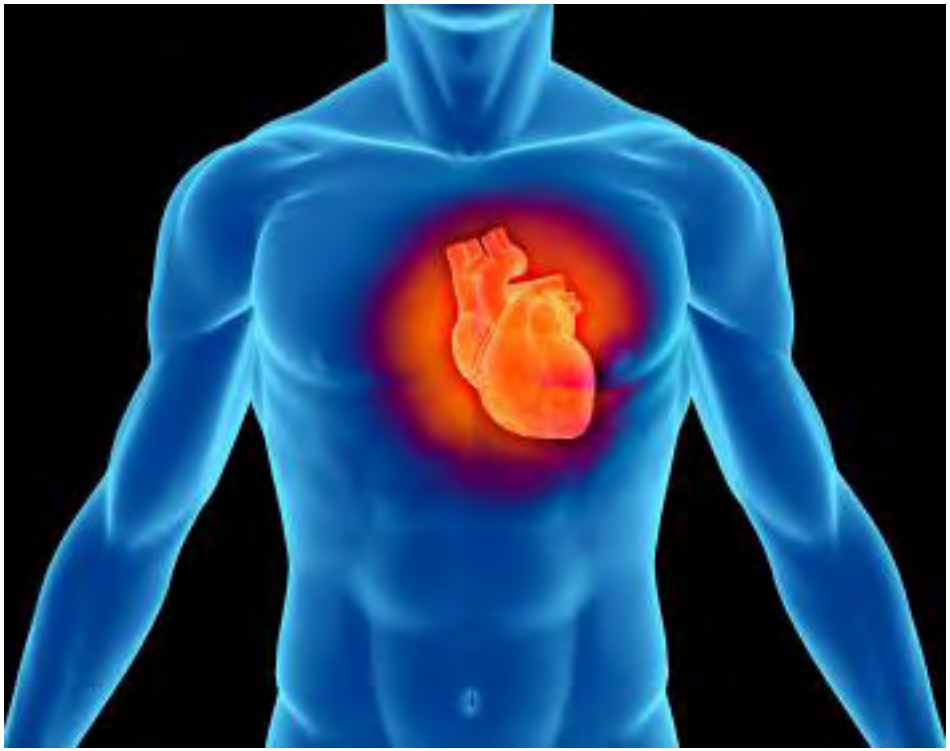
Inflammation in Heart Failure



Denise Hilfiker

Kardiologie & Angiologie

MHH, Hannover

Germany

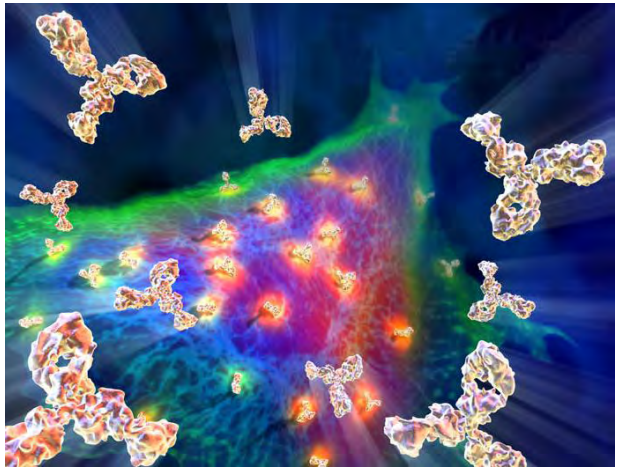
Inflammatory diseases of the heart involve inflammation of the heart muscle and/or the tissue surrounding it.

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

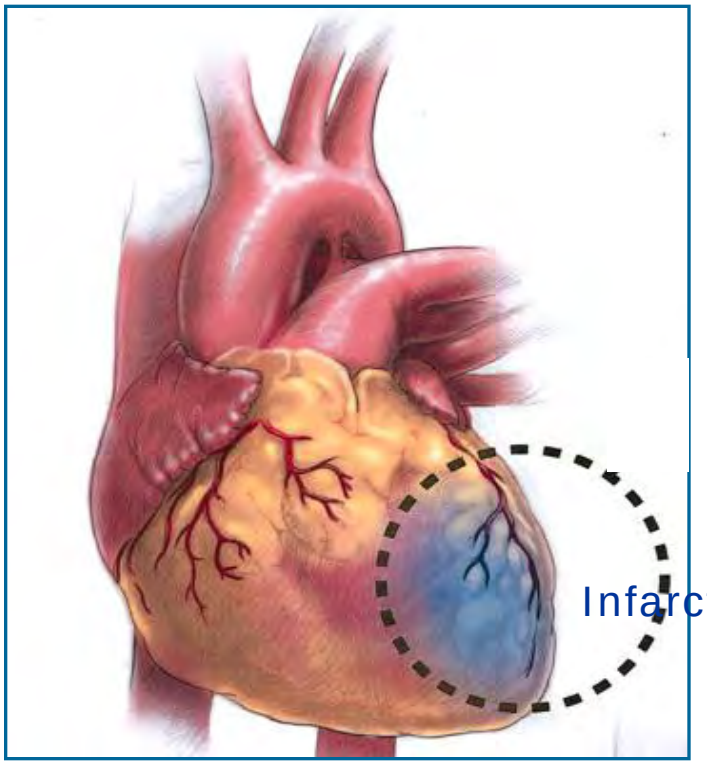
Ischemic heart disease - myocardial infarction.

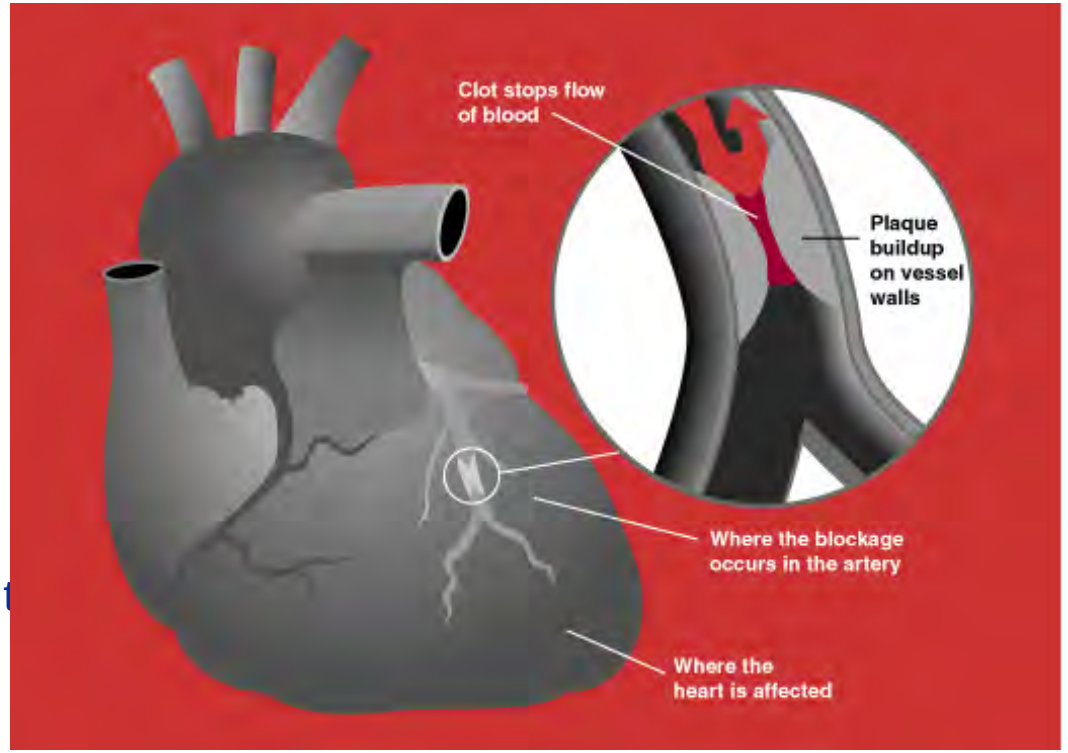
Endocarditis - inflammation of the inner layer of the heart, the endocardium. The most common structures involved are the heart valves.

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

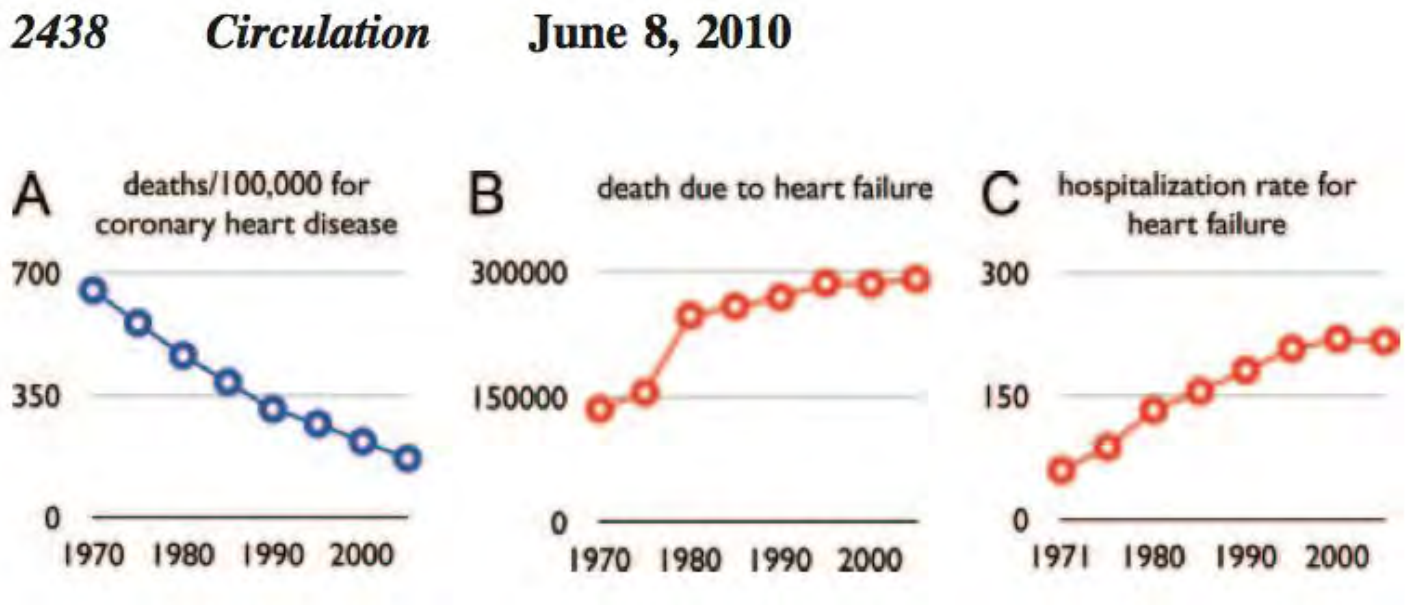
Inflammation

hypertrophy

fibrosis

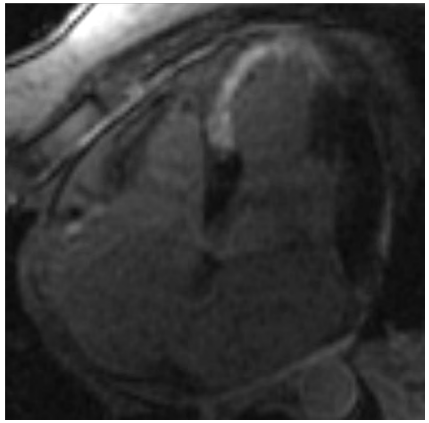
Advances in interventional therapies have led to a significant decline in mortality during the acute phase of MI, especially in patients with large MI.

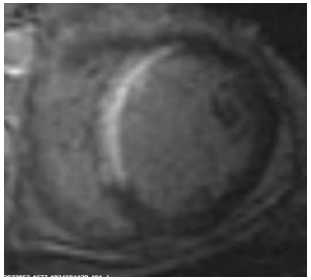
Unfortunately this decrease in mortality is paralleled by an increase in the incidence of heart failure in patients surviving with significant residual myocardial damage.



Nahrendorf et al. Circulation 2010

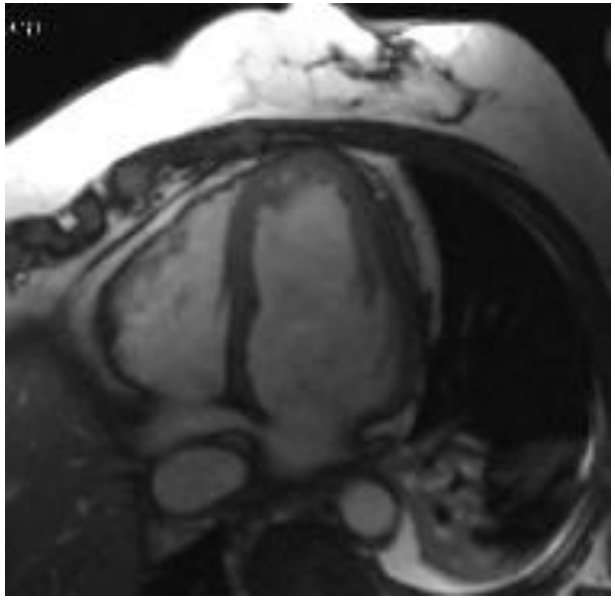
Anterior Myocardial Infarction: *the Remodeling Process*

