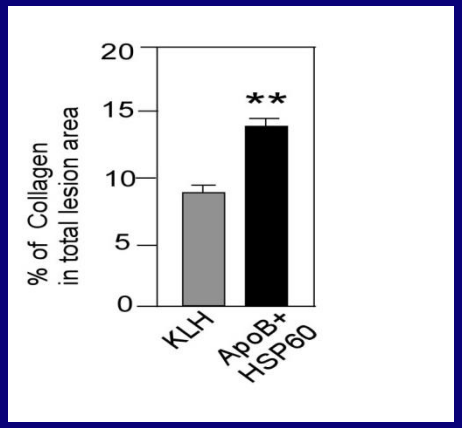
Regulation

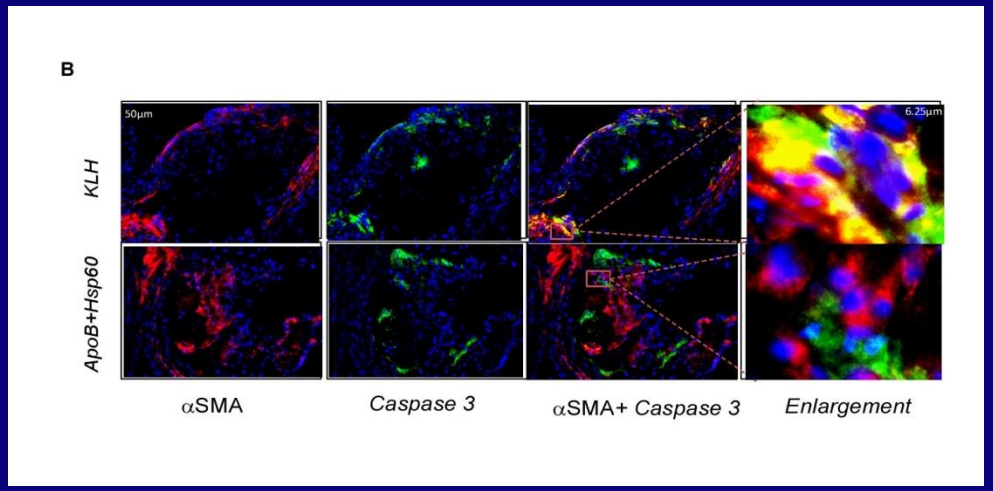
Autoimmune response

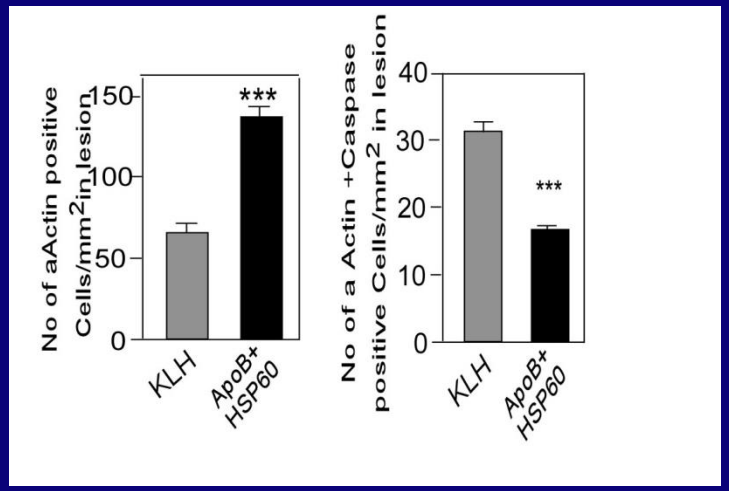

Innate immunity
Adaptive immunity
Mϕ
Dendritic cell
MHC-II
TCR
Naïve T cell
B cell
NK cell
CD80/86
CD28
CD4+ Th0 cell
CD8+ T cell
IL-12, INF-γ
IL-4
TGFβ IL-6
TGFβ, IL-2
Th1
Th2
Th17
Treg Foxp3+
CD25
Tc
INF-γ, IL-2, TNF
IL-4, IL-5, IL-6 IL-10, IL-13
IL-17, IL-6 IL-22, IL-1, TNF
IL-10, TGFβ
Macrophage activation
IgE production
Acute inflammation
Immune suppression

