Inflammation and extracellular proteinases
Plaque rupture 75% of MI (heart attack)

Thin fibrous cap (<65 µm), large lipid core, no collagen, macrophages, T-cells

Davies, M Circulation 1996; Falk, E JACC 2006;47:C7-12
Long-term consequences of MI

Cardiac rupture

Too little Inflammation/repair

Heart failure

Too much inflammation

www.readcube.com

stanfordhospital.org
Foam cells make proteases that cause plaque vulnerability.
Metalloproteinases and TIMPs

23 MMPs

Many non-matrix substrates

<table>
<thead>
<tr>
<th>Numbers</th>
<th>MMP class</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMPs 1,8,13</td>
<td>Interstitial collagenases</td>
</tr>
<tr>
<td>MMPs (2),9</td>
<td>Gelatinases (A),B</td>
</tr>
<tr>
<td>MMPs 3,7,10,11</td>
<td>Stromelysins</td>
</tr>
<tr>
<td>MMP 12,19</td>
<td>Metalloelastase +</td>
</tr>
<tr>
<td>MMPs 14-7,23,25</td>
<td>Membrane-type MMPs</td>
</tr>
</tbody>
</table>
Inflammation and macrophage diversity

M1
LPS, CD40L, TNFα, IL-1, IFNγ
Invasion, Phagocytosis, Killing

Ox-LDL?

M2
M-CSF, GM-CSF
IL-4, IL-13
Exit from lesions, Antigen presentation, Fibrosis, Calcification, Angiogenesis

Effect of foam cell formation

Spann et al. Cell 2012; 151, 138–152
Are all mouse models of atherosclerosis Dinosaurs?
Many biochemical differences

- HDL rather than LDL
- SAA rather than CRP
- MMP-13 rather than MMP-1

Many MMP/TIMPs showed 10-10,000 fold Δ

Transcript Levels of MMPs/TIMPs in Mouse vs Human Macrophages
Macrophage phenotypes – the future

"I see a girl, I see a marriage, I see her not understanding you, I see a beer belly. Do you want me to go on?"
Follow the pathways!

David Hockney
# MCSF, GMCSF and OxLDL

<table>
<thead>
<tr>
<th>Old name</th>
<th>M-Mac</th>
<th>GM-Mac</th>
<th>Foam cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed name</td>
<td>MM(-CSF)</td>
<td>MGM(-CSF)</td>
<td>FM(OxLDL)</td>
</tr>
<tr>
<td></td>
<td>CSFR1/CD115</td>
<td>CSFR2/CD116</td>
<td>Scavenger Rs</td>
</tr>
<tr>
<td>Signal 1</td>
<td>ERKs, PI-3K</td>
<td>ERKs, PI-3K</td>
<td>Free cholesterol, desmosterol</td>
</tr>
<tr>
<td>TFs</td>
<td>Pu.1, Egr-1</td>
<td>Pu.1, Egr-1</td>
<td>LXRα,β SREBPs</td>
</tr>
<tr>
<td></td>
<td>AP-1</td>
<td>AP-1, STAT-5</td>
<td></td>
</tr>
<tr>
<td>Markers</td>
<td>CD206</td>
<td>CD206</td>
<td>CD11c, Foamy appearance</td>
</tr>
<tr>
<td>Proposed marker</td>
<td>Nuclear STAT-5P</td>
<td>Adipophilin</td>
<td></td>
</tr>
</tbody>
</table>
Classical activation pathways

LPS

IL-1

TNFα

TRIF

MyD88

TRAF

Production of interferons
Classical activation pathways

LPS

IL-1

TNFα

TRIF

MyD88

TRAF

IFNs

JAK-2

STAT1
## Classically-activated (M1) phenotypes

<table>
<thead>
<tr>
<th>Proposed name</th>
<th>MTNFα</th>
<th>MCD30L/CD40L/Ox40L</th>
<th>MIL-1</th>
<th>MLPS (TLL)</th>
<th>MIFNγ</th>
<th>(M1) MLPS +IFNγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>TNFRSF</td>
<td>IL1R1</td>
<td>TLR-4 (TLRs)</td>
<td>IFNγR1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Signal 1</td>
<td>TRAFs</td>
<td>Myd88</td>
<td>Myd88 IRF-3</td>
<td>Jak1/2, IRF-3</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>Signal 2</td>
<td>ERKs, PI3K, PKC, IKK2</td>
<td></td>
<td></td>
<td>STAT-1</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>TFs</td>
<td>AP-1, NF-κB</td>
<td></td>
<td></td>
<td>STAT-1</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>Markers?</td>
<td>IκB, COX-2, iNOS</td>
<td></td>
<td></td>
<td>IL12, TLR4, CD14</td>
<td>Both</td>
<td></td>
</tr>
<tr>
<td>Proposed marker</td>
<td>Nuclear NF-κB</td>
<td></td>
<td></td>
<td>Nuclear STAT1-P</td>
<td>Both</td>
<td></td>
</tr>
</tbody>
</table>
Human Foam cells stimulated by LPS + IFNγ in vitro (M1)

Huang, Sala-Newby et. al. unpublished
Compare Huang, Sala-Newby et. al. PLoS ONE 2012
Human foam cells stimulated by LPS + IFN$\gamma$ (M1) in vitro

