Inflammation in Heart Failure

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Inflammatory diseases of the heart involve inflammation of the heart muscle and/or the tissue surrounding it.

Myocarditis - inflammation of the myocardium, the muscular part of the heart.

Ischemic heart disease - myocardial infarction.

Endocarditis - inflammation of the inner layer of the heart, the endocardium. The most common structures involved are the heart valves.

Acute pericarditis is an inflammation of the sac surrounding the heart --- the pericardium.
Myocardial infarction and ischemic cardiomyopathy

- Blockade of a coronary artery
- Loss of cardiac muscle (necrosis, apoptosis)

Inflammation
- Hypertrophy
- Fibrosis
Advances in interventional therapies have led to a significant decline in mortality during the acute phase of MI, especially in patients with large MI.

Unfortunately this decrease in mortality is paralleled by an increase in the incidence of heart failure in patients surviving with significant residual myocardial damage.
Anterior Myocardial Infarction: *the Remodeling Process*

**Pat. I.L., 57 years**

<table>
<thead>
<tr>
<th></th>
<th>LVEDV</th>
<th>LVESV</th>
<th>LVEF</th>
</tr>
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<tbody>
<tr>
<td>Initial</td>
<td>142ml</td>
<td>59ml</td>
<td>58%</td>
</tr>
<tr>
<td>5 Years</td>
<td>154ml</td>
<td>64ml</td>
<td>59%</td>
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Anterior Myocardial Infarction: *the Remodeling Process*

**Why substantial adverse remodelling in patient #2 but not #1?**

*Pat. S.R., 46 years*

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<tr>
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<tbody>
<tr>
<td>Initial</td>
<td>198ml</td>
<td>79ml</td>
<td>59%</td>
</tr>
<tr>
<td>5 Years</td>
<td>246ml</td>
<td>145ml</td>
<td>41%</td>
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Phases following myocardial infarction

Phase 1
Cardiac myocyte death

Phase 2
Acute inflammation

Phase 3
Granulation tissue formation

Phase 4
Scar formation
Blocking inflammation after MI?

Failed!

Deleterious effects of methylprednisolone (steroid therapy) in patients with myocardial infarction.
Enhanced expression of cardiac and systemic expression of the pro-inflammatory cytokines for example TNF-$\alpha$

- TNF-$\alpha$-serum levels increase in patients with severity of the heart failure
- Mechanical unloading improves cardiac function and reduces TNF-$\alpha$ serum levels
Cardiac failure in transgenic mice with myocardial expression of TNF-\(\alpha\)

Magnetic Resonance Imaging (MRI) and invasive evaluation of development of Heart Failure in transgenic mice with myocardial expression of TNF-\(\alpha\). Bryant et al, Circulation 1998

Serial MRI studies in the TNF-\(\alpha\) mouse model demonstrate that the rate of progression and severity of LV Dysfunction are dependant on the degree of TNF-\(\alpha\) overexpression. Franco et al, Circulation 1999
Endogenous TNF-α protects the heart against ischemic-induced cardiomyocyte death in a mouse model of acute myocardial infarction

Double knockout of the two TNF-α receptors leads to increased infarct size in acute infarction.

Kurrelmeyer et al. PNAS 2000
Therapeutic approach to neutralize TNF-α

Mechanism of action of TNF-α antagonists. The monoclonal antibody (infliximab, adalimumab) and the receptor analog (etanercept) bind to circulating TNF-α and block its interaction with membrane receptor.

Experimental studies in animals and in small patient collectives were promising.