Tolerance to ApoB and HSP60 peptides – Plaque Stabilization









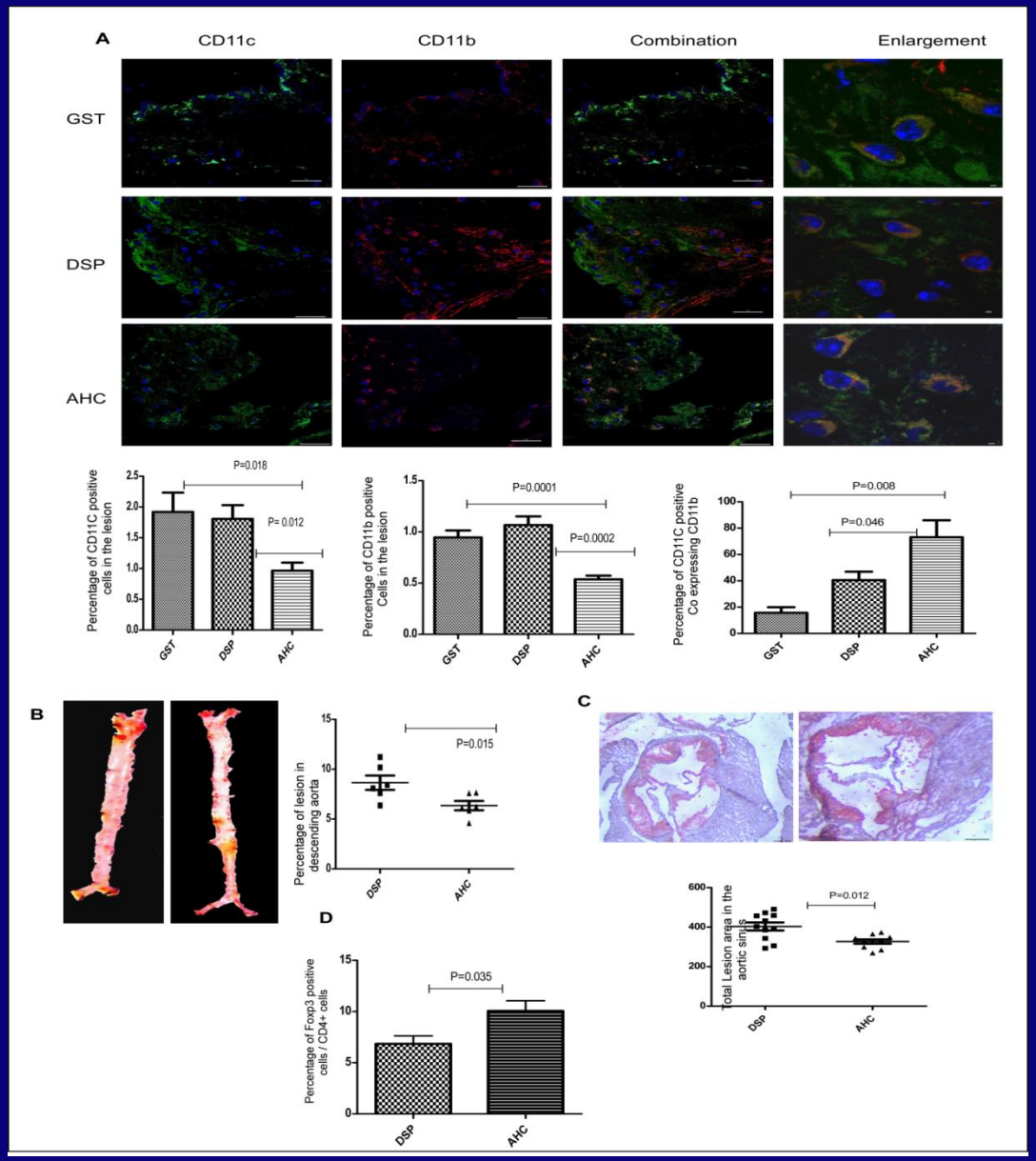
Increase in collagen content

*

Decrease in SMC apoptosis in treated animals

Mundkur et al Plos-One 2013

Oral tolerance to recombinant multi antigenic construct



Increase in $CD11c^{+}CD11b^{+}$ cells in plaque

Increase in Treg markers in plaque

Adoptive transfer of CD11c+ve cells from AHC tolerized mice

18.7 % reduction in lesion in sinus

Mundkur et al Int journal of cardiology 2014

Immunotherapy for Atherosclerosis

Several groups

- Lund University, Sweden
- Cedars-Sinai Heart Institute, USA
- Leiden, University, Netherlands.
- Karolinska Hospital Sweden
- Medical University of Vienna, Austria

- **Therapeutic approach**
  - Protein- peptide
  - DNA vaccination.
  - Passive Immunization.

Selection of antigens.

- Oxidized phospholipids
- bacterial and viral proteins
- Heat shock proteins
- Beta 2-glycoprotein – 1
- Cholesterol ester transfer protein (CETP)

Animal Models

- Plasma cholesterol of mice is primarily packaged in the HDL fraction
- Low ApoB levels
- Elimination of ligand (*Apoe*−/−) or a receptor (*Ldlr*−/−) increase ApoB levels
- High cholesterol diet increases plasma apoB−lipoprotein levels to an even greater degree, −accelerated plaque formation in the major arteries.
- Mice have apoB−48 which can clear lipoproteins other receptors in addition to the LDLR
- ApoB ^{tm2Sgy}/Ldlr $^{tm1Her/J}$ which express only ApoB 100 and are *Ldlr*−/−