Innate Immunity in Atherosclerosis

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Characteristics of Atherosclerotic Plaques Associated with Various Presentations of Coronary Artery Disease

We tend to face today’s battle prepared to fight the last war.

Is the “vulnerable plaque” a valid concept in 2015?
Challenges to the “vulnerable plaque” concept

Few thin-capped plaques actually rupture!
Only 5% of thin-cap fibroatheromas cause events at a median follow-up of 3.4 Years (PROSPECT)

Thin-cap fibroatheromas (TCFA)
Minimal luminal area (MLA)
Plaque burden (PB)

<table>
<thead>
<tr>
<th>Lesion hazard ratio (95% CI)</th>
<th>P value</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCFA (all)</td>
<td>3.90 (2.25–6.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TCFA + MLA ≤4 mm²</td>
<td>6.55 (3.43–12.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TCFA + PB ≥70%</td>
<td>10.83 (5.55–21.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TCFA + PB ≥70% + MLA ≤4 mm²</td>
<td>11.05 (4.39–27.82)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Challenges to the “vulnerable plaque” concept

The character of human plaques is changing in the statin era
Time-Dependent Changes in Atherosclerotic Plaque Composition in Patients Undergoing Carotid Surgery

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**Background**—Time-dependent trends in the incidence of cardiovascular disease have been reported in high-income countries. Because atherosclerosis underlies the majority of cardiovascular diseases, we investigated temporal changes in the composition of atherosclerotic plaques removed from patients undergoing carotid endarterectomy.

**Methods and Results**—The Atero-Express study is an ongoing, longitudinal, vascular biobank study that includes the collection of atherosclerotic plaques of patients undergoing primary carotid endarterectomy in the province of Utrecht from 2002 to 2011. Histopathologic features of plaques of 1583 patients were analyzed in intervals of 2 years. The analysis included quantification of collagen, calcifications, lipid cores, plaque thrombosis, macrophages, smooth muscle cells, and microvessels. Large atheroma, plaque thrombosis, macrophages, and calcifications were less frequently observed over time, with adjusted odds ratios of 0.72 (95% confidence interval, 0.650-0.789), 0.62 (95% confidence interval, 0.569-0.679), 0.87 (95% confidence interval, 0.800-0.940), and 0.75 (95% confidence interval, 0.692-0.816) per 2-year increase in time, respectively. These changes in plaque characteristics were consistently observed in patient subgroups presenting with stroke, transient ischemic attack, ocular symptoms, and asymptomatic patients. Concomitantly, risk factor management and secondary prevention strategies among vascular patients scheduled for carotid endarterectomy significantly improved over the past decade.

**Conclusions**—In conclusion, over the past decade, atherosclerotic plaques harvested during carotid endarterectomy show a time-dependent change in plaque composition characterized by a decrease in features currently believed to be causal for plaque instability. This appears to go hand in hand with improvements in risk factor management. *(Circulation. 2014;129:2269-2276.)*
Human plaques are getting less fatty in the statin era ▼

▲ Human plaques are getting less “inflamed” in the statin era

Figure 1. Semiquantitative plaque information on intraplaque hemorrhage and luminal thrombus (A), fat (B), macrophages (C), and calcification (D) based on the presence or degree of staining.
The character of human plaques is changing

❤️ Statin use is on the rise
ACS Treatments Changing with Time

Aspirin

ASA

Statins

Dual anti-platelet Rx

ACE-I or ARB

β-blockers

Swedish Registry: Hospital Survivors Receiving Specific Medications

Jernberg et al. JAMA 2011;305:1677-1684
Previous Use of Medication on an Outpatient Basis


Yeh RW et al.
Figure 1. Percentage of adults aged 40 and over who reported using a prescription cholesterol-lowering medication: United States, 2003–2012

1Significant linear trend (p < 0.01).
NOTE: Age-adjusted by direct method to the year 2000 projected U.S. population.
Challenges to the “vulnerable plaque” concept

The risk profile and demographics of ACS patients is shifting worldwide (global burden, younger patients, more women, more insulin resistance/diabetes, more hypertriglyceridemia, less LDL excess)

Statin treatment and other preventive measures have begun to modify atherosclerotic disease
The Changing Face of the Acute Coronary Syndromes

What mechanisms beyond plaque rupture may contribute to the residual burden of events in the current era?
The Acute Coronary Syndromes are Changing Before Our Eyes

Fewer STEMI, more NSTEMI

Less rupture, more erosion?

The Changing Face of the Acute Coronary Syndromes

Is innate immunity involved in superficial erosion?
Potential mechanisms of plaque erosion

Plaque erosion associates with disturbed flow

Figure 1. Typical longitudinal section of a carotid plaque showing upstream and downstream parts.

| Apoptosis of Luminal Endothelial Cells in Upstream and Downstream Parts of Human Carotid Atherosclerotic Plaques |
|---------------------------------------------------|------------------|
|                                                   | Upstream | Downstream |
| Counted endothelial cells                        | 360±64    | 331±68     |
| Apoptotic cells                                  | 10.4±3.9  | 69.3±16.0  |
| Percentage of apoptotic cells                    | 2.7±1.2   | 18.8±3.3*  |

Values are expressed as means±SEM.
*P<0.001 (n=8).

