The Autoimmune Hypothesis of Atherosclerosis

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Outline

• Autoimmunity in atherosclerosis
• Recall responses in the lesion
• Th1, Th17, Treg
• Immunization
Autoimmunity

• *Direct evidence* requires *transmissibility* of the characteristic lesions of the disease from human to human, or human to animal. In the real world, such evidence is attainable at this time only for diseases mediated by autoantibody, since we do not have the means for reliably studying T lymphocyte-mediated autoimmune diseases by transfer to animals.

Johns Hopkins Autoimmune Research Center
Autoimmunity

- *Indirect evidence* requires re-creation of the human disease in an *animal model*. The majority of autoimmune diseases fit in this category. For example, the autoimmune basis of systemic lupus erythematosus is well accepted because of the availability of several genetically determined mouse models which, while not simulating lupus as seen in the clinic, do very closely replicate the serological features and some pathological features.  

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Autoimmunity in Atherosclerosis

- **Autoantigens:** ApoB100, oxLDL, hsp60
- **Antibody responses:** IgM, IgG
- **T cells:** Th1, Th17, Treg
- **Caveat:** Both pro- and anti-atherogenic effects of antibodies and T cells
Göran Hansson, Karolinska

Discovered T cells in human plaque in 1986

$Ldlr^{-/-}$ mice transgenic for human ApoB100 vaccinated with human oxLDL show oligoclonal T cells (TRBV31) reactive to unmodified ApoB100

Raising antibodies to these T cells by immunizing with a TRBV31 peptide reduces atherosclerosis

$Ldlr^{-/-}$ mice transgenic for human ApoB100 show smaller lesions when immunized with dendritic cells pulsed with human oxLDL

Intranasal vaccination with an ApoB100 fusion protein is atheroprotective, possibly through Tr1 cells
Joe Witztum, UCSD

Oxidation-specific epitopes (OSEs) generated on oxLDL

oxLDL found in atherosclerotic lesions

IgM and IgA antibodies to oxidized phospholipids in
oxLDL, T cell-independent and made by B1a cells

These antibodies are thought to be atheroprotective,
because they inhibit oxLDL binding to macrophages in
vitro by about 20%

In humans, IgM titers to oxLDL are inversely related with
cardiovascular disease
Andrew Lichtman, Harvard

Absence of CD80 and CD86 reduces atherosclerosis in $Ldlr^{-/-}$ mice, but this is controversial (Mallat)

Knocking out the Th1 transcription factor T-bet reduces atherosclerosis in $Ldlr^{-/-}$ mice

Mice lacking ICOS show defects in Tregs and enhanced atherosclerosis

$Ldlr^{-/-}$ mice lacking inhibitory co-stimulators PDL1 and 2 show more atherosclerotic lesions

In vitro, Tregs reduce endothelial activation
Peptides from ApoB100 are recognized by autoantibodies in human plasma

Immunization of transgenic mice with these peptides (including p210) conjugated to BSA reduced atherosclerosis in Apoe^{-/-} mice

This protection is thought to work through Treg induction, because Treg numbers are negatively correlated with oil red O lesion area and an antibody to CD25 abrogated the Treg induction and the atheroprotective effect of vaccination with p210

An intranasal vaccine was later developed based on p210

However, alum itself (without peptide) showed atheroprotective effects, because alum without antigen induces FoxP3+ Treg cells

Antigen specificity of the Tregs was not addressed

Intranasal vaccines targeted to oxLDL or heat shock protein-60 are also atheroprotective
Natural regulatory T cells (nTregs) are thought to be atheroprotective, based on the protective effect of infusion of T cells into “empty” Apoe<sup>-/-</sup>Rag2<sup>-/-</sup> mice.

Mice lacking both CD80 and 86 or CD28 lack natural Tregs and show larger lesions, although this is controversial (Lichtman).

An antibody to CD25 increases lesion size in Apoe<sup>-/-</sup> mice, presumably by reducing Tregs. Mice carrying a dominant negative version of TGFβRII in CD4 T cells do not show this effect, suggesting that TGFβ signaling is required.

Infusing mice with ApoB100 peptides without adjuvant for 2 weeks reduced atherosclerosis at 8 weeks. This was attributed to Tregs, because an antibody to CD25 abrogated the protective effect.

B cell depletion by a CD20 antibody reduced atherosclerosis in Apoe<sup>-/-</sup> and Ldlr<sup>-/-</sup> mice.
N-glycolyl neuraminic acid (Neu5Gc) as a xenoantigen
From milk and red meat
Incorporates into endothelial, epithelial cells
Endothelial cells in vitro show Neu5Gc-dependent antibody binding, complement deposition, endothelial activation, selectin expression, increased cytokine secretion, and monocyte binding
Neu5Gc expressed in endothelium over atherosclerotic lesions
Antibody production requires uptake by haemophilus influenzae
All adults have IgG, IgA and IgM antibodies to Neu5Gc
Not in cord blood; in infants at 12 months similar to mothers
Not known whether these antibodies are pro-atherogenic
Most leukocytes in aortic arch

