Accelerated Atherosclerosis in Autoimmune Rheumatic Diseases

Andrea Doria
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Endothelial Dysfunction
Fatty-Streak Formation
Formation of an advanced lesion
Unstable fibrous plaques

Pathogenetic Steps in Atherosclerosis
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Triggers and Common Pathway in Atherosclerosis

Key Features of Innate and Adaptive Immunity in Atherogenesis

Innate Immunity
- Rapid immune response to pathogen
- Antigen recognition driven by pattern recognition receptors which bind to highly conserved structures expressed by pathogens
- Effectors
  - Phagocytes
  - Pattern recognition receptors
  - Pentraxins (CRP, PTX3)
  - Complement
  - Cytokine (TNFα, IL-1, IL-6)
  - Chemokines (MCP-1)

Adaptive Immunity
- Require three to five days to mature
- Antigen recognition driven by T and B cell receptors; very specific antigen recognition
- Effectors
  - T and B cell
  - Antibodies
  - Cytokine (TNFα, IL-2, INFγ)
  - Chemokines
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Immunocytochemistry for CRP Distribution Within the Atherosclerotic Plaque

Odds Ratios for Coronary Heart Disease Among 2459 Patients with Coronary Heart Disease and 3969 Controls

Unstable Carotid Plaque (CARS)

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An Unusual Subset of T-Cells, CD4+CD28null, Is Clonally Expanded in the Peripheral Blood and Infiltrates Coronary Plaques in UA

CD4+CD28null T cells expansion in UA

Infiltration of selected CD4+CD28null T cells clones into the unstable plaque

Liuzzo G, Circulation 1999; 100: 2135-9

Stable Angina Unstable Angina

P < 0.001

CD4+CD28null Lymphocytes

• Undergo clonal proliferation
• Form long-lived clonal population

• Produce IFN-γ high levels
  (Liuzzo G, Circulation 1999; 100: 2135-9)

• Coexpress the CD57 NK cell marker

• Have cytolytic activity
  (Nakajima T, Circulation 2002; 105: 570-5;
  Nakajima T, Circ Res 2003; 93: 106-113;
  Nakajima T, Circulation 2002; 105: 570-5)

• Are autoreactive
• Enhanced number with increasing age in the blood of healthy subjects
  (Warrington KJ, Blood 2003; 101: 3543-9)

Frequency of CD4+CD28 - T Cells in 87 RA and 30 Control Subjects


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Common Carotid IMT in RA Patients Subdivided According to the Presence or Absence of CD4+CD28- Cell Expansion

Controls     All RA  CD4+CD28+  CD4+CD28-

Common carotid IMT (mm)


Atherosclerosis and Autoimmunity

- Anti-oxLDL
- Anti-β2GPI
- Anti-HSP60/65
- Anti-oxLDL
- Anti-ECA
- Anti-HDL
- Anti-APO A-1
- Anti-LPL


Oxidized LDL and other lipoproteins can be actively taken up by scavenger receptor mediated endocytosis, transforming macrophages into the so called foam cells

Some anti-oxLDL can enhance oxLDL uptake by macrophages through the involvement of the Fcγ receptors rather than via the usually employed scavenger receptor favouring foam cell formation and macrophage activation

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Anti-oxLDL and Atherosclerosis
- Raised levels in some conditions linked to coronary artery disease in humans
- Immunization with oxLDL of animals results in suppression rather than aggravation of early atherogenesis:
- Anti-oxLDL mAbs inhibit uptake of oxLDL by macrophages:

Anti-oxLDL and Atherosclerosis
- Protective
- Pathogenetic
  - Help to clear the high levels of oxLDL
  - Aid the uptake of oxLDL by macrophages turning them into foam cells within the atherosclerotic plaques
  - IgM anti-oxLDL
  - IgG anti-oxLDL

Antiphospholipid Antibodies
- Generally they are directed to phospholipids binding proteins (β2 glycoprotein I, prothrombin, etc.)
- Detected by different tests as:
  - Lupus anti-coagulant
  - Anti-cardiolipin antibodies
  - Anti β2GPI
  - Anti prothrombin
  - Anti ethanolamine
  - Anti annexin
  - Anti phospholipids
- Major cause of thromboembolic disorder in patients with autoimmune diseases and otherwise healthy subjects (antiphospholipid syndrome)
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The Primary Structure of Human β2GPI and Location of Attachment Sites for Oligosaccharide Chains

