Presenter Disclosure Information Elements

Esther Lutgens, MD PhD
Immune checkpoint regulators in Atherosclerosis

FINANCIAL DISCLOSURE:
No relevant financial relationship exists
Jeffrey M. Hoeg (1952-1998)

Great Scientist & clinician

Superb mentor

Active member of many societies
(ATVB, AAAS, ACC)
Immune Checkpoint Regulators in Atherosclerosis

Esther Lutgens, MD PhD

Dept of Medical Biochemistry
University of Amsterdam, the Netherlands
E.Lutgens@amc.uva.nl

Institute for Cardiovascular Prevention (IPEK)
LMU, Munich, Germany
Esther.Lutgens@med-uni-muenchen.de

ATVB meeting, May 5-7, 2016, Nashville, TN, USA
Atherosclerosis is a lipid-driven immune disease

Cholesterol- Triglycerides

Immune system

IMMUNE CHECKPOINT REGULATORS
Co-stimulatory molecules
Co-stimulation warrants proper immune reactions

- Signal 2 in T-cell/APC interactions: proliferation and polarisation
- Endothelial cell activation
- Platelet activation
CD40L-CD40 interactions drive atherosclerosis

Inhibition of CD40L or CD40 as therapy for atherosclerosis??

Short term anti-CD40(L) antibody treatment has been tested in phase I/II trials in MS, Crohn’s disease, and (hematologic) malignancies

but..

Long-term blockage of CD40-CD40L will result in immune-suppression…
CD40-CD40L as therapeutic target in atherosclerosis: knowledge gaps

• Which *signaling pathways* are involved?

• Which *cell types* that express CD40(L) are involved in atherosclerosis?

• How does the *co-stimulatory interactome* work in atherosclerosis?
SIGNALING
Identification of CD40-downstream pathways in vascular disease
CD40-TRAF interactions: mouse model
CD40-TRAF6 signaling in MHCII+ cells drives atherosclerosis
CD40-TRAF interactions in atherosclerosis

MHCIId dependent CD40-TRAF6, but not CD40-TRAF2/3/5 signaling inhibits atherosclerosis

CD40-TRAF6 deficiency omits the Ly6C<sup>high</sup> monocyte population and polarizes macrophages towards an alternatively activated anti-inflammatory phenotype.

CD40-TRAF6 but not CD40-TRAF2/3/5 interactions drive macrophage activation
CD40-TRAF6: a novel therapeutic target?
Top-SMIs: TRAF-STOP

TRAF-STOPS decrease CD40-induced monocyte recruitment and macrophage activation
TRAF-STOPs reduce established atherosclerosis

ApoE-/-> 6 wks  ApoE-/-> 22 wks  ApoE-/-> 30 wks

TRAF-STOP treatment

TRAF-STOP: delayed treatment

NaCl  Vehicle  6877002  6860766

6877002  6860766
TRAF-STOP rHDL-nanoparticles

\[ \text{[Gd-dye-S]-rHDL} + \text{[S]-rHDL} + \text{rHDL} = \text{TRAF-STOP-rHDL} \]
Does TRAF-STOP-HDL treatment reduce atherosclerosis???

ApoE--/
6 wks

ApoE--/
12 wks

ApoE--/
18 wks

TRAF-STOP-rHDL treatment

Seijkens et al.. In preparation
Does TRAF-STOP-rHDL treatment reduce established atherosclerosis???

FMT-CT: Protease activity

Macrophage counts (FACS)

Lameijer, Duivenvoorde, Nahrendorf, Mulder et al.. In preparation
Conclusions

• Macrophage CD40-TRAF6 interactions drive atherosclerosis

• Small molecule mediated inhibition (nanoparticles) of CD40-TRAF6 interactions is a promising therapeutic strategy for the treatment of atherosclerosis

• ...but also obesity, EAE, sepsis, peritonitis

• Promising future for TRAF-STOP
CELLS
<table>
<thead>
<tr>
<th>(Plaque) cell type</th>
<th>CD40L</th>
<th>CD40</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>B-cell</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>DC</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>macrophage</td>
<td>+-</td>
<td>++</td>
</tr>
<tr>
<td>platelet</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>VSMC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endothelial cell</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Bone marrow transplantation
Adoptive transfer
Transgenic mice

