At the crossroads of immunology and atherosclerosis – clinical implications

Roland Klingenberg, MD, FESC
Conflict of Interest - Disclosures

There are no financial interests to report within the past 12 months that may pose a potential conflicts of interest.

Current employer: Kerckhoff-Klinik, Bad Nauheim, Germany
Diversity of immune cells in atherosclerosis

CD4+ T helper cell subsets

- Th17 cells – dual role
- Th1 cells – pro-atherogenic
- Th2 cells – dual role
- Treg cells – anti-atherogenic


CD4+ Th1 cell subset in atherosclerosis

- Major T cell subset in atherosclerotic lesions

- Bona fide transcription factor T-bet and signature cytokine IFN-γ
- Drives arterial inflammation and promotes lesion progression

- Signalling via the CD40 costimulation pathway is essential for the development of atherosclerotic lesions

- Differentiation into the Th1 cell subset is promoted by antigen presentation through APC in the context of a CD40-CD40L costimulatory signal
Regulatory T cells come in distinct flavors

**Natural**

- Thymic
  - CD4+ CD25+ Foxp3+

**Antigen-induced Treg cells in atherosclerosis**

- TGF-β
  - CD4+ Foxp3+
- Low-dose antigen
  - CD4+ Foxp3+
- Pathogen
  - IL-10
  - CD4+ IL-10
  - CD4
  - CD8
  - DN

Modified from Shevach EM. Immunity. 2006; 25:195-201

- Klingenberg R, et al.
  - JCI 2013; 123:1323-1334

**CD4+ CD25+ natural Treg**
- Ait-Outella H, et al.
- Mor A, et al.
  - ATVB. 2007; 27:893-900

**BUT: CAUSALITY (Foxp3)?**

**MECHANISM OF ACTION?**

**Transcription factor FoxP3:**
- Key lineage marker and master switch in regulation and development

- Fontenot, JD, et al.
- Hori, S, et al.
  - Science. 2003; 299:1057-1061
- Khattri, R, et al.
  - Nat Immunol. 2003; 4:337-342
Depletion of transgenic Treg cells propagates atherosclerosis

Increased necrotic core with few cells but no increase in vascular inflammation

Depletion of Treg cells promotes hypercholesterolemia (VLDL/CMR fraction)

Impaired VLDL/CMR lipoprotein clearance upon Treg cell-depletion

Increased activity of LPL, HL and PLTP, decreased Sort1 protein

Plaque regression upon natural Treg expansion by anti-CD3 Ab in Ldlr−/− mice

1) Administration of anti-CD3 Ab mediated:
   - Increase in Treg cells in spleen, lymph node and atherosclerotic lesion
   - Decrease in macrophages in atherosclerotic lesion
   - Increase in collagen in atherosclerotic lesion

2) Depletion of Treg cells by co-administration of anti-CD25Ab:
   - Abrogated effect of anti-CD3 Ab on lesion regression

Increased infarct size in Treg-cell–ablated Foxp3DTR mice compared with WT

Expansion of natural Treg cells improves survival and reduces LV ruptures

Diversity of regulatory T cells in atherosclerosis

**Natural**

**Antigen-induced Treg cells by vaccination**

- **Thymic**
  - CD4$^+$ Foxp3$^+$

- **TGF-β**
  - CD4$^+$ Foxp3$^+$

- **Low-dose antigen**
  - CD4$^+$ Foxp3$^+$

- **Pathogen**
  - IL-10
  - CD4$^+$ IL-10

Modified from Shevach EM. Immunity. 2006; 25:195-201

All used apoB-100 peptide p210 as vaccine antigen

BUT: different route and adjuvants effecting distinct mechanism of action
Intranasal p210-CTB reduces atherosclerosis in apoe−/− mice

Increased IL-10+ Tr1 (Treg) cells and apoB-100-specific Treg cell activity in spleen

Human setting – Treg cells in clinical atherosclerosis

Clinical data are scarce:


Thrombus and blood to analyze T cell subsets and Antigen-specificity in ACS

Thrombus

FACS analysis

DNA analysis

+ peripheral blood

Identification of V-J rearrangements at the genomic level
276 gene segment combinations possible = 100%

V-J gene segments encode variable chains
one cell = one rearrangement

Analysis: ImmunTraCkeR® PCR technology
Predominance of Treg cells in coronary thrombi vs. peripheral blood in ACS

Klingenberg R et al. Eur Heart J. 2015; 36:1041-8
Reduced TCR diversity in coronary thrombus vs. peripheral blood in ACS

Intra-individual comparisons of TCR diversity expressed as percentage of 276 possible V(D)J gene segment rearrangements in the human TCR β chain (hTRB)

Klingenberg R et al. Eur Heart J. 2015; 36:1041-8
Reduced TCR diversity in peripheral blood (ACS vs. healthy)

Klingenberg R et al. Eur Heart J. 2015; 36:1041-8
Approaches how to enhance inherent atheroprotective immunity (Treg cells)

Macrophages
T lymphocytes
Dendritic cells

Expansion of Treg cells in vivo:
- Vaccination

Clinical stage
Asymptomatic
Asymptomatic or angina (CCS I-IV)
ACS

1° prevention

2° prevention

Anti-inflammatory therapies:
Current:
Statins
Experimental:
Antigen-specific expansion in vivo:
- Vaccination

Expansion of Treg cells in vivo:
CLINICAL
- IL-2 (Saadoun D, et al. NEJM 2011;365:2067-2077)

EXPERIMENTAL
- Anti-CD3 antibody therapy
- Modulation of co-stimulatory molecules (ICOS, PD-1, OX40L, and CD137)
- Cytokine administration (IL-10, IL-2)
- Calcitriol (Vitamin D3)
- Viral proteins (Measles virus, BCG)
- Vaccination (antigen-specific)

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