The Primary Structure of Human β2GPI and Location of Attachment Sites for Oligosaccharide Chains

β2GPI Adhered on Endothelial Cells Is a Target for Anti-Phospholipid Antibodies

- β2GPI adhesion to endothelium in vivo (McIntyre 1993, La Rosa 1994)
- β2GPI binds endothelium through the PL-binding site located at the fifth domain and through annexin II (Del Papa 1998; Keying 2000)
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Two Step Complex Formation Between OxLDL and β2GPI

Serum OxLDL/β2GPI Complexes May Be a Marker of Vascular Inflammation

The Role of β2GPI in Atherogenesis
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Immunolocalization of β2GPI (Apolipoprotein H) in Human Atherosclerotic Plaques: Potential Implications for Lesion Progression
George et al., Circulation 99:2227, 1999

- A and B: rabbit anti-β2GPI antibody
- C: normal rabbit IgG
- D: hematoxylin-eosin
- E: anti-CD4
- F: anti-CD8

Immunization with Human β2 Glycoprotein I

- In LDL-receptor-deficient mice or Apo-E knock-out mice immunization with β2GPI enhances atherosclerotic lesions which contain abundant CD4+ cells.
  - George J et al., Circulation 1998
  - Afek A et al., Pathobiology 1999
- The transfer of lymphocytes from mice immunized with β2GPI to mice of similar genetic background increases atherosclerotic lesions compared to those occurring in animals transferred with lymphocytes from control mice.

Antiphospholipid Antibodies in Patients with Clinical and Subclinical Atherosclerosis

- The presence of antiphospholipid antibodies was found to be a risk factor for various manifestations of coronary artery disease such as angina, myocardial infarction or cardiac death, as well as being associated with subclinical atherosclerosis.
  - Vaarala O et al., Circulation 1995;91;23: Sherer Y, Cardiology 2001, 95; 20
  - Sherer Y et al., Am J Cardiology 2005; 96:1306-1311
  - Vaarala O et al., Thromb Haemost 1996; 75:456
- It has also been shown that patients with primary antiphospholipid syndrome have a high prevalence of increased carotid intima-media thickness or plaque.
  - FG Vlachoyiannopoulos et al., Rheumatol 2003; 42:645-651

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Reduction of Atherosclerosis in Low-Density Lipoprotein Receptor-Deficient Mice by Passive Administration of Antiphospholipid Antibody

- In contrast to earlier studies of aPL which were specific for oxidized forms of LDL, FBI cross-reacted with both native LDL and oxLDL.
- In vivo, passive administration of FBI significantly reduced plaque formation in atherosclerosis-prone LDLR(-/-) mice.
- These results indicate that some aPL may play a protective role in atherogenesis.


Human HSP60

- Intracellular chaperone protein
- Expressed on the surface of stressed cells
- Human HSPs are expressed on endothelial cells in response to stressors like hypertension, smoking, lipoproteins, etc.
- HSPs offer a target for autoimmunity under such circumstances
- HSP60 shows high inter-species homology

Anti-Heat Shock Protein (HSP) 60/65 Antibodies

- Patients with high levels of anti-HSP65 were found to be at increased risk of subsequent cardiovascular events and cardiovascular mortality.
  - Xu Q, et al., Circulation 100: 1169, 1999
- Subjects with sonographic evidence of carotid atherosclerotic lesions had significantly elevated levels of anti-HSP65 compared with controls.
- Immunization of animals with mycobacterial or recombinant antigen causes atherosclerotic lesions (rabbits, C57BL/6 mice fed with high cholesterol diet, or LDL receptor deficient mice).

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### Enhanced Atherosclerosis and Associated Factors in ARD

<table>
<thead>
<tr>
<th>Disease</th>
<th>Evidence for enhanced ATS</th>
<th>Factors involved in enhanced ATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>↑ Prevalence of CAD</td>
<td>↑ Prevalence of classic risk factors for ATS, drugs used for treatment</td>
</tr>
<tr>
<td>SLE</td>
<td>↑ Prevalence of CAD, increased extent of subclinical ATS</td>
<td>↑ Prevalence of classic risk factors for ATS, corticosteroid therapy, long disease duration</td>
</tr>
<tr>
<td>APS</td>
<td>↑ Prevalence, thrombosis, increased extent of subclinical ATS, APS are associated with accelerated disease, APS predict future CVD in the general population</td>
<td>Anticardiolipin antibodies support a proatherogenic role of APS</td>
</tr>
<tr>
<td>SSc</td>
<td>↑ Prevalence of macrovascular disease, few studies on ATS</td>
<td>Oxidative stress, antinuclear cell antibodies</td>
</tr>
<tr>
<td>PSV</td>
<td>Increased extent of subclinical ATS in WG, Not studied in other diseases</td>
<td>Enhanced inflammation and excessive vascular remodelling</td>
</tr>
<tr>
<td>SLE</td>
<td>Not studied</td>
<td>Unknown</td>
</tr>
</tbody>
</table>