---

\[\text{CD40}^{\text{fl}fl}\]  
\[\text{CD40l}^{\text{fl}fl}\]
Monocytes/macrophages

CD40 goes innate....
CD40 and macrophages

Ldlr<sup>−/−</sup> cd40<sup>−/−</sup> or wt Ldlr<sup>−/−</sup> chimeras

Illumina mouse WG-6 arrays

FGK-stimulated vs control foam cells

FGK (CD40 activation)

macrophages

foam cells

Isolation and culture of BMDMs

oxLDL

oxLDL + FGK (CD40 activation)

M1 polarization

CD40

TRAF6

IL-10

IL-12

TNFα

MMP9

Isolation and culture of BMDMs

- FGK

(CD40 activation)

oxLDL

oxLDL + FGK

(CD40 activation)
Macrophage CD40 activates the inflammasome

Shami, van Tiel, unpublished
Dendritic cells
**CD40: dendritic cells**

Constutively active CD40 signaling in DCs

DC-CD40ca

DC-CD40ca-ldlr-/- chimeras

Wt-ldlr-/- chimeras

![Graphs showing CD40 expression and other measurements](image)
Constitutive activation of DC CD40

CD40wt

DC-CD40ca

Colitis!!!

CD40wt

DC-CD40ca

neutrophilia

CD40wt

cd11c-LMP

DC-CD40ca

Cholesterol (mmol/L)

CD40wt

DC-CD40ca

Plaque area (μm²)

CD40wt

DC-CD40ca

Colitis!!!
Deficiency of CD40 on DCs

Shift from Th1 to Th2
CD40L: T cell
CD40L$^+$ T cells: of importance in CVD?

No effect of hematopoietic CD40L on atherosclerosis !?!?!?

Smook, Atherosclerosis 2005, Bavendieck, 2005
CD40L mediates homing of HSPCs!!

Cell cycle analysis of Lin- cells

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G0/1</td>
<td>80%</td>
</tr>
<tr>
<td>S</td>
<td>20%</td>
</tr>
<tr>
<td>G2/M</td>
<td>0%</td>
</tr>
</tbody>
</table>

Survival after 5-FU treatment

- **WT**
- **CD40L-/-**

Survival (%)

Survival over time.
Inflammatory stimuli: ↑ CD40L expression

CD40

Activation of mature immune cells

Immune activation and inflammatory disease

CD40 on HSPCs

WT

CD40−/−

Adhesion (% of control)

0

50

100

150

CD40 on BM stroma

WT

CD40−/−

Adhesion (% of control)

0

50

100

150

Non-classical receptor

Adhesion-mediated quiescence of HSPC

Securing long term hematopoiesis

Independent of CD40!!!
T cell CD40L drives atherosclerosis

Plaque area

\[ \text{Plaque area} \]

\[ \text{cd4cre}^+\text{cd40}^{cre}\text{fl}^+\text{ApoE}^{-/-} \]

\[ \text{cd40}^{cre}\text{fl}^+\text{ApoE}^{-/-} \]

\[ \text{cd4cre}^+\text{cd40}^{cre}\text{fl}^+\text{ApoE}^{-/-} \]

\[ \text{cd40}^{cre}\text{fl}^+\text{ApoE}^{-/-} \]

\[ \text{cd4cre}^+\text{cd40}^{cre}\text{fl}^+\text{ApoE}^{-/-} \]

CD4\(^+\) cells per plaque

\[ \text{CD4}\(^+\) cells per plaque} \]

% CD4\(^+\) cells of cells per plaque

\[ \% \text{CD4}\(^+\) \text{cells of cells per plaque} \]

Th1 response, aorta

\[ \text{Th1 response, aorta} \]