Randomized multi-center studies?
RENEWAL
All-cause Mortality and CHF Hospitalisations was not lowered by neutralizing TNF-\(\alpha\)
Check points involved in impaired healing after myocardial infarction

Nahrendorf et al. Circulation 2010
Development of monocytes, macrophages and dendritic cells
Geissmann et al. Science 2010

Fig. 2. Differentiation of DCs and macrophages in mice. In the bone marrow, hematopoietic stem cells (HSC) produce myeloid (MP) and lymphoid (LP) committed precursors. MP give rise to monocyte/macrophages and DC precursors (MDP). MDP give rise to monocytes, and to common DC precursor (CDP). Two monocyte subsets, Ly-6C<sup>−</sup> and Ly-6C<sup>+</sup> leave the bone marrow to enter the blood. CDP give rise to pre-classical dendritic cells (pre-cDC) and plasmacytoid dendritic cells (PDC). Pre-cDC circulate in blood and enter lymphoid tissue, where they give rise to CD8α<sup>+</sup> and CD8α<sup>+</sup> cDCs, and non-lymphoid tissues, where they may give rise to CD103<sup>+</sup> lamina propria DC (pDC). Under homeostatic conditions, Ly-6C<sup>−</sup> monocytes may contribute to alveolar macrophages (MΦ) and Ly-6C<sup>+</sup> monocytes can become CX<sub>3</sub>CR1<sup>−</sup> pDCs in non-lymphoid tissues. During inflammation, Ly-6C<sup>−</sup> monocytes give rise to monocyte-derived DCs, e.g. TNF and iNOS-producing dendritic cells (TipDC), inflammatory macrophages, and may contribute to myeloid-derived suppressor cells (MDSC) associated with tumors. They are also suspected to contribute to microglia and Langerhans cells in selected experimental conditions. Microglia and Langerhans cells can renew independently from the bone marrow (curved arrow). HSC can also leave their bone marrow niche and enter peripheral tissues, where they differentiate to myeloid cells during inflammation. It is unclear at this time if LP contribute significantly to PDC and cDCs (dashed arrow).
Identification of Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites
Swirski et al. Science 2009
Targeted Deletion of CC Chemokine Receptor 2 Attenuates Left Ventricular Remodeling after Experimental Myocardial Infarction

Figure 1: A, Representative examples of Masson's trichrome and van Gieson staining at 7 days after experimental MI. Stained slides demonstrate that necrotic centers of infarcted region remain devoid of fibrosis in CCR2-/- mice, with less accumulation of collagen in CCR2-/- mice than in WT mice. B and C, Infarct size (B) and infarct area (C) in WT and CCR2-/- mice. D, Collagen volume fraction from van Gieson-stained myocardium as a percentage of stained tissue in muscle areas and connective tissues in visual fields of the sections. *P < 0.01 versus CCR2-/- mice.

Kaikita et al. American Journal of Pathology, 2004
Figure 2. Immunohistochemical detection of FA-11-positive macrophages in infarcted regions from WT (left) and CCR2−/− (right) mice at 1, 3, 7, 14, and 28 days after experimental MI. Scale bars, 100 μm.

Figure 3. Differences in numbers of FA-11-positive macrophages (A), Gr-1-positive granulocytes (B), B220-positive B cells (C), and Ly-1-positive T cells (D) in infarcted regions from WT and CCR2−/− mice. Data points represent the number of positive cells per 1 mm² in infarcted tissues. * P < 0.01 versus CCR2−/− mice. † P < 0.05 versus WT mice.
CCR2-KO mice display lower MMP activity after infarction that is associated with less dilatation and better cardiac function.
Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure

S. Heymans¹, A. Luttun¹, D. Nuyens³, G. Theilmeier¹, E. Creemers², L. Moons¹,
G.D. Dyspersin², J.P.M. Cleutjens², M. Shipley¹, A. Angellilo¹, M. Levi¹, O. Nube⁵,
A. Baker⁶, E. Keshet⁷, F. Lupu⁸, J-M Herbert⁹, J.F.M. Smits³,
S.D. Shapiro¹, M. Baes¹, M. Borgers⁹, D. Collen¹,
M. J.A.P. Daemen³ & P. Carmeliet¹

Heymans et al. Nature Medicine 1999
Deletion of CCR2 attenuates angiogenesis in injured skeletal muscle and reduces the angiogenic potential of endogenous cardiac progenitor cells.

