Presenter Disclosure Information Elements

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Atherosclerosis: Targeting the immune system

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Atherosclerosis is a lipid-driven immune disease

Modulation of immune system

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


Lutgens et al, J Exp Med 2010
Inhibition of CD40L or CD40 as therapy for atherosclerosis??

CD40L-CD40 actions are crucial for maintaining proper immunity.

Short term 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...
Identification of CD40-downstream pathways in vascular disease
..some focus was needed
..and a model to study CD40-TRAF interactions
CD40-TRAF6 interactions drive atherosclerosis...

Lutgens et al, J Exp Med 2010
...by reducing leukocyte recruitment and by inducing anti-inflammatory macrophages ...
CD40-TRAF interactions in atherosclerosis

Leukocyte 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.

Lutgens et al JEM 2010
Identifying TRAF6-CD40 interactions
Virtual ligand screen and validation

Chatzigeorgiou, Seijkens et al. PNAS 2014; Zarzyka et al. J Chem Inf Mod 2015

ChemBridge collection
400,000 unique compounds

ADME/tox filtering

Filtered database
271,759 compounds

3D Multiple conformer generation

1.36*10^7 conformers

Rigid docking

40,000 compounds

Flexible docking

800 compounds

Cell-based assay

>50% of inhibition at 10 μM

51 hits

b  IL1b

Fold Induction

Concentration (μM)

0  0.001  0.01  0.1  1  10  100

0.00  0.25  0.50  0.75  1.00  1.25

b  IL6

Fold Induction

Concentration (μM)

0  0.001  0.01  0.1  1  10  100

0.00  0.25  0.50  0.75  1.00  1.25

6877002

6860766

6877002

6860766

6877002

500 μM

300 μM

150 μM

75 μM

37.5 μM

18.8 μM

9.4 μM

100 μM

50 μM

25 μM

12.5 μM

6.3 μM

3.1 μM

1.6 μM
Top-SMIs: TRAF-STOP

Chatzigeorgiou, Seijkens et al. PNAS 2014; Zarzyka et al. J Chem Inf Mod
TRAF-STOPs decrease CD40-induced macrophage inflammation

![Graphs showing the fold induction of CCL-2, CCR-2, CCL5, and CCR-5](image)

- **CCL-2**: Vehicle vs. 6877002 vs. 6860766
- **CCR-2**: Vehicle vs. 6877002 vs. 6860766
- **CCL5**: Vehicle vs. 6877002 vs. 6860766
- **CCR-5**: Vehicle vs. 6877002 vs. 6860766
TRAF-STOPs decrease CD40-induced macrophage inflammation
TRAF-STOPs decrease myeloid cell recruitment....
....decrease atherosclerosis....
….and plaque inflammation.

- CD45+ cells
- T-cells
- Macrophages
- Neutrophils
TRAF-STOPs also reduce existing atherosclerosis

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

TRAF-STOP treatment

TRAF-STOP: delayed treatment

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<th>NaCl</th>
<th>Vehicle</th>
<th>6877002</th>
<th>6860766</th>
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NaCl
Vehicle
6877002
6860766
Atherosclerosis

Intracellular

CD40

Extracellular

CD40L

SMI

TRAF6

NFκB

Leanocyte recruitment
Foam cell formation
Cytokines

Atherosclerosis
Treatment in patients???
TRAF-STOP HDL-nanoparticles

\[ \text{[Gd-dye-S]-rHDL} + \text{[S]:r-IDL} + \text{[rHDL]} = \text{Dio-6877002-HDL} \]

Apoe-/- mice, 18 wks NC, 24 hrs after treatment

W. Mulder, MSSM, NYC
TRAF-STOP-HDL in myeloid cells

- **Ly6C\(^{hi}\) monocyte**
- **Ly6C\(^{lo}\) monocyte**
- **Neutrophil**
- **Macrophages**

**Blood**

**Spleen**

PBS

DiO-6877002-rHDL
TRAF-STOP-HDL in aortic root and aorta
TRAF-STOP-HDL targets plaque macrophages

![Images showing HDL targeting macrophages in plaque]

Nuclei
HDL
MΦ
HDL
Nuclei
MΦ
HDL

200 μm

Monocytes
Macrophages

Aorta

Count

DiO-x-HDL

DiO-6877002-rHDL
TRAF-STOP-HDL preferentially arrives at the aorta
Does TRAF-STOP-HDL treatment reduce atherosclerosis???
Conclusions

• CD40L-CD40-TRAF signaling is an important immune-modulatory pathway
• Atherosclerosis is dependent on CD40-TRAF6 interactions
• CD40-TRAF6 interactions are a promising therapeutic target for inflammatory diseases
• Small molecule mediated inhibition (nanoparticles) of CD40-TRAF6 interactions is a promising therapeutic strategy for the treatment of atherosclerosis
• …but also DIO, EAE, sepsis peritonitis
• Promising future for TRAF-STOP
Obesity

CD40(L) plays an important role in regulating and enhancing immune reactions

CD40(L) is involved in a plethora of inflammatory diseases

Plasma sCD40L levels are elevated in obese patients

Human adipocytes carry CD40 and interact with T-cell CD40L
Deficiency of CD40L prevents diet-induced weight gain
CD40 deficiency induces insulin resistance and hepatosteatosis
CD40-TRAF signaling in obesity

CD40-Twt  CD40-T2/3/5-/-/  CD40-T6-/-

Standard Fat Diet (SFD)
(70% kcal carbohydrate, 10% kcal fat, 20% kcal protein, 3.68 kcal/g)
Or
High Fat Diet (HFD)
(35% kcal carbohydrate, 45% kcal fat, 20% kcal protein, 4.54 kcal/g)

20 weeks
Deficiency results in:

- early increase weight gain
- worsened insulin sensitivity
- increased hepatosteatosis
- AT inflammation

- late decrease in weight gain
- better insulin sensitivity
- no hepatosteatosis
- decrease in AT inflammation
- increase UCP-1 and BAT

Chatzigeorgiou, Seijkens et al PNAS 2014
TRAF-STOPs in obesity
CD40-downstream pathways ....
Are there more CD40 binding partners?

CD40-C-term as bait

Filamin A/B

Burger, van Tiel et al. submitted
Filamin A binds near the CD40 transmembrane domain
Filamin A is recruited upon CD40 activation

Anti-CD40 or IgG stained incubation

Anti-CD40
IgG

bend Filamin A
CD40

Filamin A is recruited upon CD40 activation
Confocal: maximized resolution

STED 3D + deconvolution

CD40
Filamin

CD40
Filamin
CD40-Filamin interactions are required for translocation of CD40 to the lipid rafts.
...thereby promoting Akt induced endothelial cell activation
Filamin A/B
TNF family of co-stimulatory molecules

Experimental Vascular Biology lab
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