Relative mRNA expression

\[ \text{Relative mRNA expression} \]

\[ \text{CD4} \]

\[ \text{IL2} \]

\[ \text{IL12} \]

\[ \text{IL1}\beta \]

\[ \text{CD69} \]

\[ \text{INF}\gamma \]

\[ \text{TNF}\alpha \]

Bürger, Gerdes, unpublished
CD40L & CD40: platelets
Platelets participate in inflammation by expression of cell surface molecules and secretion of soluble mediators.
Platelet CD40L mediates platelet aggregation via PI3kβ

A

Pik3cb WT/WT

Pik3cb R/R

10% 1 min

+ CD40L

+ CD40L

B

% Transmission

coll 5

+ CD40L

coll 0.5

coll 5

coll 0.5

C

Pik3cb WT/WT

Pik3cb R/R

P-Ser473

Total Akt

Relative P-Akt [Ser473]

Unstim

Coll 5

Coll 0.5

Coll 0.5 + CD40L

Unstim

Coll 5

Coll 0.5

Coll 0.5 + CD40L

Kuijpers, ATVB 2015
Platelet CD40L contributes to the progression of established plaques
Platelets participate in inflammation by expression of cell surface molecules and secretion of soluble mediators.
CD40-deficient platelets exhibit normal platelet activation secrete less PF4/CXCL4, and impair PLA formation

Gerdes et al., ATVB, 2016
Platelet transfusion model

Donor

Apoe\(^{-/-}\)

Cd40\(^{-/-}\)-Apoe\(^{-/-}\)

Recipient

i.v. every 5 d, for 12 wks.

3x10\(^7\) thrombin-activated platelets

Gerdes et al., ATVB, 2016
Platelet CD40 deficiency reduces endothelial activation and neutrophil recruitment

In vitro adhesion assay

In vitro EC mRNA expression

Gerdes et al., ATVB, 2016
Platelet CD40 contributes to atherosclerosis

Aortic arch plaque area (x 10^9 μm²)

Baseline | Apoe^−/− | Cd40^−/− Apoe^−/− | Vehicle
---|---|---|---

Baseline

Apoe^−/−

Cd40^−/− Apoe^−/−

Vehicle

Ascending aorta plaque area (x 10^6 μm²)

Baseline | Apoe^−/− | Cd40^−/− Apoe^−/− | Vehicle
---|---|---|---

n.d.

Baseline

Apoe^−/−

Cd40^−/− Apoe^−/−

Vehicle

Gerdes, ATVB 2016
INTERACTOMES OF IMMUNE CHECKPOINT INHIBITORS
My “Jeffrey Hoegs”

Prof. Mat Daemen

Prof. Christian Weber
My Scientific BFFs

Prof. Menno de Winther

Dr. Norbert Gerdes
The Amsterdam Laboratory
The Munich Laboratory
My family
Acknowledgements

Academic Medical Center, AMC Amsterdam
Tom Seijkens
Svenja Meiler
Esther Smeets
Pascal Kusters
Claudia van Tiel
Patrick Burger
Linda Beckers
Myrthe den Toom
Suzanne Aarts
Susan van den Berg
Annelie Shami
Matthijs Janssen
Marion Gijbels
Menno de Winther
Noam Zelcer

IPEK, LMU, Munich
Norbert Gerdes
Holger Winkels
Christina Bürger
Charlotte Spitz
Maiwand Ahmadsei
Sigrid Reim

Sanquin, Amsterdam
Jaap van Buul
Peter Hordijk
Martijn Nolte

Bioceros BV, Utrecht
Louis Boon

Radboud UMC, Nijmegen
Gert Vriend
Sander Nabuurs

Icahn School of Medicine, Mount Sinai, NY, USA
Jun Tan
Francois Fay
Willem Mulder

NKI, Amsterdam
Jannie Borst

VU, Amsterdam
Christien Dijkstra
Gijs Kooij

CARIM, University of Maastricht
Barbara Zarzycka
Gerry Nicolaes

European Research Council
Netherlands Organisation for Scientific Research
Hartstichting

Deutsche Forschungsgemeinschaft
Alexander von Humboldt Stiftung/Foundation