Inflammation and cardiovascular disease

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What do these diseases have in common?
What do these diseases have in common?

Immune related diseases
Diseases are multifactorial

- Immune system is in the basis of many diseases

- Targeting the immune system may be potentially useful for the treatment of these diseases

- A diversity of models available to test biological questions
Cardiovascular diseases are multifactorial

- Genetics
- Development
- Metabolism
- Vascular remodeling
- Inflammation
- Immunity
- Epigenetics
- Mechanobiology

Genetics
- Lifestyle
- Dyslipemia
- Tissue repair
- Oxidative stress
- Hypertension
- Angiogenesis

atherosclerosis
- Coronary artery disease
- Cerebrovascular disease
- Peripheral arterial disease
- Aneurysm
- Cardiomyopathy
- Hypertensive heart disease
- Heart failure
- Pulmonary heart disease
- Dysrhythmias
- Inflammatory heart disease
- Valvular heart disease
- Rheumatic heart disease

Coronary artery disease
Cerebrovascular disease
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Rheumatic heart disease
Cardiovascular diseases are multifactorial

Genetics
Lifestyle
Dyslipemia
Tissue repair
Oxidative stress
Hypertension
Angiogenesis

Development
Metabolism
Vascular remodeling
Inflammation
Immunity
Epigenetics
Mechanobiology

ATHEROSCLEROSIS
Coronary artery disease
Cerebrovascular disease
Peripheral arterial disease
Aneurysm
Cardiomyopathy
Hypertensive heart disease
Heart failure
Pulmonary heart disease
Dysrhythmias
Inflammatory heart disease
Valvular heart disease
Rheumatic heart disease
<table>
<thead>
<tr>
<th></th>
<th>Infectious</th>
<th>Non-infectious</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation</strong></td>
<td>Innate response to infection</td>
<td>Chronic inflammation</td>
</tr>
<tr>
<td><strong>Immunity</strong></td>
<td>Adaptive response to infection</td>
<td>Autoimmunity</td>
</tr>
</tbody>
</table>

**Immunity and Inflammation**

**Immune response**

**Immunity**

**Inflammation**
Which cells control immunity and inflammation?

Dendritic cells and macrophages

Source: Donny Bliss, National Library of Medicine
Dendritic cells & macrophages: at the basis of immunity and inflammation

**TRIGGER**
(sterile or microbial)

**Inflammation**
- Dendritic cells
- Macrophages
- Proinflammatory cytokines
- Monocytes
- Macrophages
- granulocytes
- Innate lymphoid cells

**Immunity**
- Foreign antigen or neoantigen
- Dendritic cells
- NKT cells
- CD4 (Th1, Th2, Th17, Treg)
- CD8 (CTL)
- B cells

**EFFECTOR IMMUNE RESPONSE**
- Atherosclerosis
- Coronary artery disease
- Cerebrovascular disease
- Peripheral arterial disease
- Inflammatory heart disease
- Rheumatic heart disease
How is initiated the immune response?  
The immunologist’s dirty little secret

The antigen is not enough

to mount an adaptive immune response
However, inflammation or immunity can occur in the absence of pathogens. e.g., autoinflammatory / autoimmune diseases.
How do danger signals initiate/modulate the inflammatory and immune response?

“danger” signals are preformed endogenous adjuvants sequestered inside healthy cells and exposed or released upon necrotic cell death.

Our research questions

- Pathogens & microbiota
  - Iborra & Martínez-López et al. 2016. *Immunity*

- How do DC & MF sense tissue damage and microorganisms?
  - Iborra et al. 2016. *Immunity*

- Tissue damage & Dysregulation

- Relevance in vivo
  - Can we target metabolism for manipulating DC function?
  - Innate response
  - Inflammation
  - Immunity
  - Adaptive Response
  - Mechanisms Function

- What is the specialized function of different DC subsets in initiating immunity?
Atherosclerotic lesion in a human artery: Panel A shows a cross-sectioned coronary artery from a patient who died of a massive myocardial infarction. It contains an occlusive thrombus on a lipid-rich atherosclerotic plaque. The fibrous cap covering the lipid-rich core has ruptured (area between the arrows), exposing the thrombogenic core to the blood. Trichrome stain was used, rendering luminal thrombus and intraplaque hemorrhage red and collagen blue. Panel B is a high-power micrograph of the area in (the asterisk indicates cholesterol crystals).
Autoinflammation

Genetic factors

Infection and environmental exposure

Immune regulation

Autoimmunity
Autoinflammation
Development of atherosclerosis
Atherosclerosis and autoimmunity

(a) Atherosclerotic plaque vs. normal artery
- Smooth muscle cells
- Lumen
- Intima
- Media
- Adventitia
- Necrotic/lipidic core
- Foam cell
- Macrophage
- Lymphocyte
- Endothelial cells

