Immunomodulation therapy for atherosclerosis

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Disclosure: Jan Nilsson is signed as co-inventor on patents for immunomodulation of atherosclerosis assigned to CardioVax, CA
Lipoprotein autoimmunity

- Macrophage
- oxLDL
- Dendritic cell
- INF-γ
- Th1 cell
- Foam cell
- Activated endothelium
- T cell
- LDL
- Monocyte
- Blood
- Necrotic core
- Apoptotic foam cell

Nilsson et al, Expert Rev Vaccines 2013
LDL autoimmunity – evidence for a functional target

- Oxidized LDL-specific autoantibodies are common in humans and are associated with cardiovascular disease (Palinski et al 1989)
- Oxidized LDL-specific T cells are present in the circulation (Frostegård et al 1992)
- Atherosclerotic plaques contain autoantibodies against oxidized LDL (Ylä-Herttualala et al 1994)
- 10-15% of T cells in human atherosclerotic plaques are specific for oxidized LDL (Stemme et al 1995)
- Apo B autoreactive T cells contribute to plaque formation (Hermansson et al, J Exp Med 2010)
How does immunization with oxidized LDL affect atherosclerosis?

- Several investigators observe reductions in atherosclerosis also when immunization with unmodified LDL
Nilsson et al, Expert Rev Vaccines 2013
Identification of immune targets in oxidized LDL

- Oxidized LDL
- LDL
- Peptide 210 (aa 3136-3155)
- Peptide 45 (aa 661-680)
- Peptide 2 (aa 16-35)
- Phospholipids
- Apolipoprotein B-100

Fredrikson et al, ATVB 2003
Atherosclerosis vaccines based on apo B peptides inhibit disease development in apo e−/− mice

Tse et al, Front Immunol 2013

Subcutaneous infusion of apo B100 peptides to Apoe−/− mice reduces development of atherosclerosis and inhibits progression of established disease

Depletion of regulatory T cells abolishes the protective effect of ApoB100 peptides

ApoB100 – mix of p210, MDA-p210 and p240

Malmö Diet and Cancer Study (MDSC) cohort

All 45-73 old men and women living in the city of Malmö, Sweden (n=70000)

Participating in baseline examination of the MDCS cohort (n=28449)

Enrolled in CVD substudy (n=6103)

Baseline clinical examination and questionnaire

Blood sampling on average 8 months after clinical examination

Mononuclear cells isolated and frozen at -140°C

Plasma and mononuclear cells thawed and analyzed

Follow-up until 2008 2009

- No loss in cell numbers (Trypan Blue)
- 95% viable (7-AAD exclusion)
- Can be stimulated to proliferate and release cytokines
Low levels of Tregs are associated with increased risk for AMI

- Mononuclear leukocytes were isolated from 700 subjects 1991-94 and stored at -140°C
- 95% of cells viable
- Leukocytes respond with cytokine release when activated
- Tregs (CD4+FoxP3+ T cells) analyzed by FACS
- 84 incident AMI registered during 15 years follow-up

Wigren et al, ATVB 2012
Oral tolerance induction to oxLDL inhibits atherosclerotic plaque formation

Intranasal administration of p210-CTB reduces atherosclerosis in ApoE−/− mice

Klingenberg R et al.
Arterioscler Thromb Vasc Biol 2010
Immunization can modulate immune responses against LDL
Apo B-100 p45 and p210 autoantibodies and AMI in the Malmö Diet and Cancer cohort (5211 subjects with 15 years follow up)

<table>
<thead>
<tr>
<th>ApoB-100 Abs</th>
<th>Non-cases</th>
<th>CVD cases</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio#</td>
<td>n = 4567</td>
<td>n = 644</td>
<td></td>
</tr>
<tr>
<td>Nat p45 IgM</td>
<td>0.449±0.497</td>
<td>0.403±0.498</td>
<td>0.003</td>
</tr>
<tr>
<td>MDA p45 IgM</td>
<td>0.515±0.358</td>
<td>0.472±0.364</td>
<td>0.005</td>
</tr>
<tr>
<td>Nat p210 IgM</td>
<td>0.671±0.219</td>
<td>0.650±0.220</td>
<td>0.022</td>
</tr>
<tr>
<td>MDA p210 IgM</td>
<td>0.742±0.201</td>
<td>0.712±0.204</td>
<td>0.001</td>
</tr>
<tr>
<td>Nat p210 IgG</td>
<td>0.432±0.227</td>
<td>0.403±0.224</td>
<td>0.002</td>
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</tbody>
</table>

# Ratio of the individual plasma sample and the control plasma; Skewed variables were log transformed before analysis; t test
High levels of Nat p210 IgG autoantibodies are associated with an decreased risk of coronary events.