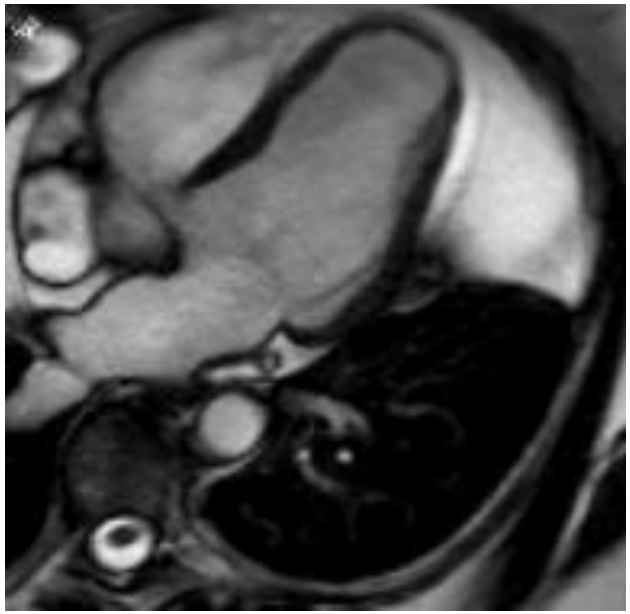




Pat. I.L., 57 years

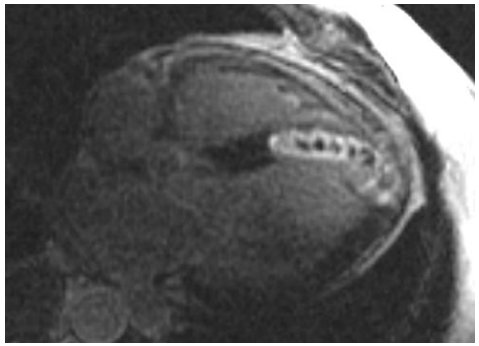
	LVEDV	LVESV	LVEF
Initial	**142ml**	**59ml**	**58%**
5 Years	**154ml**	**64ml**	**59%**

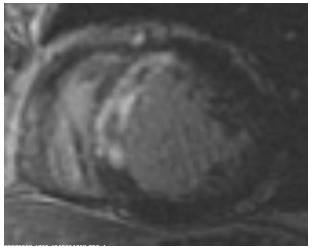




Anterior Myocardial Infarction: *the Remodeling Process*

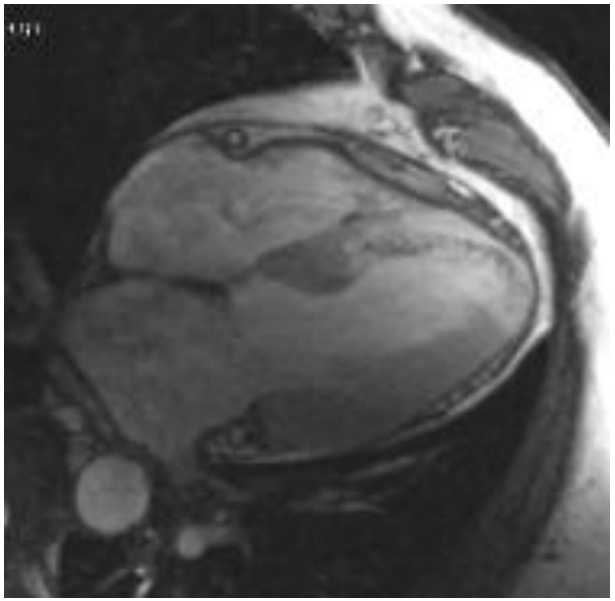
Why substantial adverse remodelling in patient #2 but not #1 ?

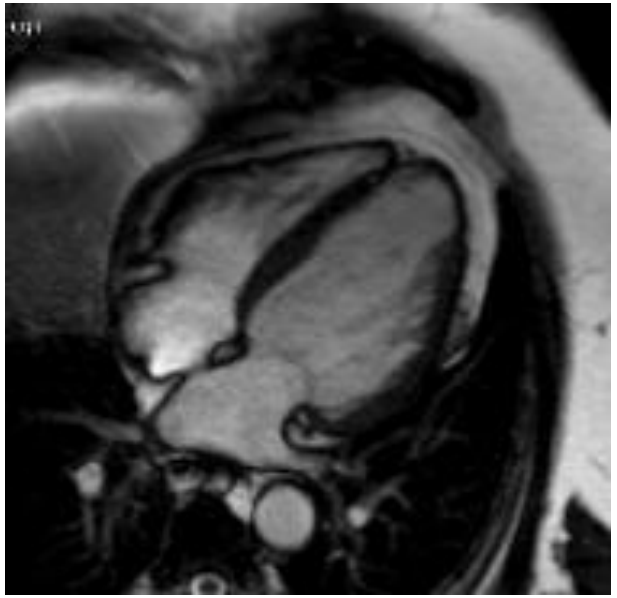




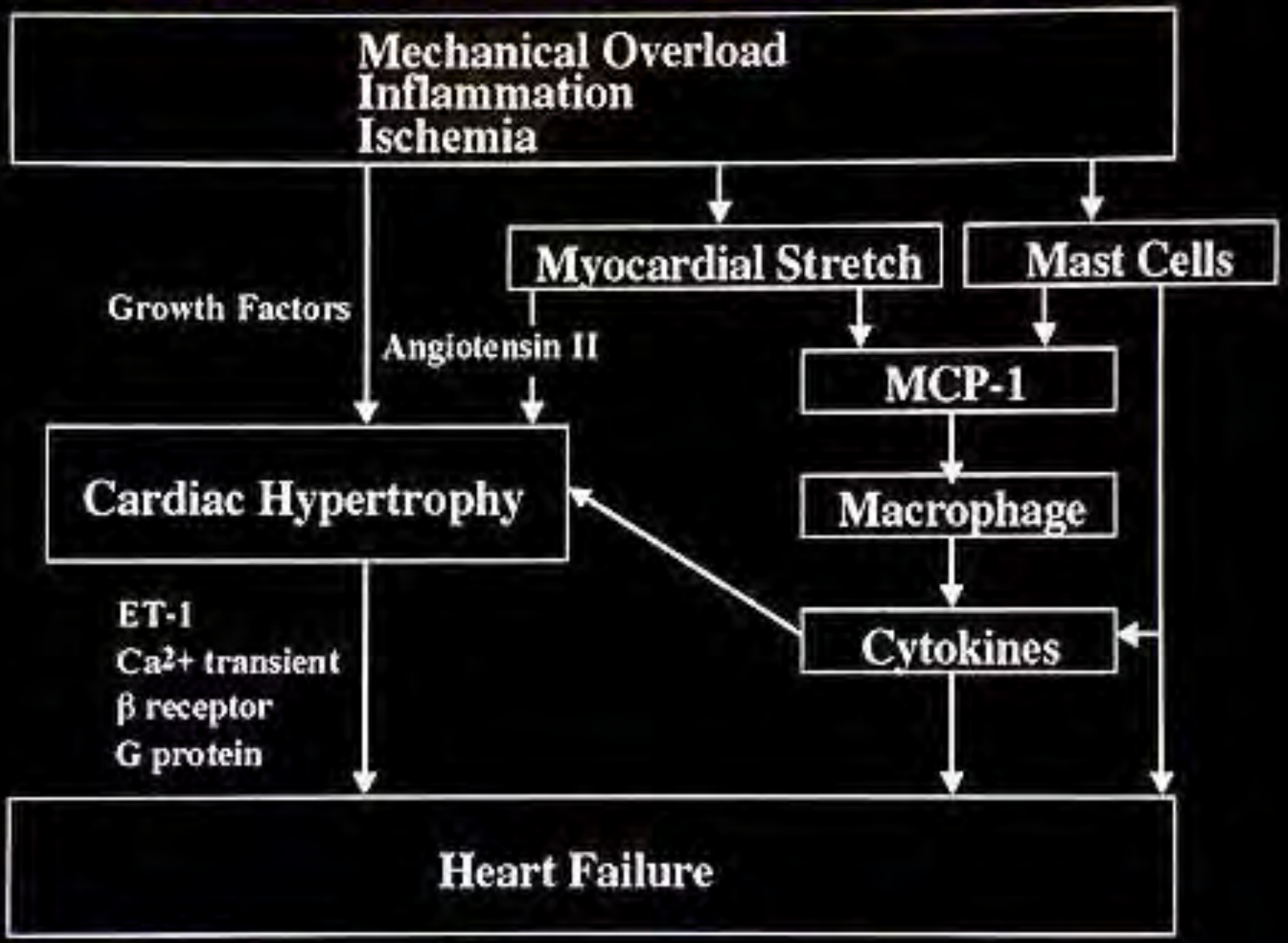
Pat. S.R., 46 years

	LVEDV	LVESV	LVEF
Initial	**198ml**	**79ml**	**59%**
5 Years	**246ml**	**145ml**	**41%**

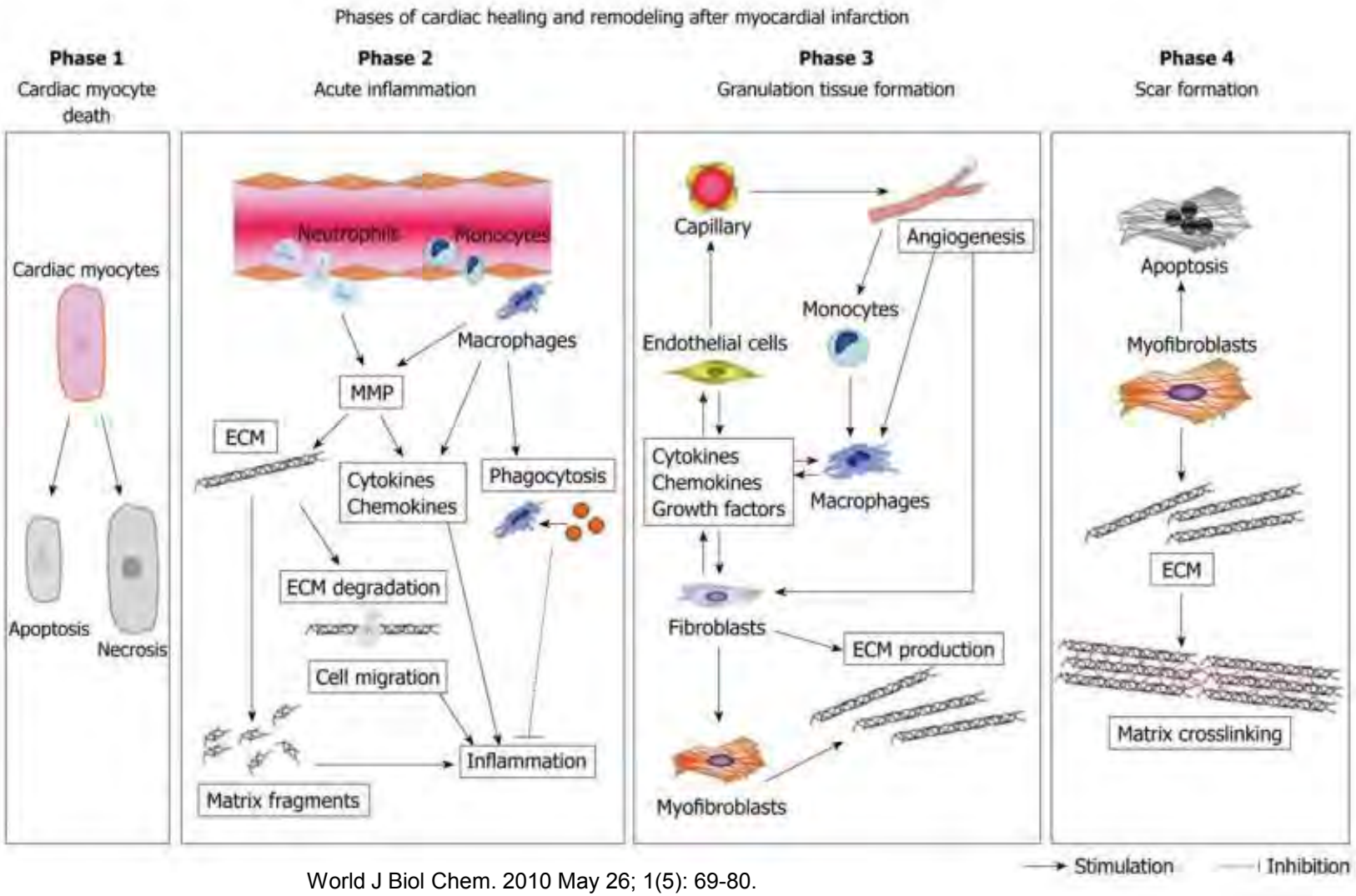




Mechanisms of Transition to Heart Failure



Phases following myocardial infarction



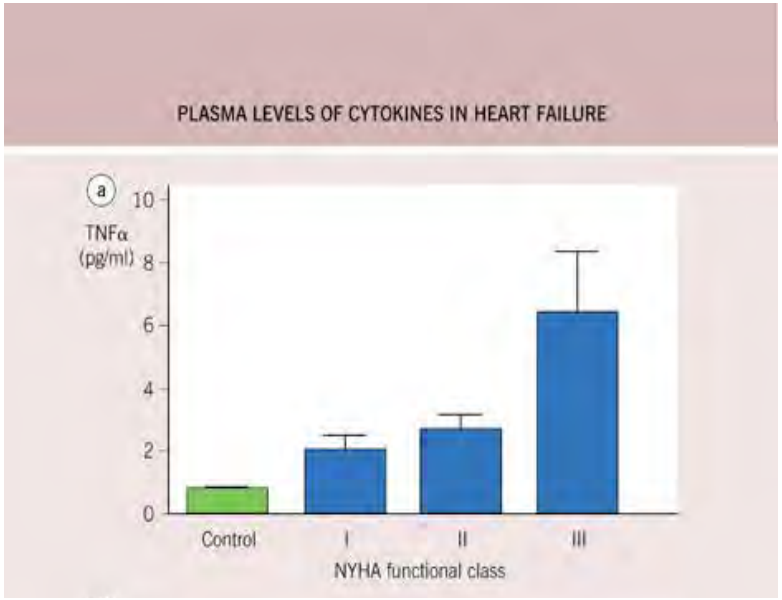
World J Biol Chem. 2010 May 26; 1(5): 69-80.

Blocking inflammation after MI?

Failed!

