Adaptive Immunity, The Janus of Atherosclerosis

Göran K Hansson
Center for Molecular Medicine
Department of Medicine
Karolinska Institute
Stockholm, Sweden
The atherosclerotic plaque – a site of immune inflammation

**Innate immunity**
- Recognition of molecular patterns
- Germline encoded receptors
  - Toll-like, NOD-like receptors, scavenger receptors
- Bind oxidized LDL or components of LDL

**Adaptive immunity**
- Highly precise recognition of specific molecules
- T cells, B cells
  - Recognize LDL as autoantigen
- Sophisticated regulation

Hansson & Hermansson
*Nature Immunol* 2011
The LDL particle - source of cholesterol, trigger of inflammation, target of immune reaction

- Endothelial activation
- Inflammasome activation in macrophage
- B cell response
- T cell response

G Cooper, The Cell
Specificity of LDL reactive T cell clones

T cells recognize apoB100 and native LDL, not oxLDL!
T cell clones were exposed to LDL preparations that had been Cu^{++} oxidized to varying extent

Hermansson, Ketelhuth et al
J Exp Med 2010
Mild oxidation improves LDL uptake in DC; native ApoB sequence is recognized by T cell
A window of mild oxidation permits uptake of LDL into antigen-presenting cells; further oxidation eliminates immunoreactivity.

Hansson & Hermansson, Nature Immunol 2011
T cells with clonotypic TCR recognize apoB100 sequence in native LDL

Transgenic mice generated that express human LDL-specific TCR (TRAV14 TRBV31) in 80% of CD4+ T cells

Libby, Lichtman, Hansson; Immunity 2013
TCR transgenic BT1 cells recognize an ApoB-derived oligopeptide in LDL.

Gisteră, Klement, Mailer et al, unpubl results 2015
Incomplete central tolerance to ApoB100 in mice carrying APOB reactive BT1 T cells

11.8 – 6.9 = 4.9 % of TRBV31+ T cells survive and can react to LDL!

Gisterå, Klement, Mailer et al, unpubl results 2015
Autoimmunity to LDL

- HuLDL/apoB specific T cells proliferate when encountering human LDL
- When encountering HuLDL from early life and onwards, most HuLDL/apoB specific T cells are deleted in thymus
- A small proportion of HuLDL/apoB specific T cells survive this selection
- These T cells are autoreactive and can promote disease development
T effector cell subsets exert different roles in inflammation

**Proatherosclerotic**
- Macrophage activation
- Inflammation

**Atheroprotective**
- Suppression
- Inflammation-repair
- Allergic inflammation

**APC**
- IL-12
- TGF-β
- IFN-γ
- IL-18
- IL-10
- IL-6
- IL-23
- IL-4

**Th1p**
- IFN-γ
- IL-18

**Th1**

**Th2p**
- IL-4

**Th2**

**Treg-p**
- TGF-β
- IL-6

**Treg**

Atheroprotective immunity!?! 

- Immunization with LDL reduces disease
  - oLDL; nLDL; ApoB100
    - Palinski et al PNAS 1995
    - Ameli, Nilsson et al ATVB 1996
- Antiinflammatory cytokines inhibit disease
  - IL-10, TGF-beta
    - Mallat et al, Circ Res 1999
    - Robertson et al, JCI 2003
- Regulatory T cells involved in atherosclerosis
  - Transfer of Treg enriched cell populations reduce disease
  - Vaccination to LDL involves Treg
    - Van Puijvelde et al, Circulation 2006
• Elimination of Treg in a mouse model

• FoxP3 transcription factor specific for Treg
• DEREG mice carry diphtheria toxin receptor under FoxP3 promoter
• Diphtheria toxin injection eliminates Treg!

K Lahl, T Sparwasser et al
Treg elimination leads to increased atherosclerosis in DEREG Ldlr^{-/-} chimeras

Klingenberg, Gerdes et al
J Clin Invest 2013
Elimination of Treg raises plasma cholesterol

Body weight, lipid profile and hematological parameters

<table>
<thead>
<tr>
<th>Treg</th>
<th>+</th>
<th>-</th>
<th>n.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>25.7 ± 0.6</td>
<td>24.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol level (mg/dL)</td>
<td>676 ± 82</td>
<td>1044 ± 122</td>
<td>*</td>
</tr>
<tr>
<td>Triglyceride level (mg/dL)</td>
<td>168 ± 31</td>
<td>156 ± 25</td>
<td>n.s.</td>
</tr>
<tr>
<td>Leukocytes (10^9/µL)</td>
<td>12.3 ± 0.7</td>
<td>14.9 ± 1.2</td>
<td>*</td>
</tr>
<tr>
<td>Monocytes (10^3/µL)</td>
<td>0.8 ± 0.2</td>
<td>2.1 ± 0.3</td>
<td>*</td>
</tr>
<tr>
<td>Lymphocytes (10^3/µL)</td>
<td>9.1 ± 0.9</td>
<td>11.2 ± 1.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Erythrocytes (10^6/µL)</td>
<td>9.2 ± 0.3</td>
<td>9.6 ± 0.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Platelets (10^3/µL)</td>
<td>1156 ± 95</td>
<td>960 ± 83</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

All values are shown as mean ± SEM. * = significant, n.s. = not significant.