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### The Prevalence of Coronary Artery Disease Is Higher in RA Patients than in General Population

- RA patients have a 2-3 fold increase in rates of MI compared to the general population
  - Solomon DHM, et al., Circulation 2003;107: 1303-7
- They are more likely to experience silent ischaemia and sudden cardiac death

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Prevalence of Coronary Artery Disease (CAD) in Three Cohort of SLE Patients and in Women of Framingham Offspring Study

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Patients, N</td>
<td>229</td>
<td>665</td>
<td>498</td>
<td>2208</td>
</tr>
<tr>
<td>Prevalence of CAD</td>
<td>8.3%</td>
<td>10%</td>
<td>6.7%</td>
<td>1.6%</td>
</tr>
</tbody>
</table>


Incidence Rates of MI per 1000 Person-Years in 498 Women with SLE, University of Pittsburgh, and 2,208 women, Framingham Offspring Heart Study, 1980-1993

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>SLE Rate</th>
<th>Framingham Rate</th>
<th>Rate ratio</th>
<th>95% CI Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-24</td>
<td>6.33</td>
<td>0.2-35.3</td>
<td>0.00</td>
<td>0.0-11.8</td>
</tr>
<tr>
<td>25-34</td>
<td>3.66</td>
<td>0.8-10.7</td>
<td>0.00</td>
<td>0.0-1.2</td>
</tr>
<tr>
<td>35-44</td>
<td>8.39</td>
<td>4.2-15.0</td>
<td>0.16</td>
<td>0.0-0.9</td>
</tr>
<tr>
<td>45-54</td>
<td>8.62</td>
<td>1.0-14.1</td>
<td>1.95</td>
<td>0.9-3.6</td>
</tr>
<tr>
<td>55-64</td>
<td>8.38</td>
<td>1.7-24.5</td>
<td>1.99</td>
<td>0.6-4.6</td>
</tr>
<tr>
<td>65-74</td>
<td>7.94</td>
<td>1.0-28.7</td>
<td>0.00</td>
<td>0.0-17.1</td>
</tr>
</tbody>
</table>


Risk Factors for Atherosclerosis in SLE

- The evaluation of risk factors for clinical atherosclerosis is difficult in SLE because of the low number of observed cardiovascular events due to the low prevalence of the disease.

- The study of subclinical atherosclerosis has the advantage of providing a higher number of lesions leading to a more suitable evaluation of risk factors.
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Non-Invasive Techniques for the Detection of Subclinical Atherosclerosis
- Brachial flow mediated vasodilatation (BFMV)
- Pulse-Wave Velocity (PWV)
- Carotid Color Doppler Ultrasonography (US)
- Electron Beam Computed Tomography (CT)
- Scintigraphy (Thallium-201)
- Single Photon Emission Computed Tomography (SPECT)
  - Thallium-201
  - Dual Isotope Myocardial Perfusion Imaging (DIMPI)

Evidence of Increased IMT in Patients with Rheumatoid Arthritis

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Similarities between Atherosclerosis and RA

- C-reactive protein
- Adhesion molecules
- Neangiogenesis
- Macrophage activation
- TNF-α
- Metalloproteinase expression
- IL-6
- Mast cell activation
- Autoantibodies (oxLDL, H5)
- Rheumatoid factor
- T-cell activation
- sIL-2 receptor
- CD3-DR+
- CD4+ CD28−
- CD4+ IFN-γ
- Th1/Th2 balance

Carotid Ultrasound Studies in SLE

- Parameter
- Mean age (years)
- Female %
- White %
- Treatmed CVD %
- Plaque definition
- Plaque %
- Mean IMT (mm)
- Risk factor evaluation


p<0.001

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Traditional and Other Metabolic Risk Factors for ATS
- Age
- Sex
- Hypercholesterolaemia
- Hypertension
- Obesity
- Diabetes
- Sedentary lifestyle
- Cigarette smoking
- Family history for CVD