Delayed angiogenesis and VEGF production in CCR2−/− mice during impaired skeletal muscle regeneration

Oscar Ochoa,1 Dongxu Sun,1 Sara M. Reyes-Reyna,1 Lindsay L. Waite,2 Joel E. Michalek,2 Linda M. McManus,3,4,6 and Paula K. Shireman1,5,6,7
Check points involved in impaired healing after myocardial infarction

Macrophages and subsequent release of cytokines are important for:
- clearing dead cells
- promote angiogenesis
- Induce fibroblast proliferation

Are there regulatory mechanisms that modulate the activity of macrophages at the site of inflammation?
Class A macrophage scavenger receptor (SR-A) is a macrophage restricted multifunctional molecule

ligand: acetyl–low- density lipoprotein)
Increased expression of pro-inflammatory cytokine TNF-α and decreased expression of the anti-inflammatory cytokine IL-10 in SR-A k.o mice

Tsujita et al. Circulation 2007
Differential expression of TIMPs and MMPs is associated with LV rupture in SR-A k.o. mice.
Check points involved in impaired healing after myocardial infarction

Macrophages and subsequent release of cytokines are important for:
- clearing dead cells
- promote angiogenesis
- Induce fibroblast proliferation

SR-A modulates the activity of macrophages at the site of inflammation.

Are there subtypes of inflammatory cells, that may serve as modulators of inflammation?
The immune system has developed mechanisms for tolerance to prevent allergy, autoimmunity etc. for which regulatory T cells (Treg) play an important role.

Regulatory T cells (Treg, sometimes known as suppressor T cells) are a specialized subpopulation of T cells suppress activation of the immune system and thereby maintaining immune system homeostasis and tolerance to self-antigens.

Treg suppress immune responses of other cells, an important "self-check" built into the immune system to prevent excessive reactions.

CD8+ Treg, CD4, CD25, and Foxp3 Treg; and other T cell types that have suppressive function.

All T cells come from progenitor cells from the bone marrow, which become committed to their lineage in the thymus.

CCR5+ foxp3+ Tregs is a CD4+ lymphocyte subset with potent anti-inflammatory properties.
No obvious difference in number of CD45+ inflammatory cells!

Figure 2. CCR5-null and wild-type (WT) infarcts exhibit comparable infiltration with macrophages. A–D: Mac2 immunohistochemistry identified macrophages in wild-type (A, C) and CCR5 KO infarcts (B, D) after 24 hours (A, B) and 72 hours of reperfusion (C, D). E: Quantitative analysis showed no significant differences in macrophage density between CCR5−/− and wild-type infarcts at all time points examined (n = 9 mice/group).
Lack of CCR5 positive inflammatory cells reduces invasion of T-regulatory cells (Tregs) into the infarcted heart

Dobaczewski et al. American Journal of Pathology 2010
Lack of anti-inflammatory Tregs enhances MMP activity and leads to dilatation and dysfunction of the infarcted heart

Dobaczewski et al. American Journal of Pathology 2010
Check points involved in impaired healing after myocardial infarction

Macrophages and subsequent release of cytokines are important for:
- clearing dead cells
- promote angiogenesis
- Induce fibroblast proliferation

SR-A modulates the activity of macrophages at the site of inflammation.

Tregs seem to play a role in modulating post infarct inflammation.

What is the impact of the kinetic of post infarct inflammatory processes and how is it regulated?
The timely regulation of inflammatory processes following infarctions seems a key event for healing versus adverse remodeling.
High serum levels of IL-6 cytokines correlate with an adverse outcome in patients with chronic myocardial infarction

Tsutamoto T. et al., JACC 1998; Tsutamoto T. et al., Eur J Heart Fail. 2007
Complex regulatory network of gp130 downstream signaling

Fischer and Hilfiker-Kleiner BJP 2008
Cardiomyocytes restricted pathway mutations at the gp130 receptor using the CreLoxP system

One wildtype gp130 allele is sufficient to ensure normal gp130 signaling
Dierssen et al. JBC 2008

Hilfiker-Kleiner et al. Circulation 2010
Severely impaired post infarct survival in $Y_{757}F$ mice due to LV rupture and heart failure

*P<0.05, **P<0.01 vs WT MI

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>$Y_{757}F$</th>
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<tbody>
<tr>
<td>EF (%)</td>
<td>39±15$$</td>
<td>22±10$$**</td>
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<tr>
<td>FS (%)</td>
<td>22±11$$</td>
<td>13±6$$*</td>
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*P<0.05, **P<0.01 vs WT MI

Hilfiker-Kleiner et al. Circulation 2010
Genetic reduction of STAT3 in CF/757 mice (by crossing in heterozygous STAT3 +/- mice) normalizes STAT3 activation and protein expression 2 weeks after MI.
Genetic reduction of STAT3 reduces post MI mortality in $Y_{757}F;\text{STAT3}_{\text{low}}$ mice compared to $Y_{757}F$ mice.