Compare Huang, Sala-Newby et. al. PLoS ONE 2012
Effects of classical activators

Huang, Sala-Newby et. al. PLoS ONE 2012
LPS on MAP and IκB kinases

<table>
<thead>
<tr>
<th>Pretreat</th>
<th>DMSO</th>
<th>LPS</th>
<th>PD</th>
<th>SB</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- **p42-44 ERK(p)**
- **P42-44 ERK**
- **p38(p)**
- **p38**
- **JNK(p)**
- **JNK**

- **SC-514**
  - -
  +

- **IκBα**

- **GAPDH**

- **LPS**
  - + +
Effect of inhibitors on LPS

Huang, Sala-Newby and Newby, PLoS One 2012
Co-localisation in human coronary macrophages

Huang, Sala-Newby and Newby, PLoS One 2012
Co-localisation in human coronary macrophages

Huang, Sala-Newby and Newby, PLoS One 2012
MMP-14 and NF-κB

Johnson, Jenkins et al, unpublished
Role of TLR2

Effect of JAK-2 inhibitor on IFNγ

Huang, Sala-Newby and Newby, PLoS One 2012
Conclusions 1

• M1 genes are regulated through NFκB in vitro and co-localize in vivo
• A subgroup may co-localize with Stat-1P (to be shown)
IL-4 and IL-13 signalling

Ingram JL, Journal of Allergy and Clinical Immunology. 2012;130:829-842
## Alternatively-activated (M2) phenotypes

<table>
<thead>
<tr>
<th>Old name</th>
<th>M2a</th>
<th>M2b</th>
<th>M2c</th>
<th>Fibrocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed name</td>
<td>MIL4/13</td>
<td>MNHR</td>
<td>MIL10</td>
<td>MTGFβ</td>
</tr>
<tr>
<td>Receptor</td>
<td>IL-4RF</td>
<td>GCR, PPAR, Nur77</td>
<td>IL10R</td>
<td>TGFβRI/II</td>
</tr>
<tr>
<td>TFs</td>
<td>STAT-6, KLF4</td>
<td>NHR</td>
<td>STAT-3</td>
<td>SMADs2/3 FosB</td>
</tr>
<tr>
<td>Markers</td>
<td>CD206, COX-1, maf-1</td>
<td>CD163</td>
<td>CD163</td>
<td>Collagen, fibronectin</td>
</tr>
<tr>
<td>Proposed marker</td>
<td>Nuclear STAT6-P</td>
<td></td>
<td></td>
<td>Nuclear SMAD2-P</td>
</tr>
</tbody>
</table>

| 7 | 8 | 9 | 10 |
Foam cells stimulated by IL-4 (M2)

Compare Huang, Sala-Newby et al. PLoS ONE 2012
IL-4 up-regulates MMP-12 via STAT6

Sala-Newby and Newby, unpublished
TIMP-3 and CD206

Johnson, Jenkins et al, unpublished
Is IL4/stat6 up-regulating CD206 in plaques?

Johnson and Newby, unpublished
Genomic studies on hypoxic macrophages

Gene fold change
SERPINB2 699.5
ANKRD1 343.5
IL1A 212.9
IL1B 148.8
LRRC50 88.6
CA12 84.1
CXCL5 69.5
MMP1 67.6
MMP10 35.8
MMP3 11.6
MMP12 3.93

Gene fold change
DDIT4 104.1
ADM 52.2
SLC2A3 30.7
TSPAN6 27.7
NR4A3 19.2
MT1X 18.7
NEFL 15.6
GSTA2 15
RGS2 14.1
IL8 13.9
MMP12 3.22
Hypoxia and MMP mRNAs *in vitro*

**Effect of hypoxia**

- MMP1
- MMP9
- MMP12
- MMP14
- COX2
- VEGFA
- IkB

**Effect of hypoxia**

- MMP1
- MMP-12

Sala-Newby, et al, unpublished
Hypoxia induced MMP-12 protein independent of Stat-6

Sala-Newby et al, unpublished
# Phenotypes related to oxidation

<table>
<thead>
<tr>
<th>Name</th>
<th>Mox</th>
<th>Mhem</th>
<th>Mhypox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>ScavengerRs</td>
<td>CD163</td>
<td></td>
</tr>
<tr>
<td>Signal 1</td>
<td>ROS</td>
<td>ROS</td>
<td>HIF1/2α, ERKs, IKK2, PI3K, wnts</td>
</tr>
<tr>
<td>TFs</td>
<td>Nrf2, low NF-κB</td>
<td>Nrf2, ATF-1, low NF-κB</td>
<td>HIF1/2α, NF-κB, TCF/Lef</td>
</tr>
<tr>
<td>Markers</td>
<td>HO-1</td>
<td>HO-1, CD163, Low MHCII</td>
<td>VEGF, Glut-1</td>
</tr>
<tr>
<td>Proposed marker</td>
<td>?</td>
<td>?</td>
<td>Nuclear HIF1α</td>
</tr>
</tbody>
</table>

14 15 16
Conclusions 2

• ‘M2’ genes are regulated through Stat-6 in vitro but do not necessarily co-localize in vivo
• There are a lot more definable phenotypes than M1 and M2
• Follow the pathways to greater wisdom!
Thanks

Buket Reel  Graciela Newby

David Huang

BHI
Bristol Heart Institute

NHS
National Institute for Health Research

British Heart Foundation