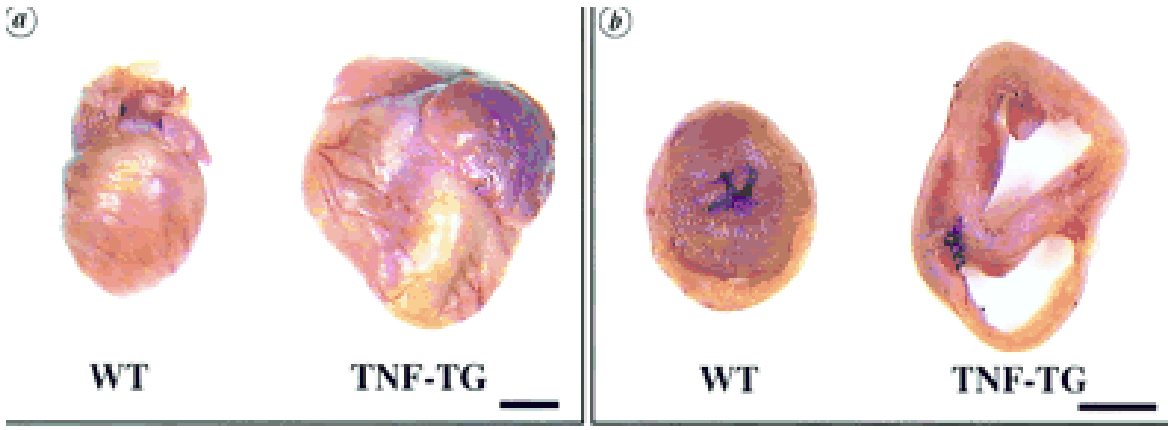
Roberts R, DeMello V, Sobel BE. *Circulation. 1976*
Deleterious effects of methylprednisolone (steroid therapy) in patients with myocardial infarction.

Enhanced expression of cardiac and systemic expression of the pro-inflammatory cytokines for example TNF-α



- **TNF-α-serum levels increase in patients with severity of the heart failure**
- **Mechanical unloading improves cardiac function and reduces TNF-α serum levels**

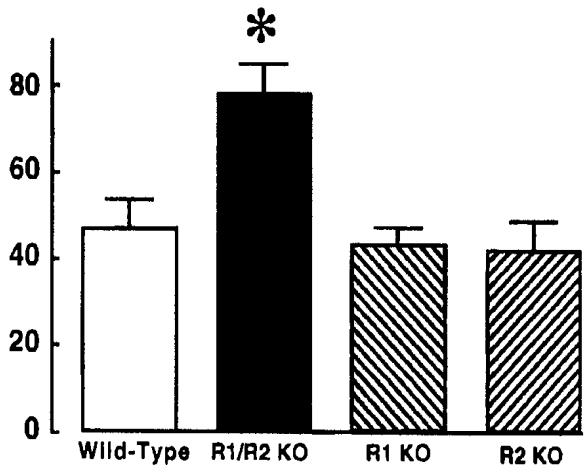
Cardiac failure in transgenic mice with myocardial expression of TNF-α



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

Serial MRI studies in the TNF-α mouse model demonstrate that the rate of progression and severity of LV Dysfunction are dependant on the degree of TNF-α overexpression. Franco et al, Circulation 1999

Endogenous TNF-α protects the heart against ischemic-induced cardiomyocyte death in a mouse model of acute mycardial infarction

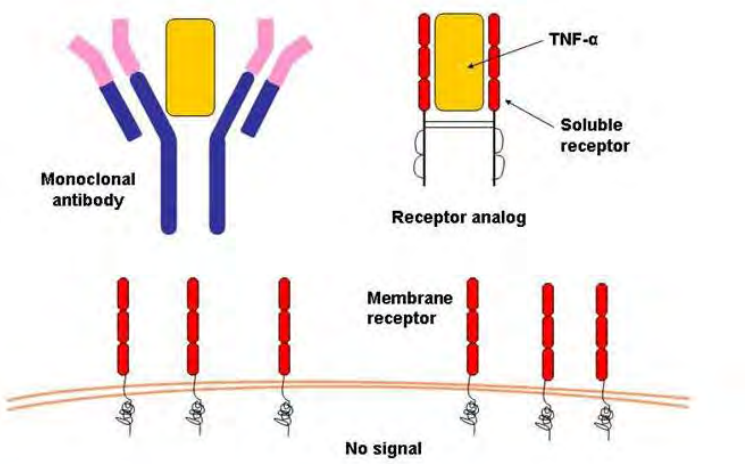


Double knockout of the two TNF-α receptors leads to increased infarct size in acute infarction.

Kurrelmeyer et al. PNAS 2000

Therapeutic approach to neutralize TNF-α

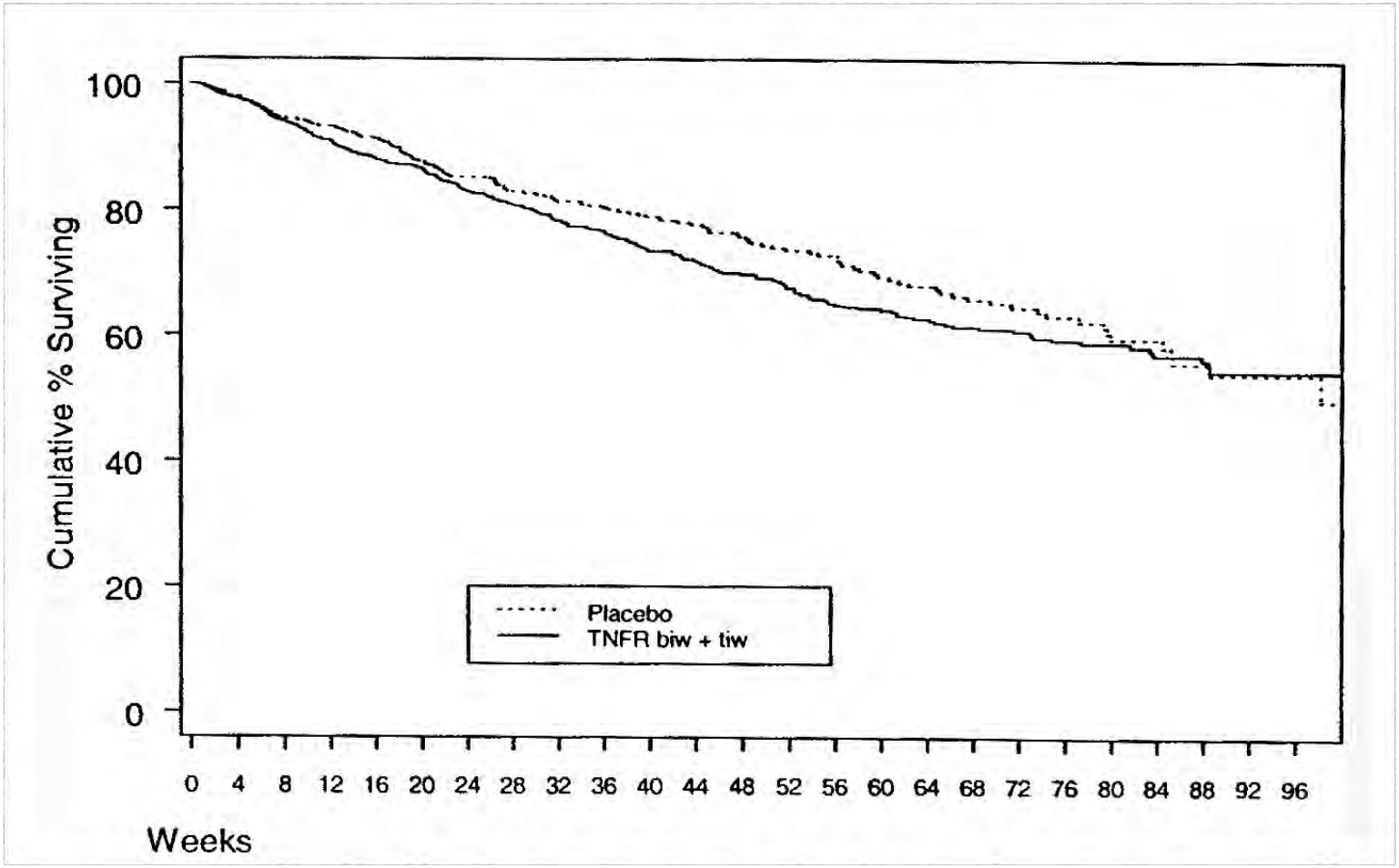
Mechanism of action of TNF-α antagonists. The monoclonal antibody (**infliximab, adalimumab**) and the receptor analog (**etanercept**) bind to circulating TNF-α and block its interaction with membrane receptor.



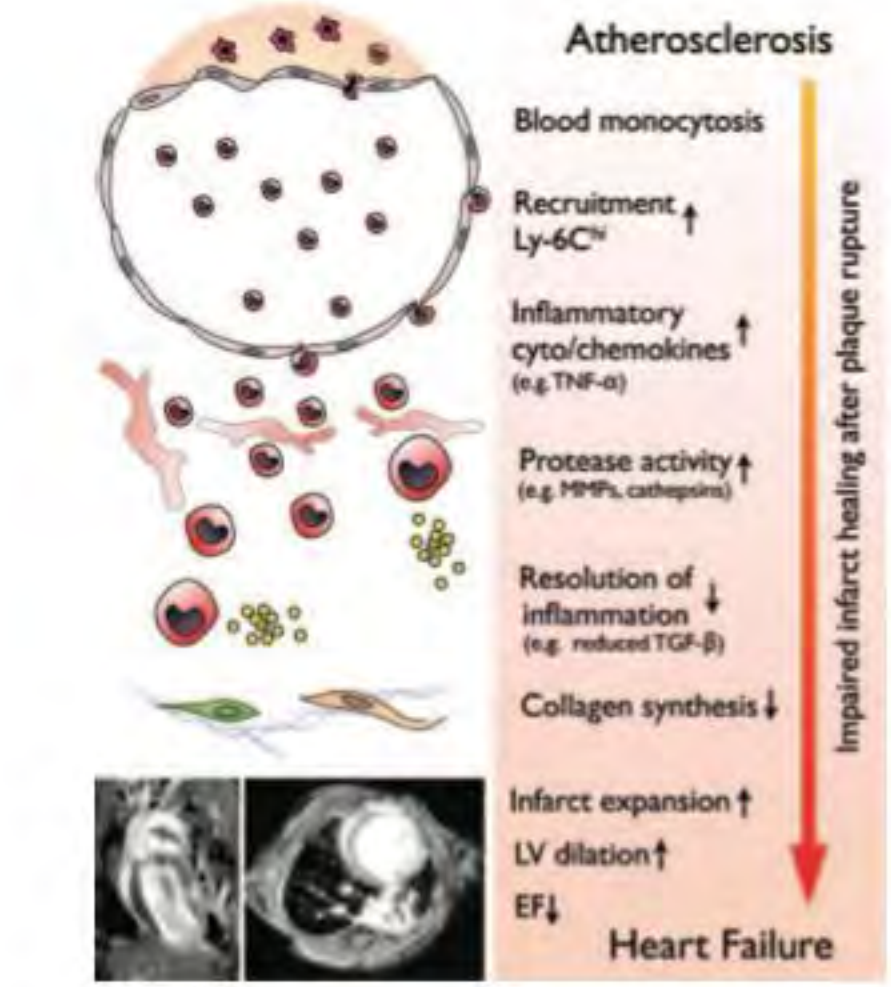
Experimental studies in animals and in small patient collectives were promising.

Randomized multi-center studies?

RENEWAL
All-cause Mortality and CHF Hospitalisations was not lowered by neutralizing TNF-α



