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
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-Herttuala 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
Identification of immune targets in oxidized LDL

Peptide 210 (aa 3136-3155)
Peptide 45 (aa 661-680)
Peptide 2 (aa 16-35)

Apolipoprotein B-100

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

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

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>
</tr>
</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

\[ P \text{ for trend} = 0.001 \]

3rd vs 1st:

\[ \text{HR [95\%CI]} = 0.74 \text{ [0.56, 0.97]} \]

\[ P = 0.03 \]

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>Subjects at risk</th>
<th>1\textsuperscript{st} tertile</th>
<th>2\textsuperscript{nd} tertile</th>
<th>3\textsuperscript{rd} tertile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years 5</td>
<td>201</td>
<td>212</td>
<td>211</td>
</tr>
<tr>
<td>Years 10</td>
<td>171</td>
<td>183</td>
<td>193</td>
</tr>
<tr>
<td>Years 15</td>
<td>113</td>
<td>135</td>
<td>163</td>
</tr>
</tbody>
</table>

Log rank p for trend =0.004

Th2 cells/µl

<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

Log rank p for trend =0.49

Stroke event free survival

Log rank p for trend =0.004

Coronary event free survival

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