New paradigms in human atherothrombotic disease

CHU X. Bichat, 75018 Paris

No conflict of interest
Topophysiology of the arterial wall

Nothing makes sense in circulation except in light of hemodynamics

- conjunctive tissue
- vasa vasorum
- leukocytes

- elastin
- avascular
- no leukocyte

EEL

IEL

Adventitia

Media

Intima

Longitudinal shear

Hydraulic conductance

Peripheral resistances

Circulating blood

Radial mass transport

Interstitial Pressure

Vessel components

Glycosaminoglycans

Hydrophobic elastin

Permeability
Evolutive Stages of Human Atheroma

Normal | Fatty streaks | Fibrolipidic streaks | Plaques | Vulnerable plaques

Stary classification | Clinical complications
Questions:
- fonctions endo/phagocytaires
- mécanisme d’oxydation
- mécanisme d’angiogénèse
- saignements intraplaques
Early lipid intimal deposits

- Fatty streaks
- FibroLipidic plaque
- Cellular Fatty Streaks
- Acellular lipid core
Fatty Streaks: Intimal GAG for cell proliferation and lipid retention

Alcian blue (GAG)  Cellular background

cell nuclei (DAPI)  SM22 α  SM myosin
From cellular fatty streaks to acellular lipid core: SM foam Cell formation and death

SM Cell death

Extracellular cholesterol crystals
### Polarized light

<table>
<thead>
<tr>
<th></th>
<th>Healthy segments</th>
<th>Fatty streaks</th>
<th>Fibrolipidic lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid crystalline lipid inclusions</td>
<td>1/18</td>
<td>17/19***</td>
<td>23/23***</td>
</tr>
<tr>
<td>Needle-like crystals</td>
<td>0/18</td>
<td>2/19</td>
<td>20/23***</td>
</tr>
<tr>
<td>Plate-like crystals</td>
<td>0/18</td>
<td>2/19</td>
<td>18/23***</td>
</tr>
</tbody>
</table>

**Images:**
- **C:** Liquid crystal
- **D:** Solid crystal (plate)
- **Before freezing:** Intimal lesion
- **After freezing:** Intimal lesion
Oxydation: Human fatty streaks
Glycophorin A, a tissue marker of RBC entosis, degradation and finally release.
Ceroids = oxidation-induced protein aggregates ± lipids

**Fatty streaks**

**Fibrolipidic plaques**

- Oil Red O fluorescence
- Peroxidation (DAB)

- Autofluorescence (550nm)
- Glycophorin A
Erythrophagocytosis: VSMC biology

DAB (diaminobenzidine)

DAB + Perl’s

CD68

Oil Red O

RBC clearance

Number of RBCs in culture supernatant (x10^6)

Time (days)

Control (no RBC)

Fresh RBC

Senescent RBC

Human VSMC

Fluorescent RBC
Spatial early angiogenesis in fibrolipidic lesions

Tanaka K et al. Atherosclerosis 2011

In mice

No angiogenesis

In human

No lesion

Lesion

In human
Mediators of angiogenesis: VEGF

- Media
- SMC myosin
- VEGF

Lesion

![Image of atherosclerotic aorta with VEGF expression and statistical analysis of VEGF levels in different conditions: Healthy aortas, Media, Lesion (Atherosclerotic aortas).](image)

Statistical analysis: NS (p=0.77), *p<0.02, *p<0.01
VEGF expression in cultured Human SMC in response to wall conditionned media

IntraPlaque Hemorrhages as the consequence of centripetal neo-angiogenesis

media

luminal  abluminal

Lipid mediator
PPAR-γ
VEGF

bleeding
immaturity
angiogenesis

sprouting of adventitial vessels

Ho-Tin-Noé B et al. Circ. Res. 2011
Intraplaque hemorrhages are the main determinant of plaque vulnerability in MRI (Takaya N & Hatsukami TS 2005).

Galen 131-201 A.D.

Cranial atheroma (atheroma = gruel)

Clinics

in MRI

(Takaya N & Hatsukami TS 2005)

Pronostic value

(Hellings WE & Pasterkamp G 2010)
Masson’s Trichrome

Perl’s Iron Stain
Conclusions:
- CML endo/phagocytes principaux
- Le trafic GR est le principal oxydant
- L’angiogénèse est dépendante des lipides
- Les IPH sont le lien de la pathologie à l'expression clinique de la maladie