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

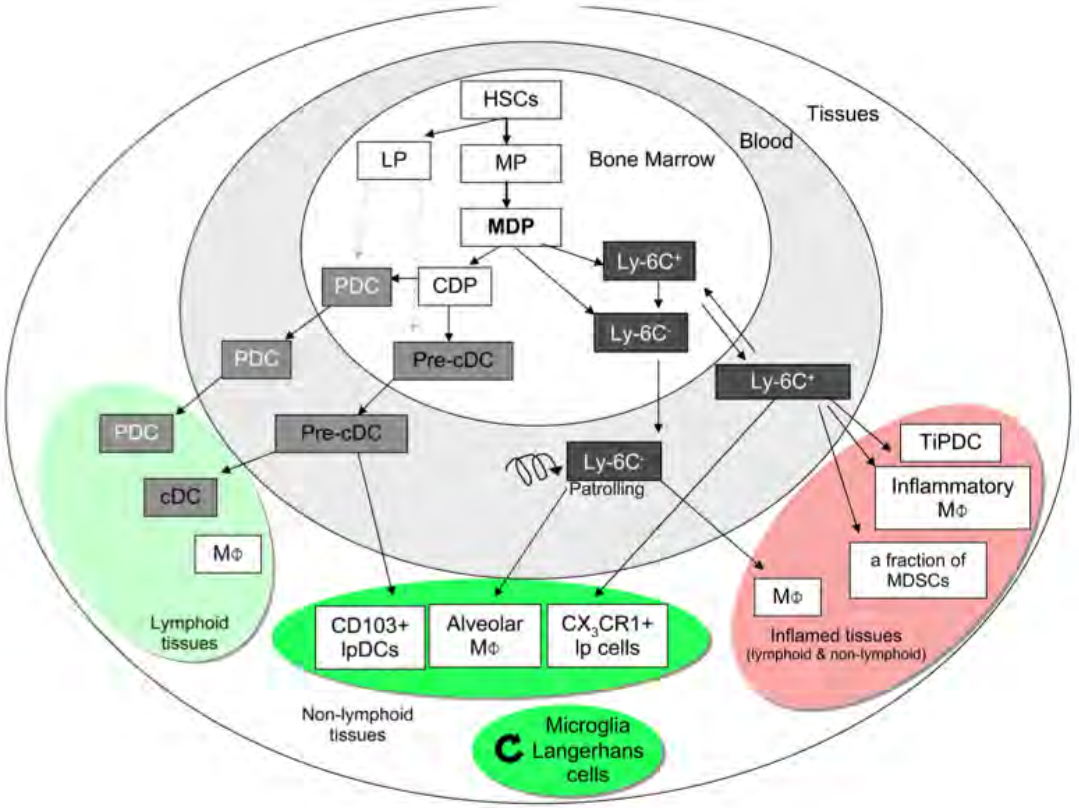
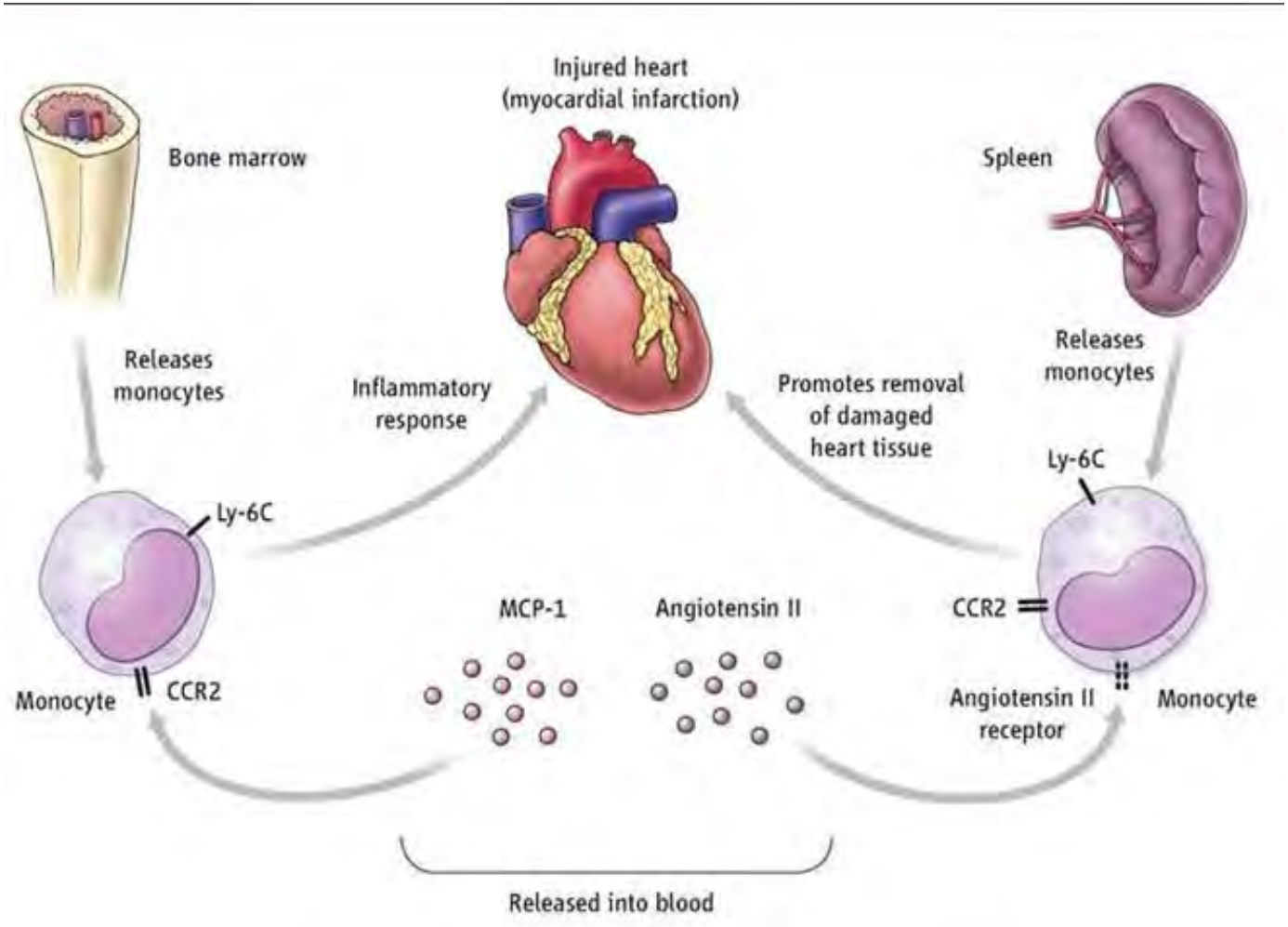


Fig. 2.
Differentiation of DCs and macrophages in mice. In the bone marrow, hematopoietic stem cells (HSC) produce myeloid (MP) and lymphoid (LP) committed precursors. MP give rise to monocyte/macrophages and DC precursors (MDP). MDP give rise to monocytes, and to common DC precursor (CDP). Two monocyte subsets, Ly-6C$^+$ and Ly-6C$^-$ 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$^+$ and CD8α$^-$ cDCs, and non-lymphoid tissues, where they may give rise to CD103$^+$ lamina propria DC (lpDC). Under homeostatic conditions, Ly-6C$^-$ monocytes may contribute to alveolar macrophages (MΦ) and Ly-6C$^+$ monocytes can become CX_3CR1^+ lpDCs in non-lymphoid tissues. During inflammation, Ly-6C$^+$ 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



American Journal of Pathology, Vol. 165, No. 2, August 2004

Targeted Deletion of CC Chemokine Receptor 2 Attenuates Left Ventricular Remodeling after Experimental Myocardial Infarction

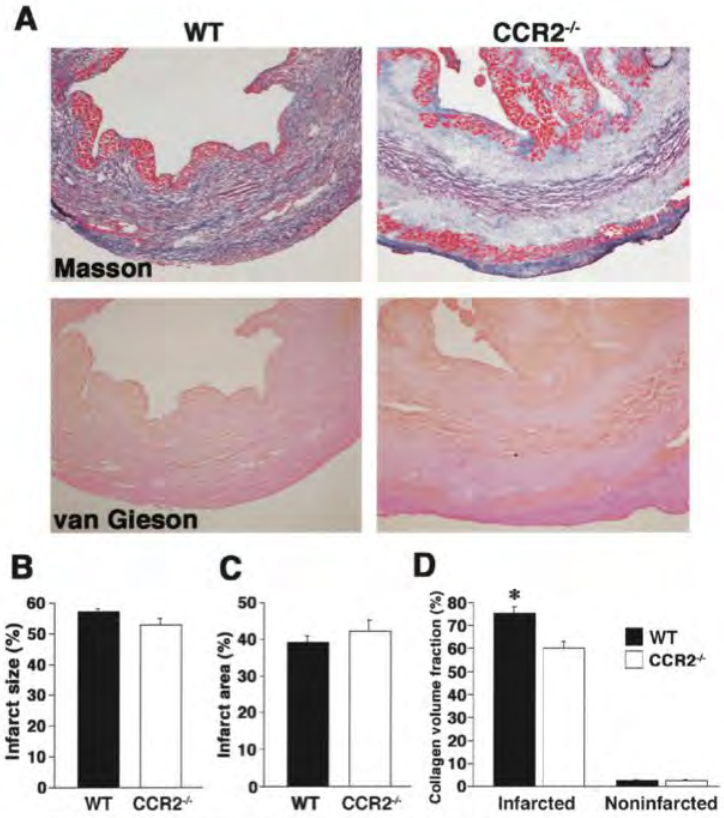


Figure 1. A: Representative examples of Masson's trichrome and van Gieson staining at 7 days after experimental MI. Stained slides demonstrate that necrotic centers of infarcted regions remain devoid of fibrosis in CCR2$^{-/-}$ mice, with less accumulation of collagen in CCR2$^{-/-}$ mice than in WT 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 tissue in visual fields of the sections. *, $P < 0.01$ *versus* CCR2$^{-/-}$ mice.

Kaikita et al. American Journal of Pathology, 2004

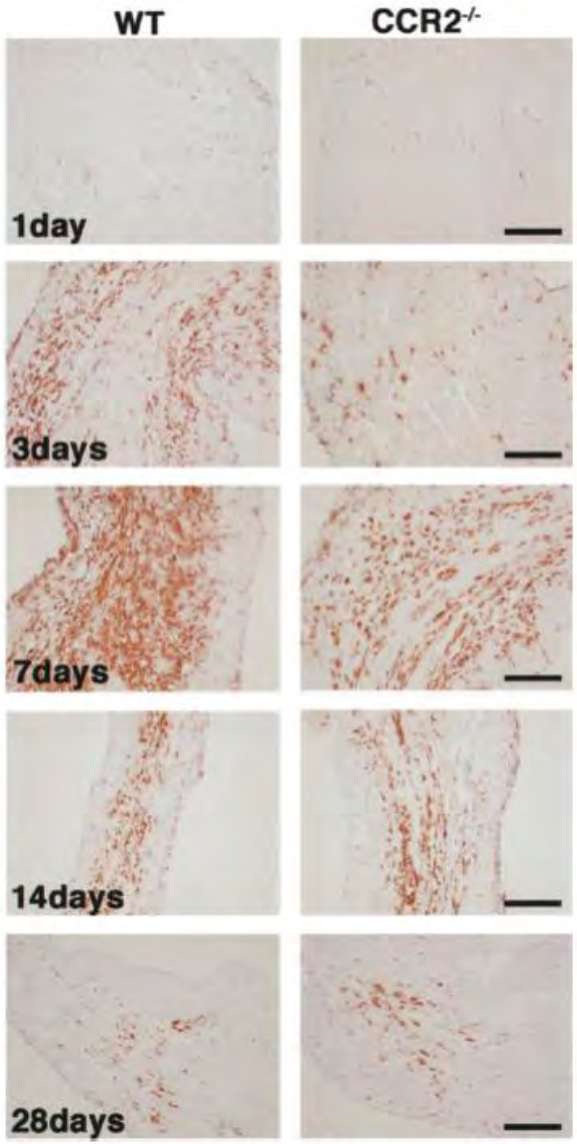


Figure 2. Immunohistochemical detection of FA-11-positive macrophages in infarcted regions from WT (**left**) and CCR2$^{-/-}$ (**right**) mice at 1, 3, 7, 14, and 28 days after experimental MI. Scale bars, 100 μm.

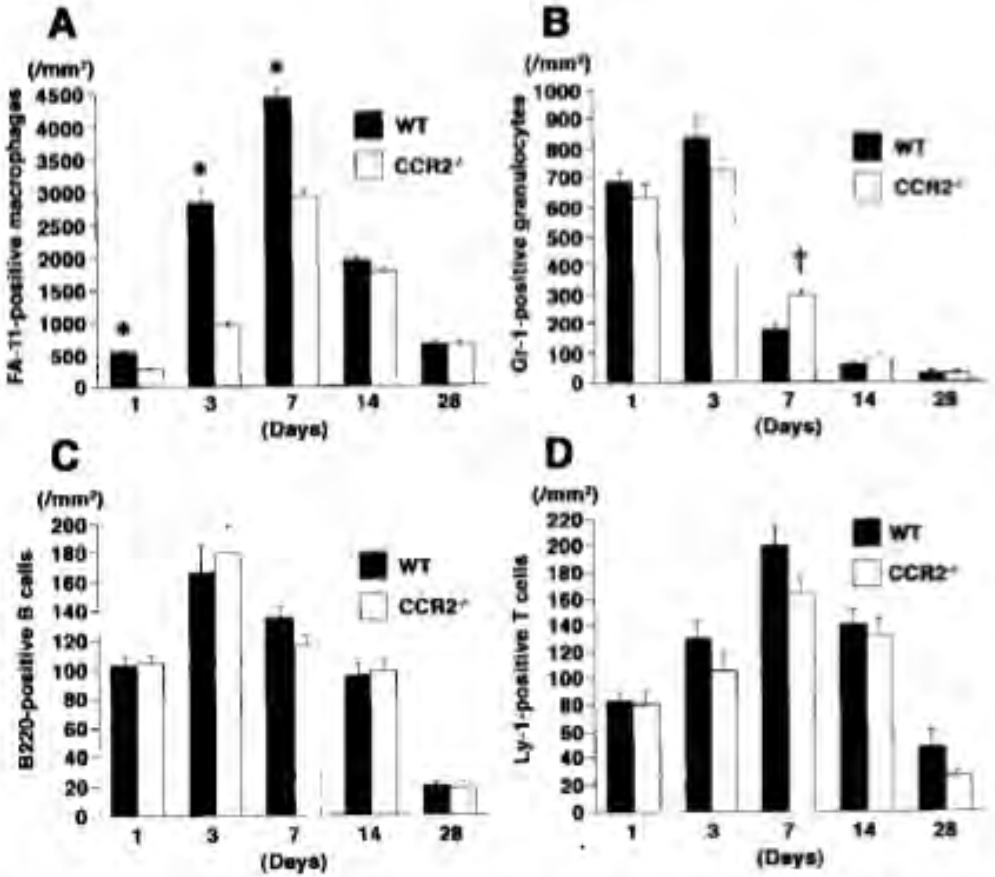
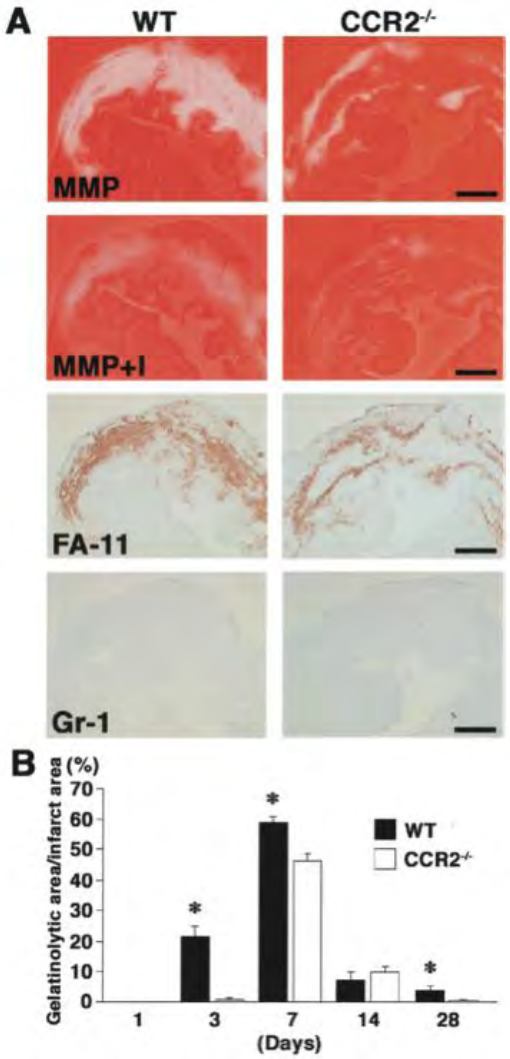
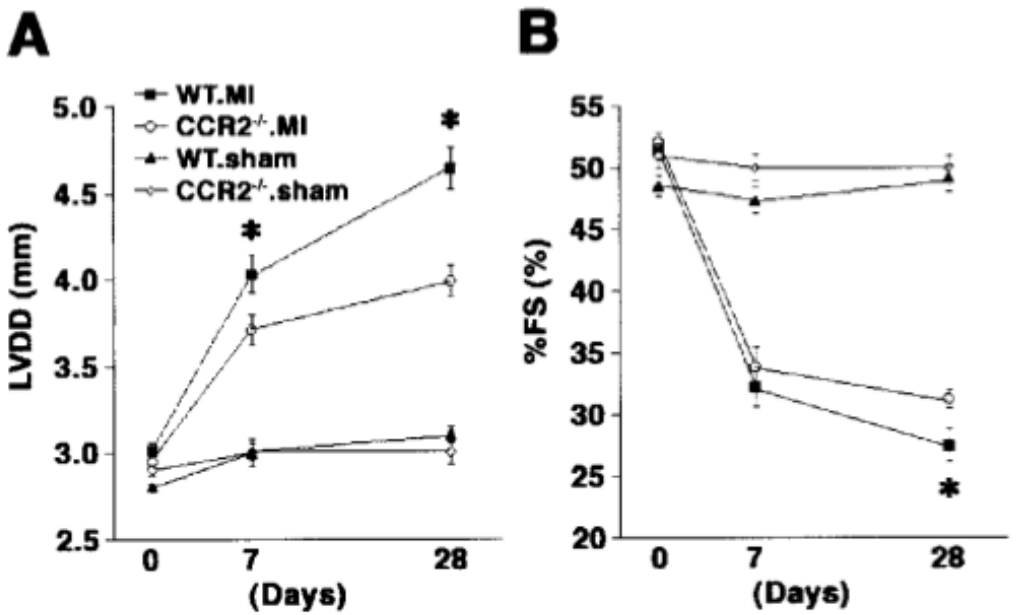