3rd vs 1st;

HR [95%CI] = 0.74 [0.56, 0.97]

$P = 0.03$

Adjusted for age, sex, LDL/HDL, SBP, triglycerides, smoking and diabetes in Cox Regression.
High levels of Nat p210 IgG autoantibodies are associated with a decreased risk of coronary events. For trend = 0.001

3rd vs 1st:

HR [95%CI] = 0.74 [0.56, 0.97]

P = 0.03

Adjusted for age, sex, LDL/HDL, SBP, triglycerides, smoking and diabetes in Cox Regression
High levels of Th2 cells are associated with lower risk of AMI

Engelbertsen et al. (2013) ATVB 33, 637-644

**Th2 cells**

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 cells/µl</td>
<td>710 ± 640</td>
<td>930 ± 720</td>
</tr>
<tr>
<td>Th2 cells/µl</td>
<td>41 ± 38</td>
<td>54 ± 47</td>
</tr>
</tbody>
</table>

*** p<0.005

**Th1 cells**
Antibodies against modified apo B peptides inhibit atherosclerosis

- Human IgG1 against MDA-peptides 45 and 210 were produced through screening of a single chain antibody fragment library and subsequent cloning into a pcDNA3 vector.

Schiopu et al, Circulation 2004 and JACC 2007
MDAp45 IgG inhibits inflammation

Diet-induced obese non-human primates

Effect mediated through activation of the inhibitory FcRII

Li et al, Molecular Metabolism 2013
Phase II study ‘Goal of oxidised Ldl and ACTivated macrophage Inhibition by Exposure to a Recombinant antibody’ GLACIER

- A Multicenter, Randomized, Double Blind, Placebo-Controlled Phase II Study involving 147 patients with stable carotid lesions
- Treatment groups were (1) single iv MLDL1278A, (2) repeated iv MLDL1278A or (3) placebo for 12 weeks
- Primary endpoint: Change in plaque inflammation as assessed by FDG PET/CT

Lehrer-Grawier et al, JACC Cardiovasc Imaging 2015
Possible reasons why the GLACIER study failed to meet its end point

- Mechanisms identified in experimental models are not valid in human atherosclerotic lesions
- The level of plaque inflammation is too low in stable patients
- Difficult to add a plaque anti-inflammatory effect on top of statins
- Problems in standardizing detection of the FDG-PET signal between different centers
MDAp45 IgG is inversely associated with markers of apoptosis and post-operative death

OxLDL and apoptosis
- Ox LDL is cyotoxic for vascular cells
- TNFR-1, TRAILR-2 and FAS are cell surface receptors that induce apoptosis through activation of caspase-8 and -3
- Ox LDL can activate FAS on endothelial cells

Association between plasma MDAp45 IgG and apoptosis markers
- TNF-R1: $r=-0.12$, p<0.05
- TRAIL-R2: $r=-0.12$, p<0.05
- FAS: $r=-0.14$, p<0.01
- IL-6: $r=0.09$, ns
- MCP-1: $r=-0.02$, ns
- RANTES: $r=-0.14$, p=0.05

Association between plaque oxLDL and apoptosis markers
- TNF-R1: $r=0.39$, p<0.001
- TRAIL: 0.42, p<0.001
- TRAIL-R2: $r=0.34$, p<0.001
- FAS: $r=0.40$, p<0.001
- Caspase 8: $r=0.30$, p<0.001
Immunomodulation therapy for atherosclerosis - conclusions

- Atherosclerosis vaccines based on apo B peptides and other antigens have shown promising results in experimental models.
- Proposed mode of action involves regulatory T cells, CD8 T cells and generation of LDL antibodies.
- Formulation and safety issues, unclear mode of action and lack of validated biomarkers to monitor therapeutic response have delayed clinical testing.
- Antibody therapy represents a promising alternative but clinical efficacy remains to be proven.
Experimental Cardiovascular Research, Lund University