Live
CD45^+
Apoe^{-/-}

- arch
- thoracic
- abdominal

CD45^+ cells

0 2000 4000 6000 8000 10000

arch  thoracic  abdominal
Aortic Wall Myeloid Cells

Live CD45+

ApoE CD  ApoE WD  B6-CD  B6-WD

B6

Apoe-/-

chow

western

CD11b

CD11c

Cell number

0 1000 2000 3000 4000 5000 6000

17 8 5 6

19 8 3 3

33 6 3 21

33 6 3 21
Accumulation of CD11b\(^+\) and CD11c\(^+\) cells in atherosclerosis

Immunofluorescence. confocal microscopy
Antigen Presentation in Mouse Aorta
CD11c\textsuperscript{YFP} cells rapidly migrate in arterial wall
Fluorescent cells in CD11c<sup>YFP</sup> Apoe<sup>−/−</sup> aorta

Koltsova et al., JCI 2012
CD4 T cell interaction with APCs

OT-II → Anti-CD3 anti-CD28 48h
CD4+ T cells
SNARF labeling
With/without Ova
12h Two-photon imaging

Koltsova et al., JCI 2012
APCs interact with antigen specific T cells in the wall of $CD11c^{YFP}(B6)$ mouse
T cells decrease migration velocity during long interaction with APC

Koltsova et al., JCI 2012
APC interact with Apoe<sup>-/-</sup> T cells in the wall of CD11c<sup>YFP</sup> Apoe<sup>-/-</sup> mouse
APC interact with Apoe<sup>-/-</sup> T cells in plaque of CD11c<sup>YFP</sup> Apoe<sup>-/-</sup> mouse

CD4 Apoe<sup>-/-</sup> T cells
CD11c<sup>YFP</sup> DC
APCs interact with \textit{Apoe}^{-/-} T cells in plaque of \textit{CD11c}^{YFP}\textit{Apoe}^{-/-} mice
Koltsova et al., JCI 2012
IFNγ Promotes Foam Cell Formation

PBS

IFNγ

IL-4

IL-17

oxLDL

CD11b

oxLDL

vehicle

oxLDL

oxLDL

oxLDL
CCL2

monocyte

IFN-γ

IL-17A

Mφ

oxLDL

Th1

T cell

Th17

CD11b⁺CD11c⁺

DC

Foam cell

Inflamed
Atheroprotective Immunization
Rationale

• Autoantigen and antigen-experienced CD4 T cells are both present in \textit{Apoe}^{-/-} mouse aortas
• T cell cytokines drive foam cell inflammation
• ApoB100 is a known atherosclerosis autoantigen
• Tregs are protective
• CD4 T cell response including Tregs requires high affinity binding of antigenic peptide to MHC-II
• Therefore, it should be possible to develop peptide-based immunization
• May be pro- or anti-atherogenic: route, dose, frequency, adjuvant
ApoB100

573 peptides (15-mers)

Structure of apoB-100 in LDL
Strategy

• Computer-based prediction of ApoB100 peptide binding to I-A\textsuperscript{b} (the MHC-II in C57BL/6 mice)
• Measure I-A\textsuperscript{b} binding affinity of candidate peptides
• Test effect of candidate peptide immunization in Apoe\textsuperscript{-/-} mice
Immunization Scheme

- Peptide in CFA s.c.
- Peptide+IFA i.p.
- Aorta, LN, spleen harvested

- Western diet

- Birth
- 8, 10, 12, 16, 20, 22, 23 weeks
En face lesion size

Lesion area, % of aortic surface

- untreated: n=9
- Pep1: *n=13
- adjuvant: *n=14
- Pep2: n=7

immunized
**Antibody response**

IgG to Pep1
- Immunized with Pep2
- Immunized with Pep1

IgG to Pep2
- Immunized with Pep2
- Immunized with Pep1

IgG to MDA-LDL
- Irrelevant peptide
- Not immunized
- Immunized with Pep1
- Immunized with Pep2

No reactivity to unmodified LDL
Dextramers to detect Antigen-specific T cells
Tregs in Aorta

CD3

B-220

CD45

Viability

SS Lin: SS

Side Scatter

FoxP3

19.2

TCR-β

Tregs in Aorta

CD3

FoxP3

TCR-β

Alexa Fluor 700

Pacfic Blue

19.2

SS Lin: SS

Side Scatter

Side Scatter

62.9

5.74

Viability

CD45

CD45
Aldefluor Assay for RALDH

Retinaldehyde dehydrogenase (RALDH)

Retinaldehyde $\rightarrow$ Retinoic acid

diethylaminobenzaldehyde

BODIPY™- aminoacetaldehyde $\rightarrow$ BODIPY™-aminoacetate
Tolerogenic DCs
Conclusions

• Activated T cells interact productively with CD11c⁺ APC in the aortic wall and produce cytokines (IFN-γ, IL-17)
• Apoe⁻/⁻ atherosclerosis antigen-experienced T cells make IFN-γ when incubated with an Apoe⁻/⁻ aorta
• IFN-γ induces a pro-inflammatory program in foam cells
• Immunization with MHC-II binding ApoB100 peptides (1x CFA, 4x IFA) is atheroprotective
• Immunization induces IgG antibody response
• Tregs and tolerogenic DCs found in atherosclerotic aorta