Figure 3. Differences in numbers of FA-11-positive macrophages (**A**), Gr-1-positive granulocytes (**B**), B220-positive B cells (**C**), and Ly-1-positive T cells (**D**) in infarcted regions from WT and CCR2$^{-/-}$ mice. Data points represent the number of positive cells per 1 mm^2 in infarcted tissues. *, $P < 0.01$ *versus* CCR2$^{-/-}$ mice. †, $P < 0.05$ *versus* WT mice.

Kaikita et al. American Journal of Pathology, 2004

CCR2-KO mice display lower MMP activity after infarction that is associated with less dilatation and better cardiac function

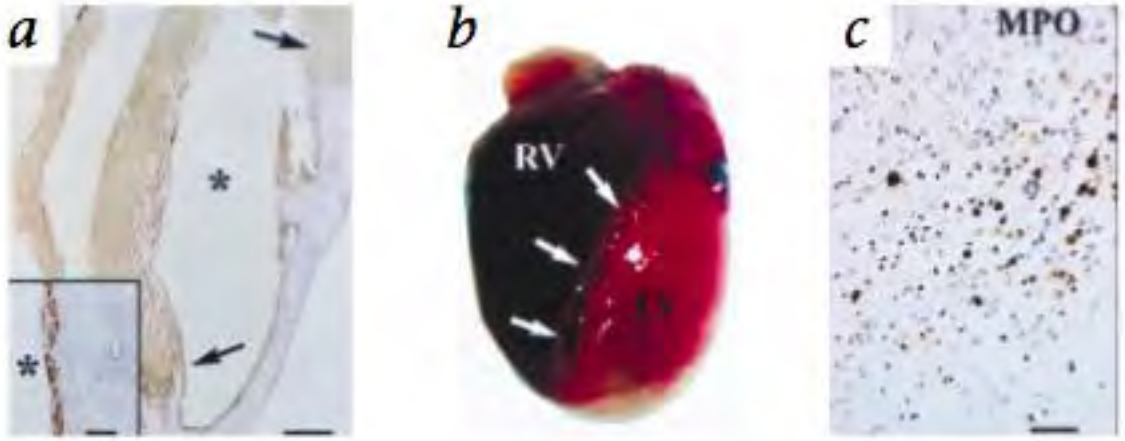




Kaikita et al. American Journal of Pathology, 2004

Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure

S. Heymans[1], A. Luttun[1], D. Nuyens[1], G. Theilmeier[1], E. Creemers[2], L. Moons[1], G.D. Dyspersin[3], J.P.M. Cleutjens[2], M. Shipley[4], A. Angellilo[1], M. Levi[1], O. Nübe[5], A. Baker[6], E. Keshet[7], F. Lupu[8], J-M Herbert[9], J.F.M. Smits[2], S.D. Shapiro[4], M. Baes[1], M. Borgers[3], D. Collen[1], M. J.A.P. Daemen[2] & P. Carmeliet[1]



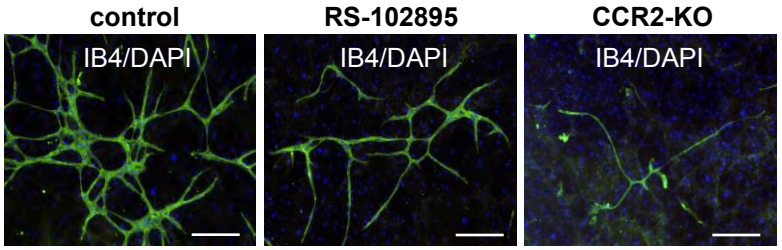
Heymans et al. Nature Medicine 1999

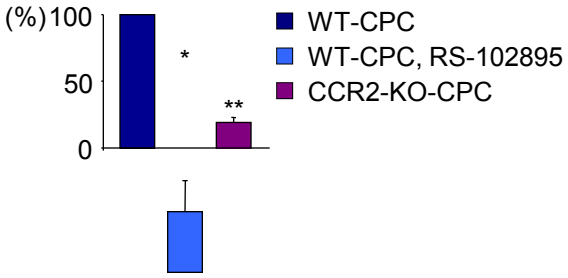
Deletion of CCR2 attenuates angiogenesis in injured skeletal muscle and reduces the angiogenic potential of endogenous cardiac progenitor cells

Am J Physiol Regul Integr Comp Physiol 293: R651–R661, 2007.
First published May 23, 2007; doi:10.1152/ajpregu.00069.2007.

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

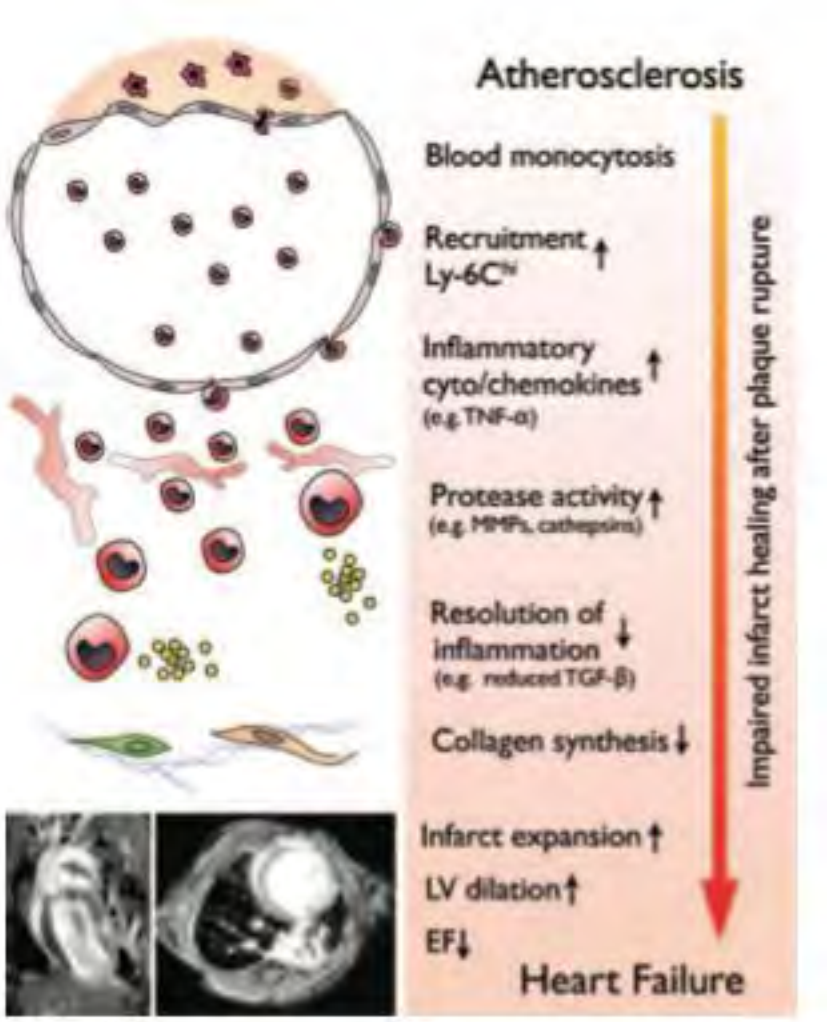
Oscar Ochoa,[1] Dongxu Sun,[1] Sara M. Reyes-Reyna,[1] Lindsay L. Waite,[2] Joel E. Michalek,[2] Linda M. McManus,[3,4,6] and Paula K. Shireman[1,5,6,7]





Hoch et al. Cell Stem Cell in revision

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 marcrophages at the site of inflammation?

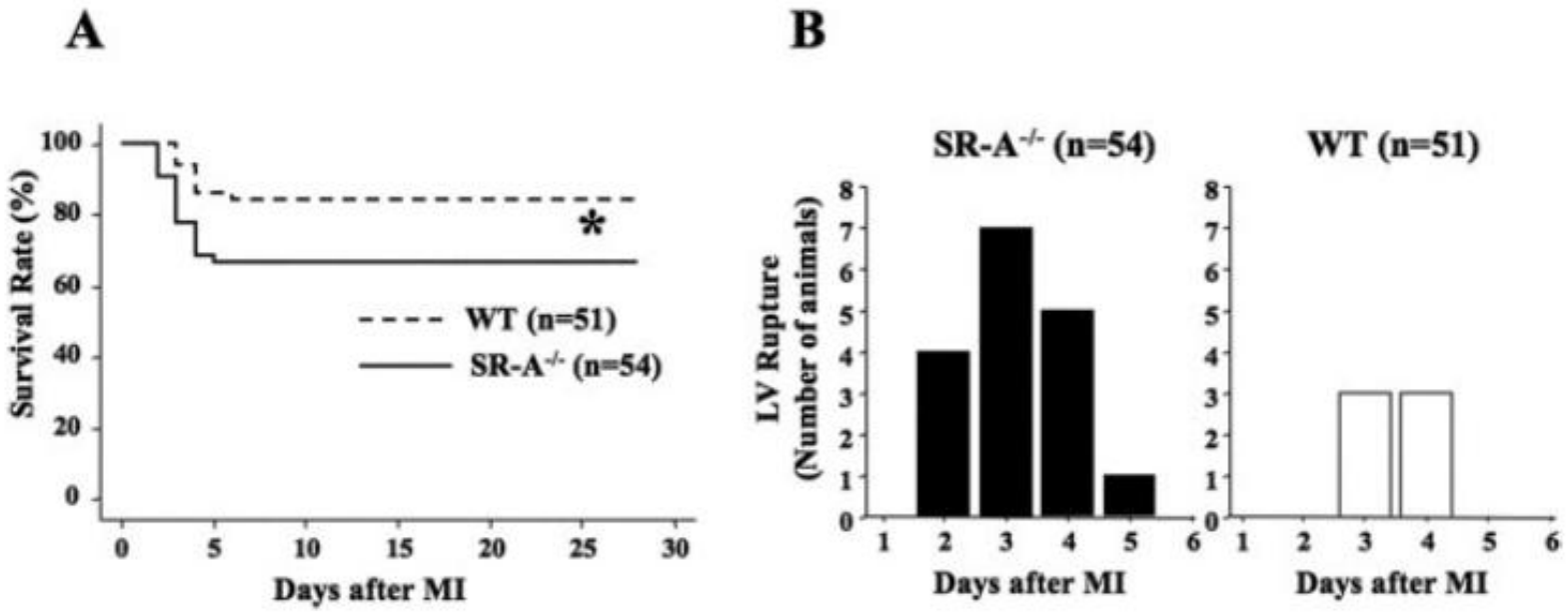
Nahrendorf et al. Circulation 2010

Targeted Deletion of Class A Macrophage Scavenger Receptor Increases the Risk of Cardiac Rupture After Experimental Myocardial Infarction

Kenichi Tsujita, MD; Koichi Kaikita, MD; Takanori Hayasaki, MD; Tsuyoshi Honda, MD; Hironori Kobayashi, MD; Naomi Sakashita, MD; Hiroshi Suzuki, PhD; Tatsuhiko Kodama, MD; Hisao Ogawa, MD; Motohiro Takeya, MD