Mean number of CV risk factors per person
SLE patients >>> General population

Traditional risk factors
- Hypercholesterolaemia
- Hypertension
- Obesity
- Diabetes
- Sedentary lifestyle
- Cigarette smoking
- Family history for CVD

↑ in SLE


Cardiovascular Risk in Lupus

Overall Risk of CHD

Low
High

SLE

> Additional factors

General population

Novel Risk Factors for Atherosclerosis
- Markers of inflammation
  - CRP
  - Serumin A
- Acute-phase proteins
- Adhesion molecules
  - ICAM-1
  - P-selectin
  - E selectin
- Cytokines
  - IL-6
  - TNFα
- Immuneological factors
  - Adaptive immunity
    - Anti-β2GPI
    - Anti-oxLDL
    - Anti-HSP-65
  - Innate immunity
    - Toll-like receptor (TLRs)
    - TLR4
    - Scavenger receptors (SR)
    - SR-A
    - CD36
- Lipoproteins and modified lipids
  - Lipoprotein (a)
  - Oxidized LDL
  - Small, dense LDL
- Abnormal coagulation factors
  - Fibrinogen
  - PAI-1
  - Homocysteine
CRP and Subclinical Atherosclerosis in SLE

- CRP was associated with plaques at univariate, but not at multivariate analysis

- CRP was not associated with plaques

Cross-Sectional Studies on the Relationship Between Autoantibodies and Atherosclerosis (ATS) in SLE Patients

Subclinical Atherosclerosis in SLE: Study Design

Traditional risk factors
- Treatment during follow-up
- Carotid ultrasound examination

- 78 SLE pts
- 5 year follow-up
- SLE clinical and laboratory features
  - SLE disease activity (ECLAM)
  - SLE damage index (SLICC D2)
  - Novel predictors:
    - CRP, anti-oxLDL, anti-β2GPI, and anti-HSP65

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Correlation Between M-IMT and IgG Anti-oxLDL Levels

\[ r = 0.344, p = 0.002 \]


Conclusions

• The IgG anti(oxLDL may play a role in the accelerated atherosclerotic process observed in SLE patients


• The role of anti(β2GPI, anti(HSP65 and CRP seems to be masked by some disease-related features particularly prednisone cumulative dosage and renal involvement

SLE-Related Risk Factors for Atherosclerosis

• SLE phenotype
• Severe/mild SLE
• Glomerulonephritis
• Arthritis
• aPL syndrome
• Other manifestations

• SLE activity
• Disease duration
• Treatment
• Corticosteroids
• Immunosuppressants
• Others

Questions Which Should Be Addressed:

1) Who are at highest risk for accelerated atherosclerosis? Severe or mild SLE patients?
   Patients with renal involvement or those with skin or joint manifestations?

2) Are corticosteroids pro or anti-atherosclerotic?
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Correlates of Accelerated Atherosclerosis in SLE


1st Multivariate analysis
Traditional risk factors
- Age
- Menopausal status
- ↑ Systolic BP
- ↑ Total cholesterol
- ↑ LDL cholesterol

SLE related features
- ↑ Age at diagnosis
- Longer SLE duration
- ↑ SLICC damage index
- Pulmonary hypertension
- ↓ Anti-RNP
- ↓ Anti-Sm
- ↓ 5-yr daily dose of PDN
- ↓ Cyclophosphamide use
- ↓ Hydroxychloroquine use

SLE related features
- Renal involv. at T0
- ECLAM ≥2 at T0
- PDN ≥40 gr
- Azathioprine

Significant in univariate and multivariate analyses

Severe or Mild SLE Patients:
Who Are at Highest Risk for Accelerated Atherosclerosis?

- In Roman’s study, patients with less severe disease were older than those with more severe disease.
- The higher prevalence of plaque in the former group of patients could not be related to milder disease, but to age itself, a factor not considered by the authors in the multiple regression analysis for disease-related factors.