Klingenberg, Gerdes et al
J Clin Invest 2013
Increased VLDL cholesterol after Treg elimination

Klingenberg, Gerdes et al
J Clin Invest 2013
Reduced clearance of VLDL and CM when Treg are depleted

* Reduced clearance of VLDL
* Reduced clearance of CM

** Hepatic uptake of Chylomicron $[^{14}C]$retinol

- FITC-VLDL (% Fluorescence)
  - PBS
  - DT

- DPM $g^{-1}$
  - PBS
  - DT

- Chylomicron $[^{14}C]$retinol clearance

- Hepatic uptake of Chylomicron $[^{14}C]$retinol
Immune regulation of lipoprotein metabolism

- Hampered VLDL clearance
- Increased plasma cholesterol
- Increased atherosclerosis

Klingenberg, Gerdes et al
J Clin Invest 2013
T effector cell subsets exert different roles in inflammation

- Macrophage activation
- Inflammation
- Suppression
- Proatherosclerotic
  - Macrophage activation
  - Inflammation
- Atheroprotective
  - Suppression
- ?
  - Inflammation-repair
- Allergic inflammation

Enhanced Th17 development increases fibrous cap of atherosclerotic lesions

Reversed by treatment with anti-IL17A antibodies

Gisterå et al, Science Transl Med 2013
IL-17A stimulates collagen production by human arterial smooth muscle cells

Gisterà et al., Transforming growth factor-β signaling in T cells promotes stabilization of atherosclerotic plaques through an interleukin-17 dependent pathway, *Science Translational Medicine* 5, 196ra100 (2013)
IL-17A and RORγt mRNA positively associate with fibrous cap markers in human atherosclerosis*

Data from Biobank of Karolinska Endarterectomy

Gisterå et al., Transforming growth factor-β signaling in T cells promotes stabilization of atherosclerotic plaques through an interleukin-17 dependent pathway, *Science Translational Medicine* 5, 196ra100 (2013)
Th17 cells stabilize plaques by secreting the fibrogenic cytokine, IL-17A

Gisterå et al, Science Transl Med 2013
T effector cell subsets exert different roles in vascular inflammation

- Th1p
  - IL-12
  - IFN-γ
  - IL-18
  - Th1
- Th1
  - Proatherosclerotic
  - Macrophage activation
  - Inflammation
- TGF-β
  - Treg-p
  - Treg
  - Treg
- IL-6
  - IL-23
  - Th17
  - Atheroprotective
  - Suppression
  - Inflammation-repair
- IL-4
  - Th2p
  - Th2
  - Plaque stabilizing
  - Inflammation-repair
  - Allergic inflammation

What about B cells?

- Antibodies to oxLDL in patients
- Natural antibodies to ox-phospholipids may be protective
- B cells in germinal centers around atherosclerotic arteries
- B cell transfer inhibits disease
Apoe-/- mice were splenectomized, then “rescued” with cells from young Apoe-/- mice or old, atherosclerotic Apoe-/- mice.

*Caligiuri et al, JCI 2002*
T-cell dependent, high affinity IgG antibody + memory

FOB

B cell

Memory B cell (BCL-6(low))

GC (BCL-6^{hi})

Pre-plasmablast

Plasmablasts (short lived)

Plasma cells (long lived)

T cell precursor

MZB

B-1

Natural antibodies

Natural antibodies

PAX5 (function)

IRF4^{low} XBP1^{low}

IRF4^{hi} XBP1^{hi}

Nature Reviews Immunology
MZ B cells and germinal centers increase in atherosclerosis

Grasset, Karlsson et al PNAS 2015
oxLDL accumulates in the marginal zone of the spleen

Accumulation in marginal zone B cells and macrophages

Grasset, Karlsson et al PNAS 2015
Atheroprotective humoral immunity by sterile inflammation that induces atheroprotective anti-PC antibodies.
# Immunology of atherosclerosis

## Atherogenic

- Innate immunity
  - M1 macrophages
- Th1 adaptive immunity
  - Th1 cells
- Proinflammatory cytokines
- Proinflammatory eicosanoids
- CD8+ T cells
- B cell subsets?

## Atheroprotective

- Treg cells
  - IL-10, TGF-beta
  - Reduces inflammation and cholesterol metabolism
- Th17 cells
  - Profibrotic, plaque stabilizing
- Humoral immunity to LDL
  - MZB cells, B1 cells
  - Lowers LDL cholesterol
  - Sterile inflammation
Former fellows:
Pina Caligiuri
Antonino Nicoletti
Andreas Hermansson
Norbert Gerdes
Roland Klingenberg
Anna-Karin Robertson

Collaborators:
Tim Sparwasser
Mats Rudling
Stefan Nilsson
Richard Flavell
Emilie Grasset
Mikael Karlsson

Funding:
Swedish Research Council
Heart-Lung Foundation
Foundation for Strategic Research
European Union
(AtheroRemo, Atheroflux, VIA)

Cardiovascular Research Laboratory
Center for Molecular Medicine, Karolinska Institutet