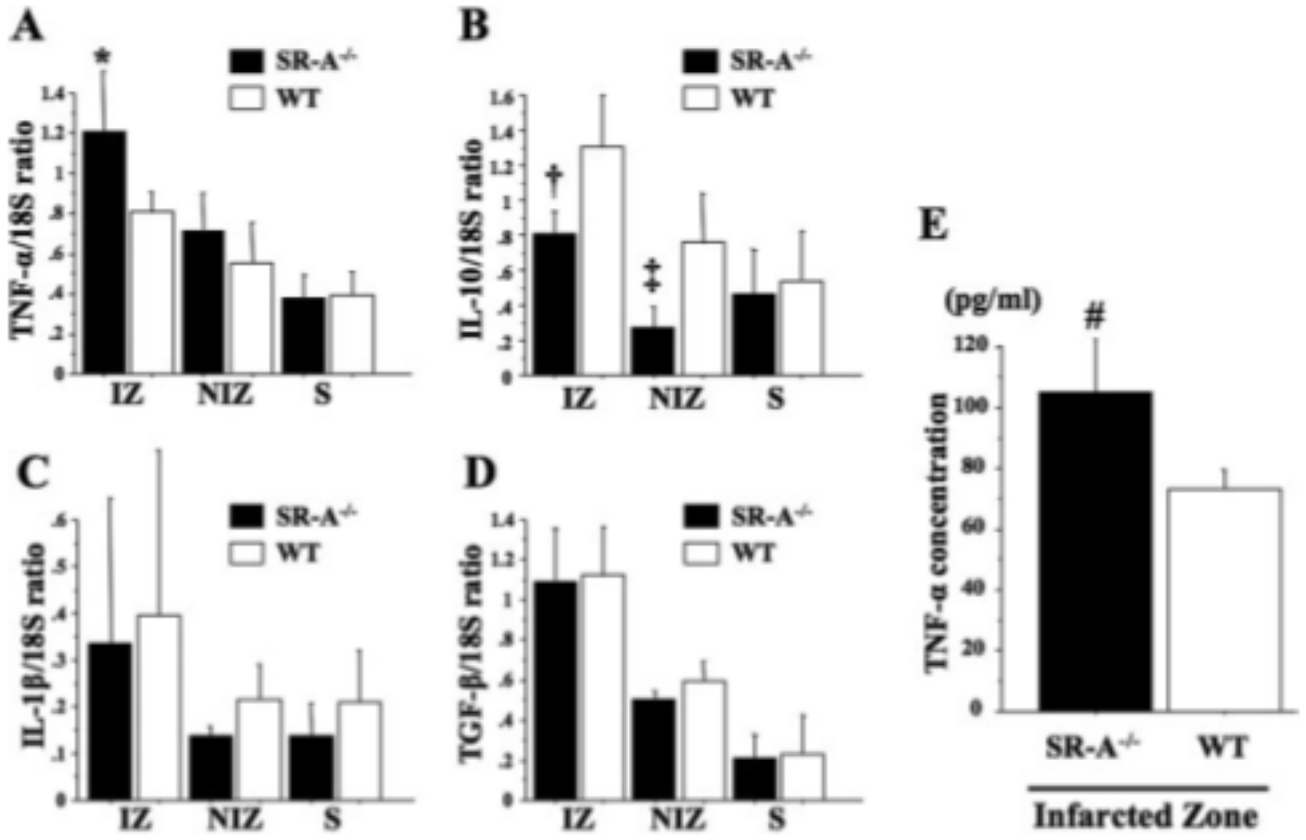
Class A macrophage scavenger receptor (SR-A) is a macrophage restricted multifunctional molecule

ligand: acetyl–low- density lipoprotein)



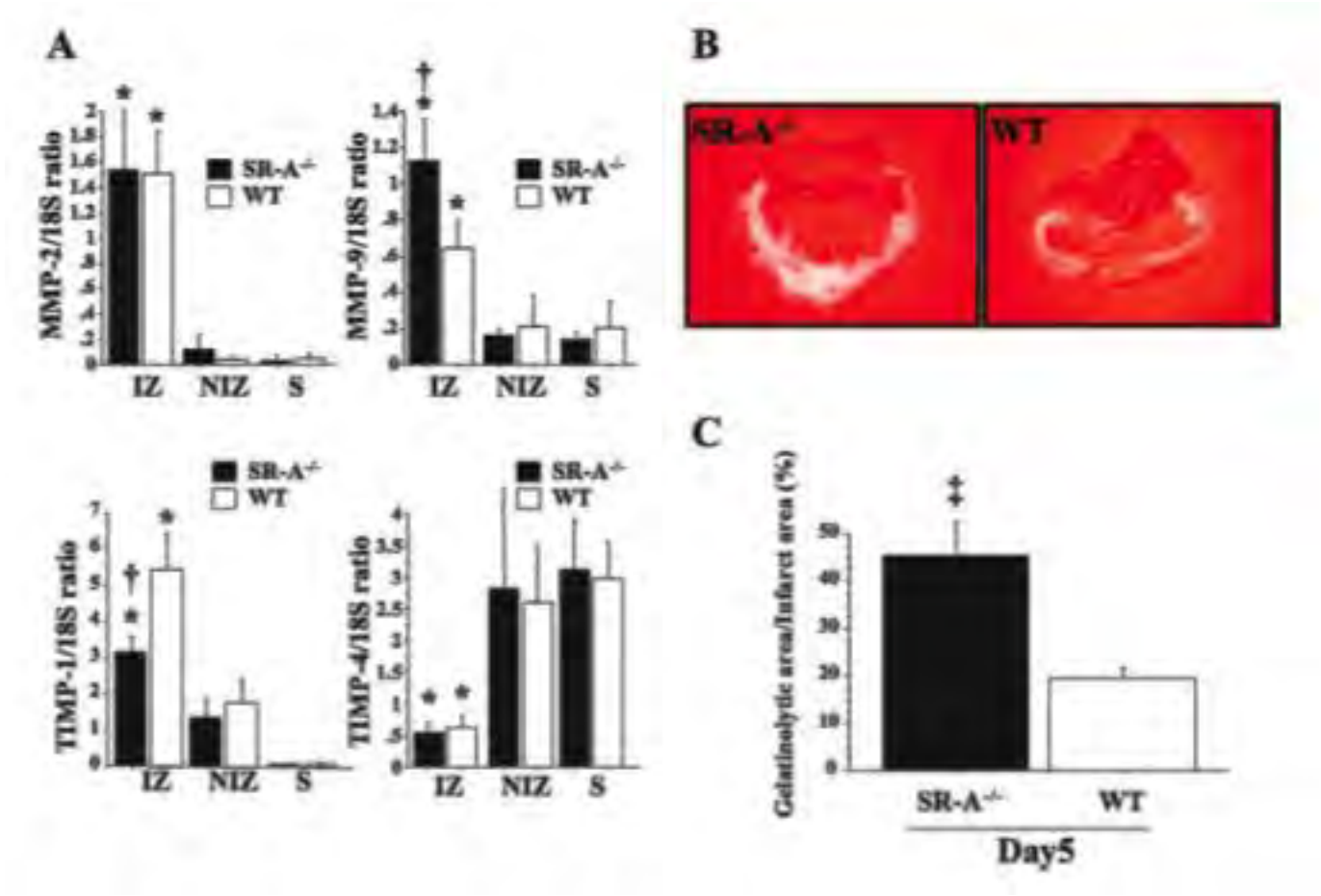
Tsujita et al. Circulation 2007

Increased expression of pro-inflammatory cytokine TNF-α and decreased expression of the anti-inflammatory cytokine IL-10 in SR-A k.o mice



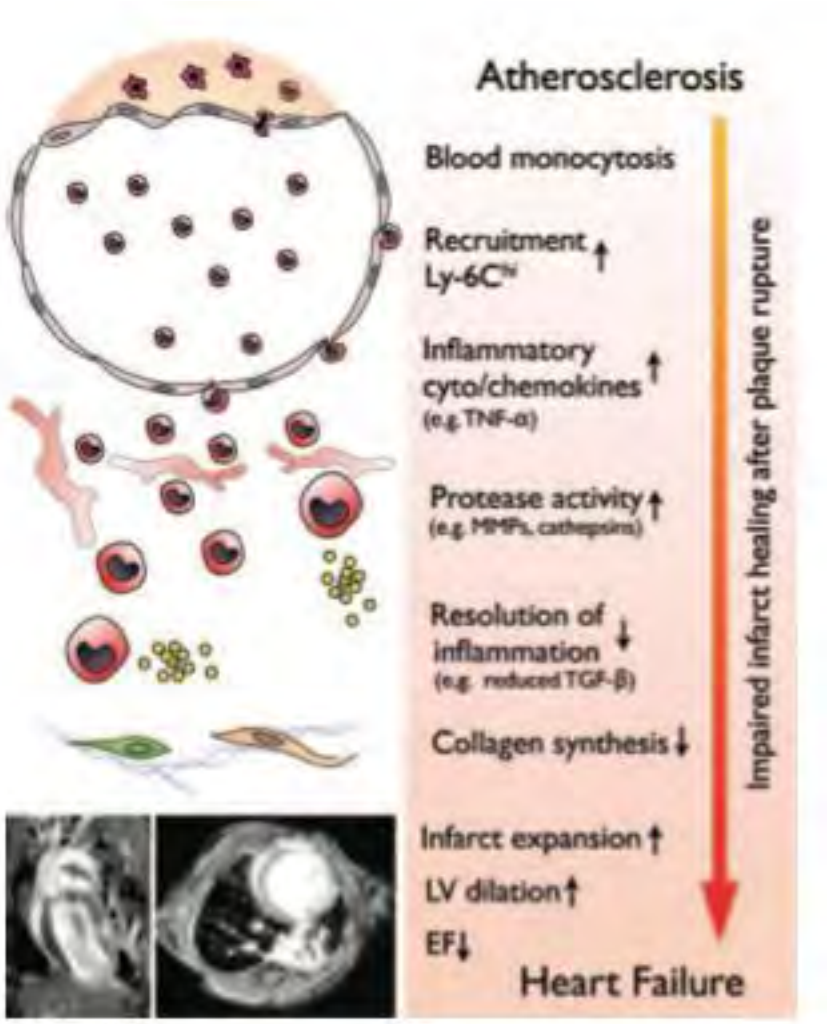
Tsujita et al. Circulation 2007

Differential expression of TIMPs and MMPs is associated with LV rupture in SR-A k.o. mice



Tsujita et al. Circulation 2007

Check points involved in impaired healing after myocardial infarction



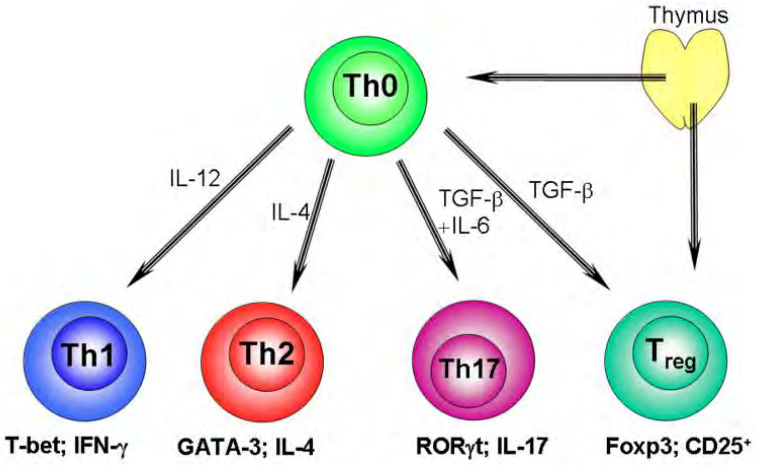
Nahrendorf et al. Circulation 2010

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

SR-A modulates the activity of marcrophages 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

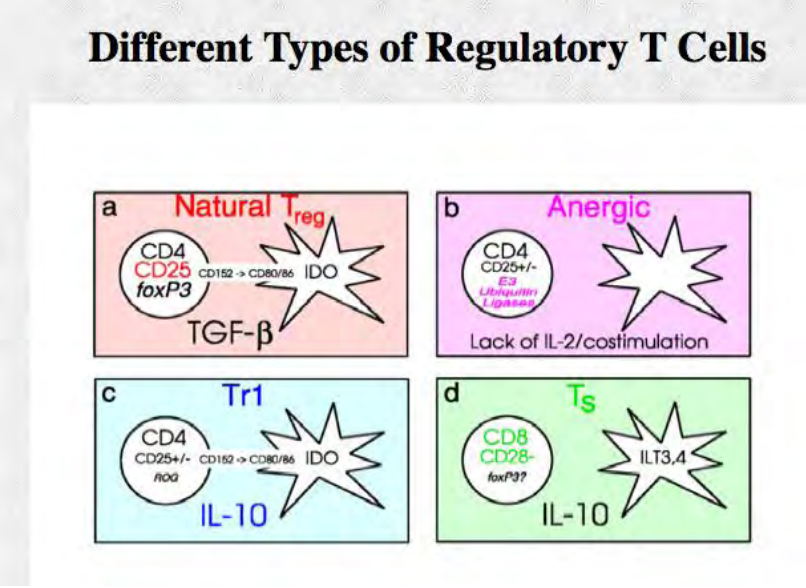


Regulatory T cells (**Treg**, sometimes known as suppressor T cells) are a specialized subpopulation of T cells **suppress activation of the immune system** and thereby maintaining immune system homeostasis and tolerance to self-antigens.

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.

The American Journal of Pathology, Vol. 176, No. 5, May 2010

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Cardiovascular, Pulmonary and Renal Pathology

CCR5 Signaling Suppresses Inflammation and Reduces Adverse Remodeling of the Infarcted Heart, Mediating Recruitment of Regulatory T Cells

No obvious difference in number of CD45+ inflammatory cells!