Risk Factors for Carotid Plaques in SLE

Doria A et al., Ann Rheum Dis 2003; 62: 1071-77

Multivariate analysis
Traditional risk factors
- Age
- Hypertension
- Hypercholesterolemia

SLE related features
- Renal involv. at T0
- ECLAM ≥2 at T0
- PDN ≥40 gr
- Azathioprine

Significant in univariate and multivariate analyses
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78 SLE Patients

- Traditional risk factors
- Lipoprotein subclasses
- SLE variables
- Novel risk factors

Prednisone cumulative intake

% of

<18 g
18-38 g
>38 g

3 Groups:
A
B
C

Doria A et al., Arthritis Rheum 2004; 50: S191-S192

Mean (±SD) Prednisone (PRD) Cumulative Dosage in Patients with and Without Carotid Plaque

* Adjusted for traditional risk factors including BP and lipoprotein subclasses

Mean (±SD) Prednisone (PRD) Cumulative Dosage in Patients with and Without Carotid Plaque

* Adjusted for traditional risk factors and renal involvement

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The Bimodal Mortality Pattern of SLE

<table>
<thead>
<tr>
<th>Years After Diagnosis</th>
<th>Active SLE</th>
<th>CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>75</td>
<td>63</td>
</tr>
<tr>
<td>10%</td>
<td>63</td>
<td>53</td>
</tr>
</tbody>
</table>

Long-Term Prognosis in SLE

- Severe disease: 97, 92, 84, 72, 72
- Mild disease: Severe disease + 72, 72

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Take Home Message

Maximum effort should be made:

- To adequately treat severe lupus manifestations, particularly glomerulonephritis, using the lowest possible dosage of corticosteroids associated with corticosteroid sparing therapy
- To manage modifiable traditional risk factors for ATS, first of all hypertension and hypercholesterolemia, as quickly as possible
- To consider the use in these patients of low-dose aspirin, hydroxychloroquine, and ACE inhibitors

Triggers and Common Pathway in Atherosclerosis

- Infections
- CV risk factors
- Chronic inflammatory diseases

Innate/adaptive immunity

Endothelial dysfunction

Atherosclerosis/inflammation

The Role of the Endothelium in the Maintenance of Vascular Homoeostasis

- Vasodilation
- Anti-inflammatory
- Anti-thrombotic
- Anti-coagulant
- Pro-fibrinolytic

- Vasoconstriction
- Pro-inflammatory
- Pro-thrombotic
- Pro-coagulant
- Anti-fibrinolytic

Endothelium

Vessel Lumen

Intima
Assessment of Endothelial Function

• Circulating markers of vascular wall inflammation
• Endothelial vasomotor testing (endothelial stress test)
  • Intra-arterial infusion of specific endothelium-dependent vasodilators (coronary microcirculation, brachial or femoral circulation)
• Ultrasound measurement of brachial artery reactivity to shear stress (upper arm occlusion)

Lerman, A. et al., Circulation 2005;111:363-368

Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of Brachial Artery

Evidence of Endothelial Dysfunction as Predictor of CV Events

Lerman, A. et al., Circulation 2005;111:363-368
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Endothelial-Dependent FMV of Brachial Artery in Patients with ARD
Endothelial dysfunction was reported in patients with:
- Rheumatoid arthritis
  Hartime Z et al., Circulation 2002; 106:204-7
  Van Dammen S et al., Arthritis Rheum 2003; 48:72-80
- Systemic lupus erythematosus
  Liwa et al., J Rheumatol 2001; 29:230
  El-Magadmi et al., Circulation 2004; 110:399
  Johnson SR et al., Circulation 2004; 110:399
- Primary antiphospholipid antibody syndrome
  Mercanoglu F et al., J Clin Exp 2004; 58:102-7
- Sjögren syndrome
  Pirildar T et al., Rheumatol 2005; 44:860-67
- Primary systemic vasculitis
  Booth AD et al., Circulation 2004; 109:1718-23
  Ozdemir R et al., Am J Cardiol 2003; 94:522-37;
  Raza K et al., Circulation 2000; 102:1470-2

Why Patients with Autoimmune Rheumatic Diseases Have a Diffuse Endothelial Dysfunction?

Vascular Endothelium at the Site of the Primary Inflammatory Response and at Distant Sites

Local inflammation .......................... Distant effects
- Leucocyte recruitment
- Systemic TNF-α
- Stromal/endothelial interactions

Systemic TNF-α/CRP etc.

Endothelial dysfunction

Buckley, C. D. et al., Rheumatology 2005; 44:860-67
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Acknowledgments

- Anna Ghirardello
- Luca Iaccarino
- Sandra Zampieri
- Silvia Arienti
- Maria Elisa Rampuda
- Nicola Bassi
- Yheuda Shoenfeld
- Paolo Pauletto
- Pier Luigi Meroni
- Roberto Gerli
- Angela Tincani