(b) Normal joint vs. rheumatoid arthritis
- Neutrophil
- Lymphocyte
- Macrophage
- Fibroblast-like synoviocytes
- Synovial fluid
- Synovial membrane
- Capsule
- Neo vessels
Chronic inflammation is positively linked to CVD
- Elevated hs-CRP, proinflammatory cytokines {IL-6 and TNF-alpha} and chemokines
- Increased incidence of CVD in those with:
  - Overt inflammatory conditions
    - Autoimmune disease, renal disease, allergic disease, coeliac disease, periodontal disease and inflammatory bowel disease
  - Fatty infiltration of liver
    - Persistent elevated GGT is predictive of metabolic syndrome
  - Insulin resistance ↔ obesity
    - Adipocyte production of cytokines and chemokines.
<table>
<thead>
<tr>
<th>Reference (year)</th>
<th>Study design</th>
<th>RA definition</th>
<th>n</th>
<th>Estimated risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>[18] (2001)</td>
<td>Prospective cohort</td>
<td>ACR 1987 criteria</td>
<td>236</td>
<td>3.86-fold of combined CV events (MI + revascularization + stroke)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.48-fold risk for stroke</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RA &gt;10 years: 3-fold risk for MI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6-fold risk for unrecognized MI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2-fold risk sudden death</td>
</tr>
<tr>
<td>[85] (2007)</td>
<td>Retrospective cohort</td>
<td>ACR 1987 criteria</td>
<td>239</td>
<td>0.1% to 0.3%/year MI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.07%/year stroke</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.9% prevalence stroke</td>
</tr>
</tbody>
</table>

ACR, American College of Rheumatology; CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction; RA, rheumatoid arthritis.
## Lupus and cardiovascular risk

<table>
<thead>
<tr>
<th>Reference (year)</th>
<th>Study design</th>
<th>SLE definition</th>
<th>n</th>
<th>Estimated risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>[11] 1997</td>
<td>Prospective cohort</td>
<td>ACR criteria for SLE</td>
<td>498</td>
<td>5-fold risk for MI 50-fold risk in ages 35 to 44 years</td>
</tr>
<tr>
<td>[86] 1999</td>
<td>Retrospective cohort</td>
<td>Rheumatologist diagnosis</td>
<td>8,742</td>
<td>2.27-fold risk for MI 3.8-fold risk for chronic heart failure</td>
</tr>
<tr>
<td>[87] 2004</td>
<td>Cross-sectional, prospective</td>
<td>ACR criteria for SLE</td>
<td>202 (cross-sectional), 47 (prospective)</td>
<td>1.4-fold risk for CHD 0.6-fold risk for stroke 8.5% CHD events 10% stroke follow up</td>
</tr>
<tr>
<td>[88] 2004</td>
<td>Case control</td>
<td>Physician diagnosis</td>
<td>770</td>
<td>1.46 risk for MI</td>
</tr>
</tbody>
</table>

ACR, American College of Rheumatology; CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction; SLE, systemic lupus erythematosus.
Atherosclerosis evolution
Innate immunity by macrophages is crucial!

Macrophages are heterogeneous

The macrophage balance in atheroma formation and evolution

- **PRO**
  - ROI, MMPs, IL-1β, TNFα, cholesterol uptake
  - NF-κB
  - TLR, oxLDL

- **ANTIL**
  - IL-10, ARG1, PTX3, cholesterol efflux
  - LXR
  - Oxysterols

EXPERIMENTAL PROBLEM:
1. WHAT WOULD YOU EXPECT IN CONDITIONS OF REDUCED EFFEROCYTOSIS???
2. HOW WOULD YOU ADDRESS THIS?
Resolution of inflammation by macrophages

**a Early atherosclerosis**
- Inflammation resolution and decreased plaque progression
  - No secondary necrosis
  - ↑ Anti-inflammatory cytokines
  - Macrophage clearance
- Apoptotic macrophage
- Efferocyte
- TGFβ and IL-10
- Endothelial cell
- Intima
- Smooth muscle cell

**b Advanced atherosclerosis**
- Secondary necrosis
- Pro-inflammatory factors and necrotic core formation
- No inflammation resolution and vulnerable plaque formation
  - ↑ Anti-inflammatory and pro-inflammatory factors
  - Secondary macrophage necrosis leading to necrotic core formation
- Disabled efferocyte
Failure in inflammation resolution by macrophages
Failure in efferocytosis contributes to atherosclerosis

Adaptive immunity in atherosclerosis

Antigen presentation and induction of antigen-specific pathogenic and regulatory T cells in the context of atherosclerosis