Tricot et al, Circ, 2000
Potential mechanisms of plaque erosion
Biomechanics, proteoglycan, and hyaluronan in erosion

Mechanical Strain Induces Specific Changes in the Synthesis and Organization of Proteoglycans by Vascular Smooth Muscle Cells*

Received for publication, November 22, 2000, and in revised form, January 11, 2001
Published, JBC Papers in Press, January 29, 2001, DOI 10.1074/jbc.M010556200

Richard T. Lee‡,§, Chika Yamamoto¶, Yajun Feng‡, Susan Potter-Perigo¶, William H. Briggs‡, Katherine T. Landschulz¶, Thomas G. Turi¶, John F. Thompson¶, Peter Libby‡, and Thomas N. Wight¶

Differential Accumulation of Proteoglycans and Hyaluronan in Culprit Lesions
Insights Into Plaque Erosion

Frank D. Kolodgie, Allen P. Burke, Andrew Farb, Deena K. Weber, Robert Kutys, Thomas N. Wight, Renu Virmani

Lee et al. JBC, 2001
Kolodddie et al. ATVB 2002
Potential mechanisms of plaque erosion

Hyaluronan and TLR2 function in endothelium under disturbed flow in atherosclerosis

Hyaluronan Fragments Act as an Endogenous Danger Signal by Engaging TLR2

Kara A. Scheibner,* Michael A. Lutz, † Sada Boodoo,* Matthew J. Fenton, 2‡ Jonathan D. Powell, † and Maureen R. Horton 3*
TLR2 and neutrophils potentiate endothelial stress, apoptosis and detachment: implications for superficial erosion

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Received 9 December 2014; revised 30 January 2015; accepted 1 February 2015
Hyaluronan promotes endothelial apoptosis and inflammation
Potential mechanisms of plaque erosion

TLR2 and neutrophils potentiate endothelial stress, apoptosis and detachment: implications for superficial erosion

Thibaut Quillard¹,², Haniel Alves Araújo¹, Gregory Franck¹, Eugenia Shvartz¹, Galina Sukhova¹, and Peter Libby¹*

Quillard et al. EHJ, 2015
TLR2 LIGATION AND NEUTROPHILS IMPAIR ENDOTHELIAL ADHERENCE

Quillard et al. EHJ, 2015
TLR2 agonists retard repair of wounded monolayer of human endothelial cells

Quillard et al. EHJ, 2015
Activated ECs can induce nets and become susceptible to NETosis-mediated cell death

Neutrophil extracellular traps (NETs)  

*Fuchs et al, ATVB, 2012*
Do NETs associate with human plaques with features of erosion?

Tissue Bank Carotid endarterectomy
n=295

Plaques with no procedural damage during surgery/tissue manipulation
n=209

Plaques without thrombosis
n=163

Excluded
Damaged tissue
(n = 86)

Excluded
Thrombus
(n = 46)

Excluded Other morphologies
(n = 104)

Lesions with:
≥+++ Macrophages
≤+ SMC
n=21

Macrophage rich SMC poor plaques
n=21

“Fibrous” plaques
n=22

≤1.0 apoptotic luminal Ecs/μm (×10⁻³)
≤1% apoptotic luminal Ecs

Lesions with:
≤+ Macrophages
≥+++ SMC
n=38

“Erosion-like” plaques
n=16

≥1.0 apoptotic luminal Ecs/μm (×10⁻³)
≥10% apoptotic luminal Ecs
Human plaques with erosion characteristics associate with NETS

« Stable »

« Erosion-prone »

« Rupture-prone »

Neutrophil elastase
Citrullinated histones
CD31
TLR2

Quillard et al. EHJ, 2015
EC apoptosis associates with luminal PMNs and TLR2 expression only in SMC-rich lesions

<table>
<thead>
<tr>
<th></th>
<th>SMC-rich plaques</th>
<th>Rupture-prone plaques</th>
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</thead>
<tbody>
<tr>
<td><strong>PMN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN/μm</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>EC apoptosis (%)</td>
<td>0.0000 - 0.0010</td>
<td>0.0000 - 0.0008</td>
</tr>
<tr>
<td>r</td>
<td>0.22</td>
<td>0.01</td>
</tr>
<tr>
<td>p</td>
<td>0.01</td>
<td>ns</td>
</tr>
<tr>
<td><strong>NETs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NETs/μm</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>EC apoptosis (%)</td>
<td>0.0000 - 0.0003</td>
<td>0.0000 - 0.0002</td>
</tr>
<tr>
<td>r</td>
<td>0.59</td>
<td>0.11</td>
</tr>
<tr>
<td>p</td>
<td>0.02</td>
<td>ns</td>
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<tr>
<td><strong>TLR2</strong></td>
<td></td>
<td></td>
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<tr>
<td>TLR2 (%)</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>EC apoptosis (%)</td>
<td>0.0000 - 0.0200</td>
<td>0.0000 - 0.0100</td>
</tr>
<tr>
<td>r</td>
<td>0.28</td>
<td>0.008</td>
</tr>
<tr>
<td>p</td>
<td>0.006</td>
<td>ns</td>
</tr>
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Quillard et al. EHJ, 2015
Neutrophils localize in erosion-like lesions

Quillard et al. EHJ, 2015
Neutrophil Extracellular Traps (NETs) in Human Atherosclerotic Plaques

Quillard et al. EHJ June 2015
The frond-like processes extending into the lumen from the intimal surface of a human carotid atheroma exemplify neutrophil extracellular traps (NETs) derived from DNA extruded by dying granulocytes. This merged immunofluorescent micrograph shows neutrophil elastase (green), citrullinated histone-4 (red), and nuclei (blue). See figure legend on page 0000.
The innate immune receptor TLR2 promotes endothelial functions related to superficial erosion. Quillard et al. EHJ 2015
Implication of the Innate Immune Receptor TLR2 in Plaque Superficial Erosion

- Promotes endothelial (EC) death
- Promotes EC desquamation
- Impairs EC monolayer healing
- Sets the stage for formation of neutrophil extracellular traps
The Changing Face of the Acute Coronary Syndromes

- Superficial erosion may be on the rise
- Innate immunity may drive superficial erosion