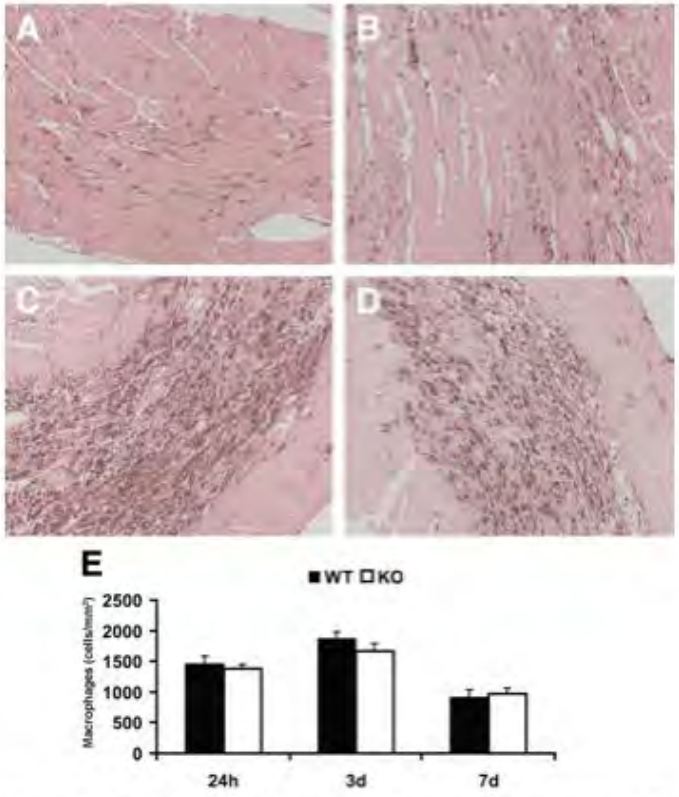
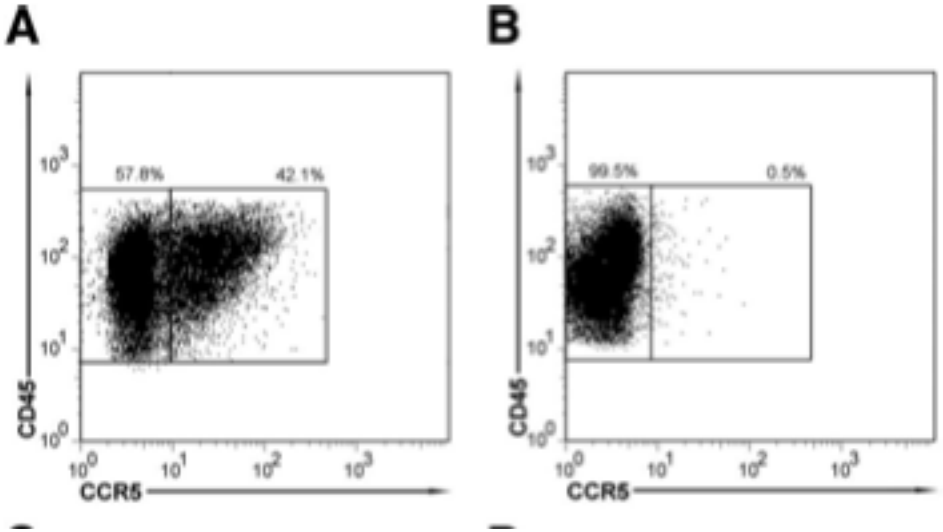
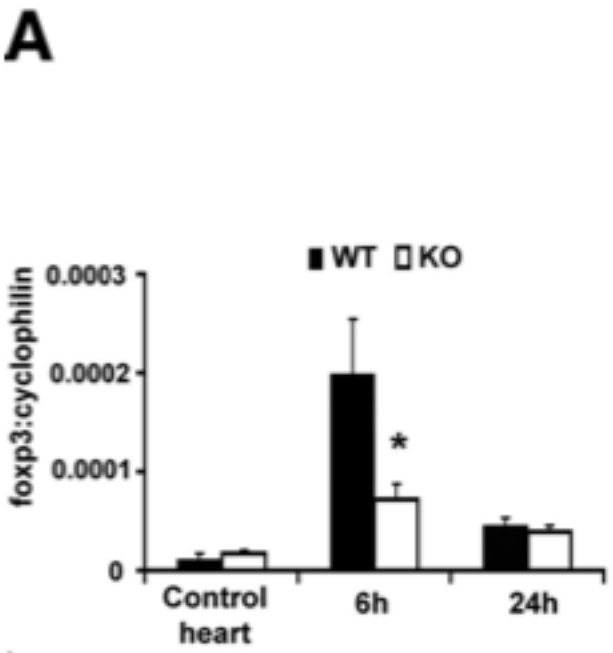


Figure 2. CCR5-null and wild-type (WT) infarcts exhibit comparable infiltration with macrophages. **A–D:** Mac2 immunohistochemistry identified macrophages in wild-type (**A**, **C**) and CCR5 KO infarcts (**B**, **D**) after 24 hours (**A**, **B**) and 72 hours of reperfusion (**C**, **D**). **E:** Quantitative analysis showed no significant differences in macrophage density between $CCR5^{-/-}$ and wild-type infarcts at all time points examined (n = 9 mice/group).

Dobaczewski et al. American Journal of Pathology 2010

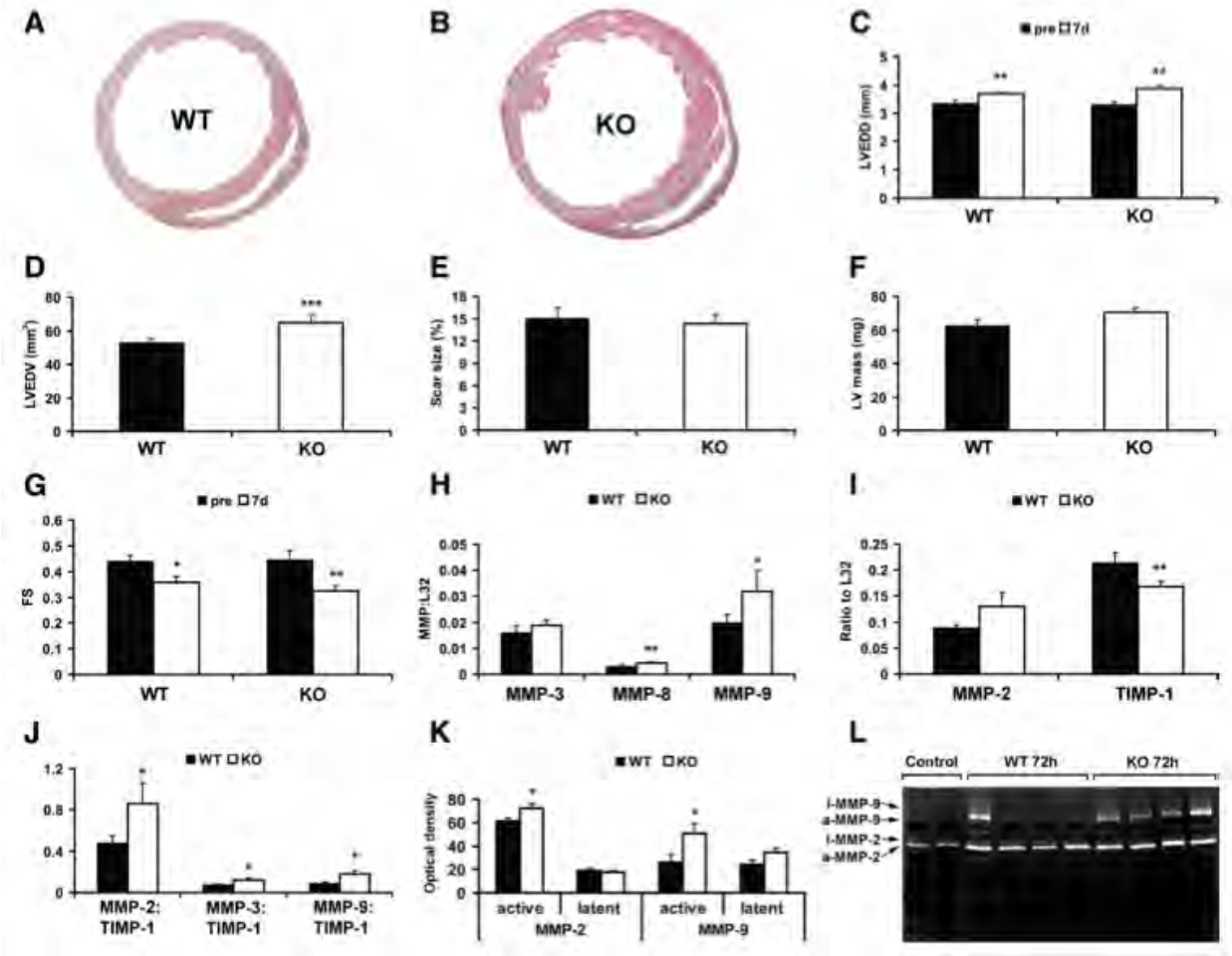
Lack of CCR5 positive inflammatory cells reduces invasion of T-regulatory cells (Tregs) into the infarcted heart





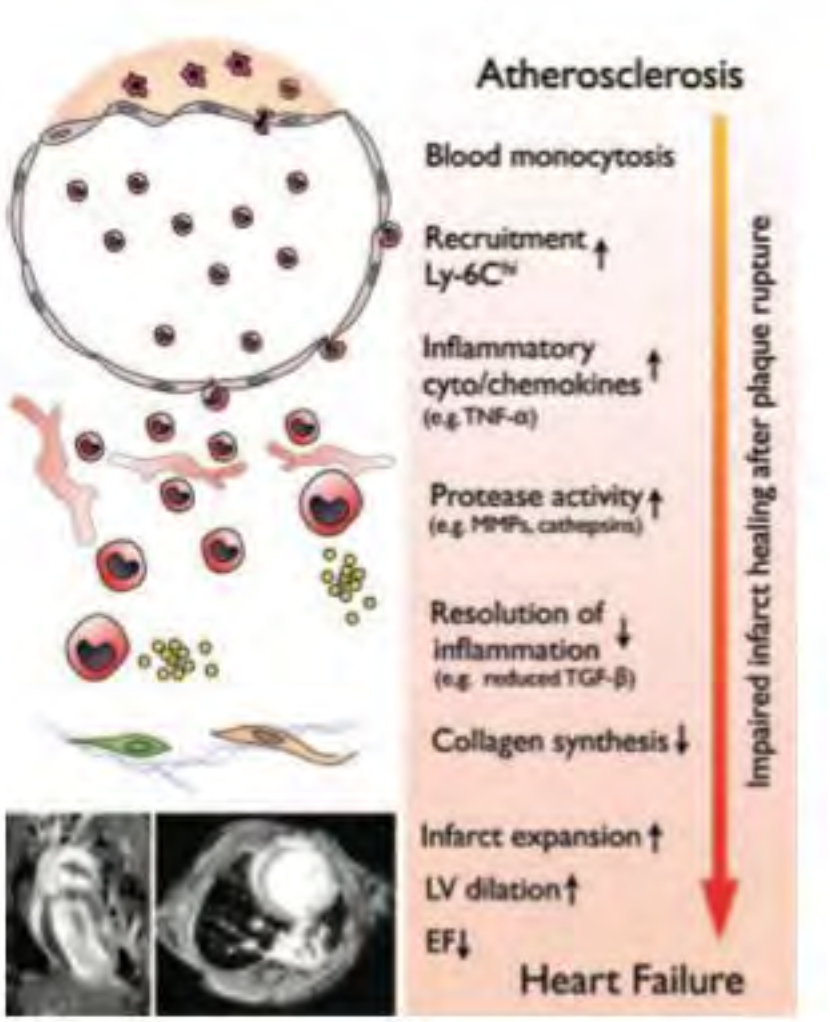
Dobaczewski et al. American Journal of Pathology 2010

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



Nahrendorf et al. Circulation 2010

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

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

Tregs seem to play are role in modulating of 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

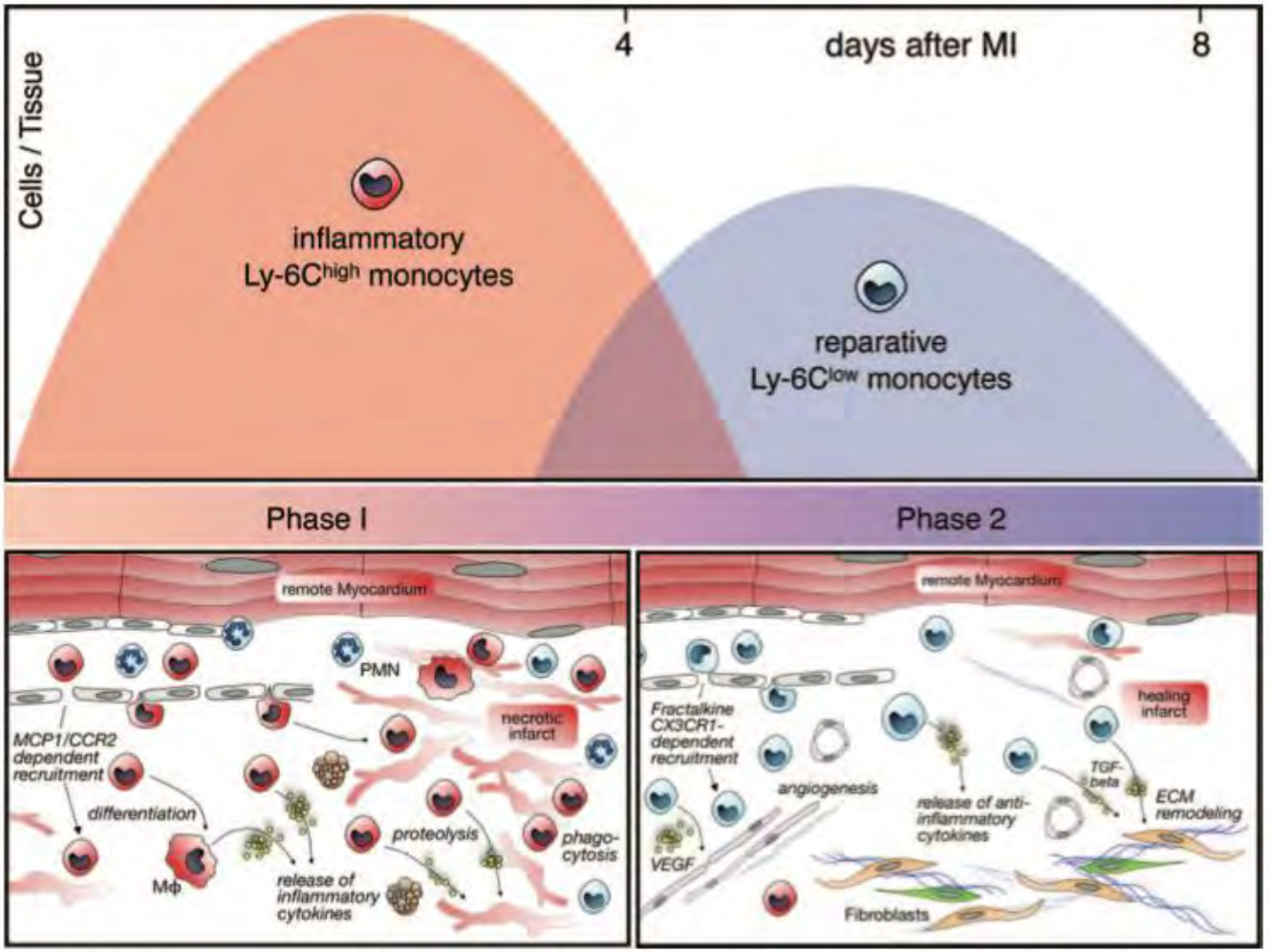
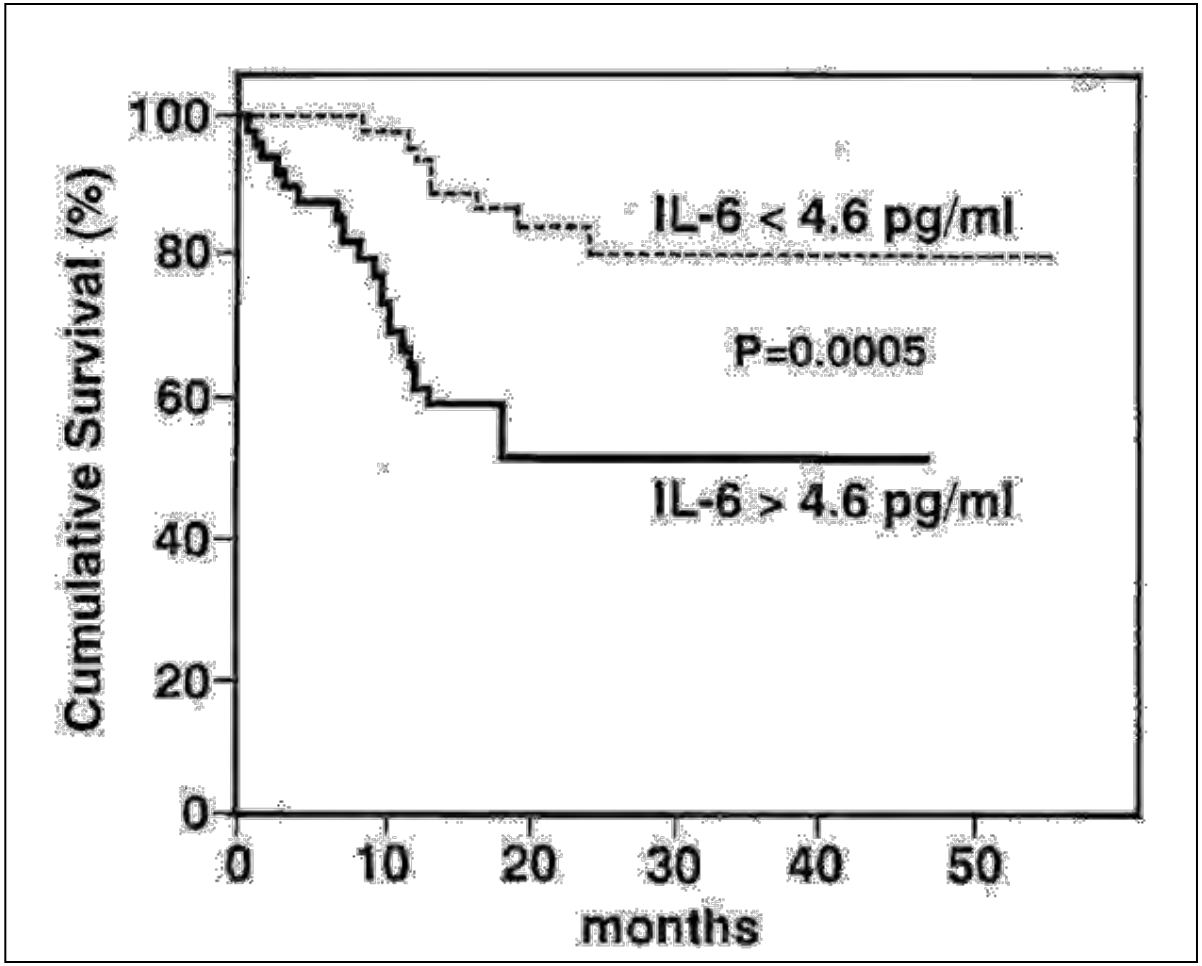