**P<0.01 vs WT MI

Hilfiker-Kleiner et al. Circulation 2010
Inflammation and remodeling of the infarcted heart in
dependence of STAT3 levels

CD45+ macrophages

Hilfiker-Kleiner et al. Circulation 2010
Inflammation and remodeling of the infarcted heart in dependence of STAT3 levels: Collagen content of the infarct scar

Hilfiker-Kleiner et al. Circulation 2010
What is the link between unrestricted and up-regulated STAT3 activation and adverse outcome after MI?
Overlay of micro array data STAT3 “gain-of-function” CF/757 versus “hypomorphimic” CF/∆ reveals up-regulation of the MBL-C/complement system

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<tr>
<th>Gene</th>
<th>Fold Change</th>
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<tr>
<td>Es1</td>
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<tr>
<td>Es1</td>
<td>8.34</td>
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<tr>
<td>Kn1</td>
<td>-12.37</td>
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<td>Mbl-C</td>
<td>-4.98</td>
</tr>
<tr>
<td>Mbl-C</td>
<td>6.03</td>
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Reatime PCR of MBL-C mRNA in RM

Hilfiker-Kleiner et al. Circulation 2010
The lectin (MBL) pathway of complement activation
The Mannose-binding Lectin and complement activation: Host defense, innate immunity, cell damage

- Necrosis and apoptotic cells
- Transformed (tumor) cells
- Anoxic endothelial cells

Additional genes up-regulated in infarcted LVs from CF/757 mice: MBL-A (3.3-fold), MASP-2 (3.3-fold), C3 (2.5-fold), C3ar1 (3.3-fold), C4bp (8-fold), C6 (3.8-fold), C8b (7.7-fold) and C8g (2.5-fold)
MBL-C is down-regulated in infarcted WT mice. Activated STAT3 re-induces expression on MBL-C in the sub-acute phase of MI in Y757F.
Reduction of STAT3 attenuates MBL-C and Complement-factor-3 deposition after MI in CF/757 mice

Hilfiker-Kleiner et al. Circulation 2010
Complement inhibition with cobra venom factor (CVF) starting 3 days post MI in CF/757 mice

Cobra Venom Factor (CVF) resembles the C3b degradation product C3c which is unable to form the C3/C5 convertase. As a result, the presence of CVF causes depletion of complement components, thereby exhausting the complement response.
Cobra venom factor (CVF) therapy attenuates cardiac inflammation and improves cardiac function and survival in CF/757 mice.

CD45, brown, H&E

FS in CF/757

2 weeks survival rate in CF/757
100% in CVF, n=11
64% in NaCl, n=11, P<0.01
LV rupture occurred in 18% of NaCl treated mice.

Hilfiker-Kleiner et al. Circulation 2010
Inflammation, MBL-C

LV rupture, cardiac dysfunction, high mortality

Complement activation

Fibrosis

MMP activity

Angiogenesis

Fibrosis

Cytoprotection

Eccentric hypertrophy

STAT3

gp130

Negative regulation: SOCS3, SHP2

Activation IL-6 cytokines

Cytoprotection

Increased angiogenesis

Decreased fibrosis

Adaptive hypertrophy

SOCS3, SHP2

STAT3
Check points involved in impaired healing after myocardial infarction

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SR-A modulate the activity of macrophages at the site of inflammation.

Tregs seem to play a role in modulating of post infarct inflammation.

The IL-6-gp130 signaling is an important regulator of the kinetic of post infarct inflammation. It involves both, systemic and cardiac components.