Tertiary lymphoid organs

Annu. Rev. Immunol. 27:165-97
<table>
<thead>
<tr>
<th>Types of effector T cell</th>
<th>CD8 cytotoxic T cells</th>
<th>CD4 T&lt;sub&gt;H&lt;/sub&gt;1 cells</th>
<th>CD4 T&lt;sub&gt;H&lt;/sub&gt;2 cells</th>
<th>CD4 T&lt;sub&gt;H&lt;/sub&gt;17 cells</th>
<th>CD4 regulatory T cells (various types)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTL</td>
<td>T&lt;sub&gt;H&lt;/sub&gt;1</td>
<td>T&lt;sub&gt;H&lt;/sub&gt;2</td>
<td>T&lt;sub&gt;H&lt;/sub&gt;17</td>
<td>T&lt;sub&gt;reg&lt;/sub&gt;</td>
</tr>
<tr>
<td>Main functions in adaptive immune response</td>
<td>Kill virus-infected cells</td>
<td>Activate infected macrophages, provide help to B cells for antibody production</td>
<td>Provide help to B cells for antibody production, especially switching to IgE</td>
<td>Enhance neutrophil response</td>
<td>Suppress T-cell responses</td>
</tr>
<tr>
<td>Pathogens targeted</td>
<td>Viruses (e.g., influenza, rabies, vaccinia), some intracellular bacteria</td>
<td>Microbes that persist in macrophage vesicles (e.g., mycobacteria, <em>Listeria</em>, <em>Leishmania donovani</em>, <em>Pneumocystis carinii</em>), extracellular bacteria</td>
<td>Helminth parasites</td>
<td>Extracellular bacteria (e.g., <em>Salmonella enterica</em>)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 8-1 Immunobiology, 7ed. (© Garland Science 2008)
Adaptive immunity in atherosclerosis
Two major subsets of dendritic cells (DCs) in the aorta
CD11c\textsuperscript{+} CD11b\textsuperscript{low} DC subset expresses Flt3
$\text{CD11c}^{\text{high}} \text{ CD103}^{+}$ aorta DC are Flt3 dependent
The absence of CD11c<sup>high</sup> DC in aorta increases lesion size...

Questions:
1. WHY THE ABSENCE OF DC INCREASES LESION SIZE?
2. HOW WOULD YOU TEST THIS?
The absence of CD11c^{high} DC in aorta increases lesion size...
Treg as anti-atherogenic

No Tregs in the absence of CD80/CD86 or CD28
B cells in atherosclerosis

control

+ B cells ApoE-/-

+ T cells ApoE-/-

Sham mice

splenectomized

INTERPRETATION of the experiment:
1. What is the role of B cells in atherosclerosis?
2. Why?
...but mature B cell depletion results in attenuated lesions
CD20 Ab-mediated B cell depletion preserves IgM over IgG
Adaptive immunity and atherosclerosis

Blood vessel lumen

- Pre-DC (monocyte or DC progenitor)
- T-cell priming in lymph nodes
- CX3CR1

Atherosclerotic lesion

- Naive T cell
- Immature DC
- Mature DC
- CCL19, CCL22
- FOXP3
- T-bet
- IFNγ

- Mild hypercholesterolaemia
- Severe hypercholesterolaemia
  - DC apoptosis
  - ↓ DC migration

- T1,1 cell
- T1,2 cell
- Treg cell
- Naive CD4+ T cell
- Naive CD8+ T cell

- Production of autoantibodies to modified lipids
- IgG1a production
- Production of protective IgM by B-1 cells
- Anti-atherogenic activities (tolerance and anti-inflammation)
- Production of IFNγ, TRAIL and pro-inflammatory cytokines
- Lysis of SMCs
- CD95–CD95L-mediated lysis of SMCs

Nature Reviews | Immunology
Immunotherapy in atherosclerosis?

HOW WOULD YOU PERFORM IMMUNOTHERAPY IN ATHEROSCLEROSIS?
Immunotherapy in atherosclerosis
We are not alone...

10 times more cells than in our body

500 times more genes than human genome
Li, J. et al. Nature Biotechnology (2014)
“Mucosal Firewall”

Mucus
McGuckin et al.,
*Nature Reviews Microbiology* (2011)

Antimicrobial peptides (RegIIIγ)
Hooper, L. & Macpherson, A.
*Nature Reviews Immunology* (2010)

IgA specific for commensals
Macpherson, A. & Uhr, T.

Belkaid, Y. & Hand, T. W.
*Cell* (2014)
Role of microbiota in atherosclerosis?

Basis: Metabolites generated by microbiota promote insulin resistance and atherosclerosis (e.g. branched-chain amino-acids, BCAA; trimethylamine N-oxide, TMAO)

Pedersen et al. 2016. *Nature*

Wang et al. 2015. *Cell*

Research question: Which are the microbiome patterns and gut microbiota-related metabolites linked to atherosclerosis?

Hypothesis: *Atherosclerosis progression associates to new gut microbiota-related metabolites and specific microbiome patterns*

1. Explore metabolites in the serum of PESA (Progression of Early Subclinical Atherosclerosis) volunteers. Is there association of atherosclerosis progression (or regression) with metabolites in serum? Are they microbiota-dependent?

2. Analyze microbiome in selected groups (low athero, high athero, fast progressors). Is there association of microbiota with early subclinical atherosclerosis?