Figure 2. Biphasic monocyte response after myocardial infarction in the mouse. Time course of monocyte subset recruitment and their function depicted in the lower panel are adapted from Nahrendorf et al.[30] PMN indicates polymorphonuclear neutrophil; MΦ, macrophage; and ECM, extracellular matrix.

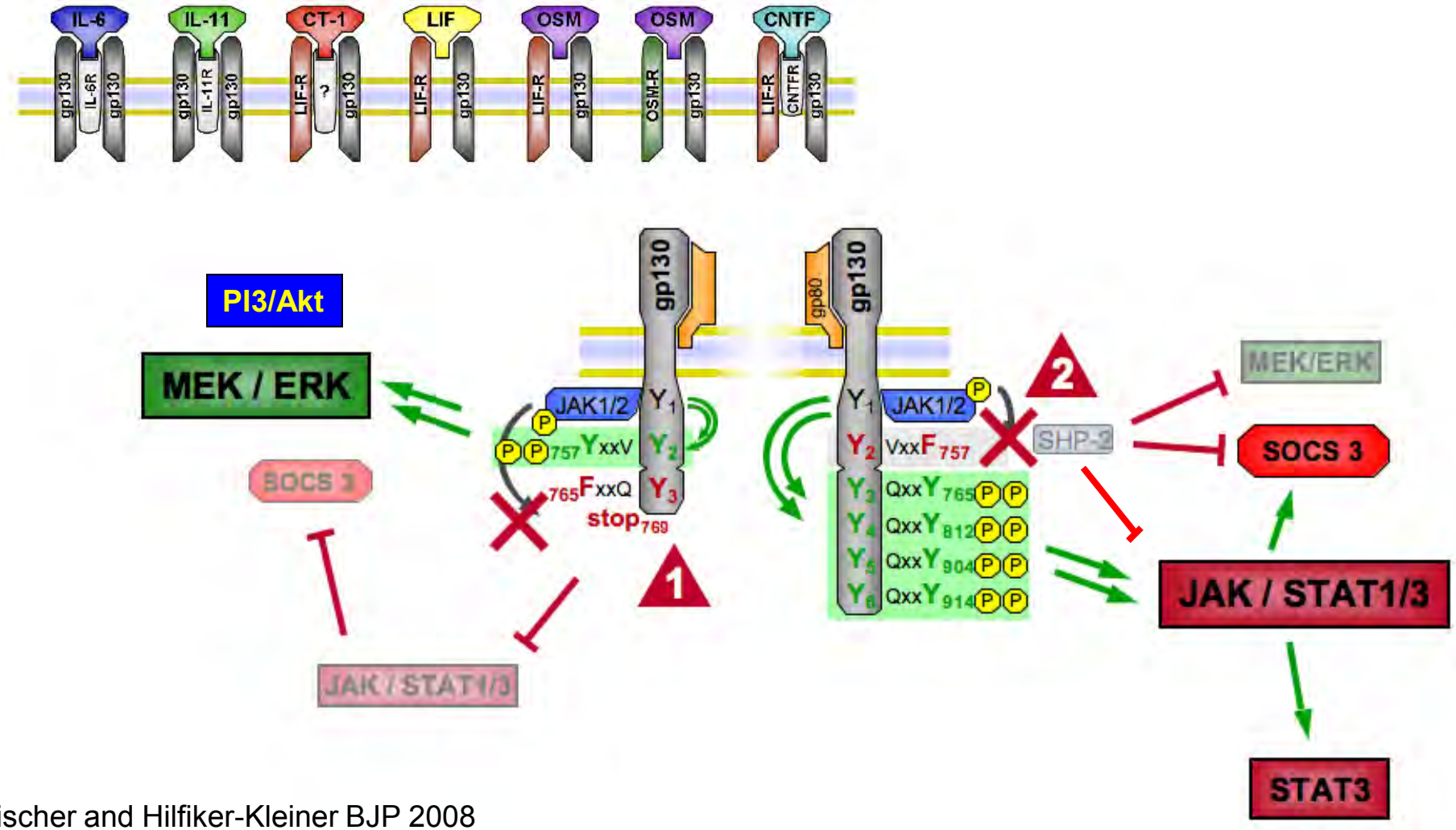
Nahrendorf et al. Circulation 2010

High serum levels of IL-6 cytokines correlate with an adverse outcome in patients with chronic myocardial infarction



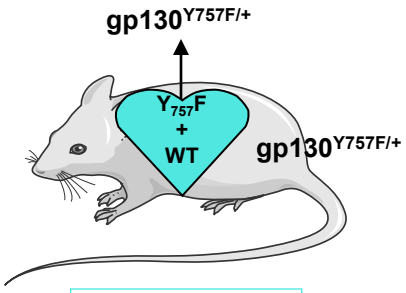
Tsutamoto T. et al., JACC 1998; Tsutamoto T. et al., Eur J Heart Fail. 2007

Complex regulatory network of gp130 down-stream signaling

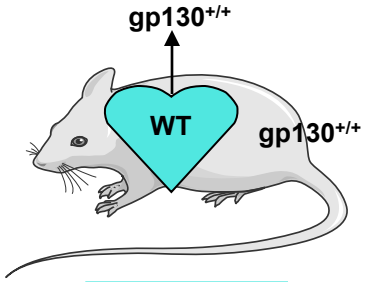


Fischer and Hilfiker-Kleiner BJP 2008

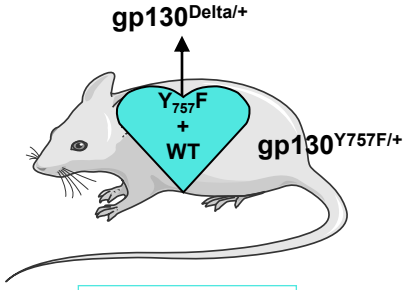
Cardiomyocytes restricted pathway mutations at the gp130 receptor using the CreLoxP system



$Y_{757}F/+$
gp130$^{Y757F/flox}$

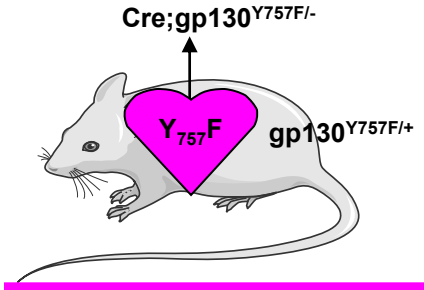


WT
gp130$^{flox/flox}$

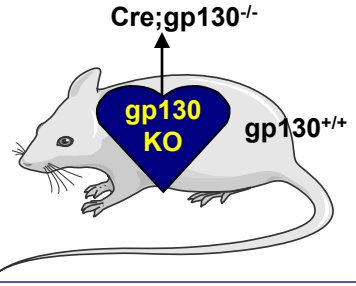


Delta/+
gp130$^{Delta/flox}$

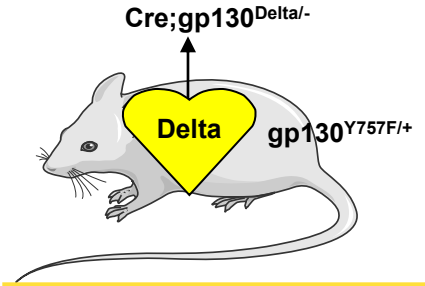
One wildtype gp130 allele is sufficient to ensure normal gp130 signaling
Dierssen et al. JBC 2008



$Y_{757}F$
α-MHC-Cre$^{tg/-}$; gp130$^{Y757F/flox}$



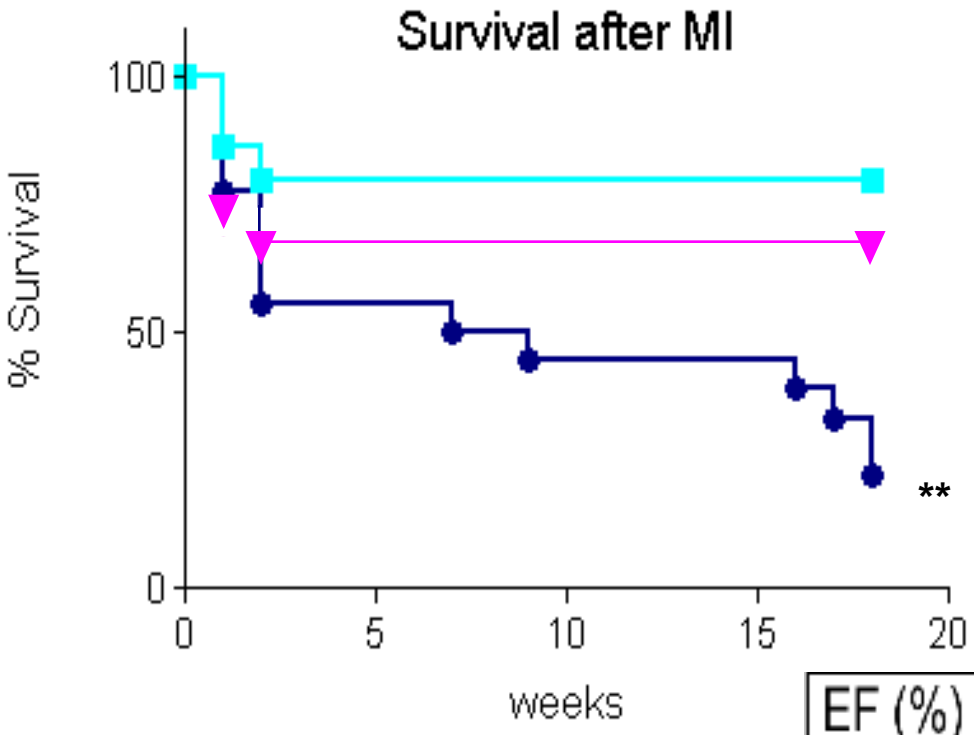
KO
α-MHC-Cre$^{tg/-}$; gp130$^{flox/flox}$

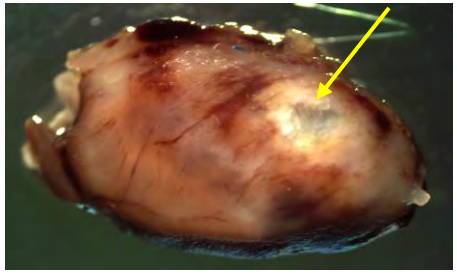


Delta
α-MHC-Cre$^{tg/-}$; gp130$^{YDelta/flox}$

Hilfiker-Kleiner et al. Circulation 2010

Severely impaired post infarct survival in $Y_{757}F$ mice due to LV rupture and heart failure



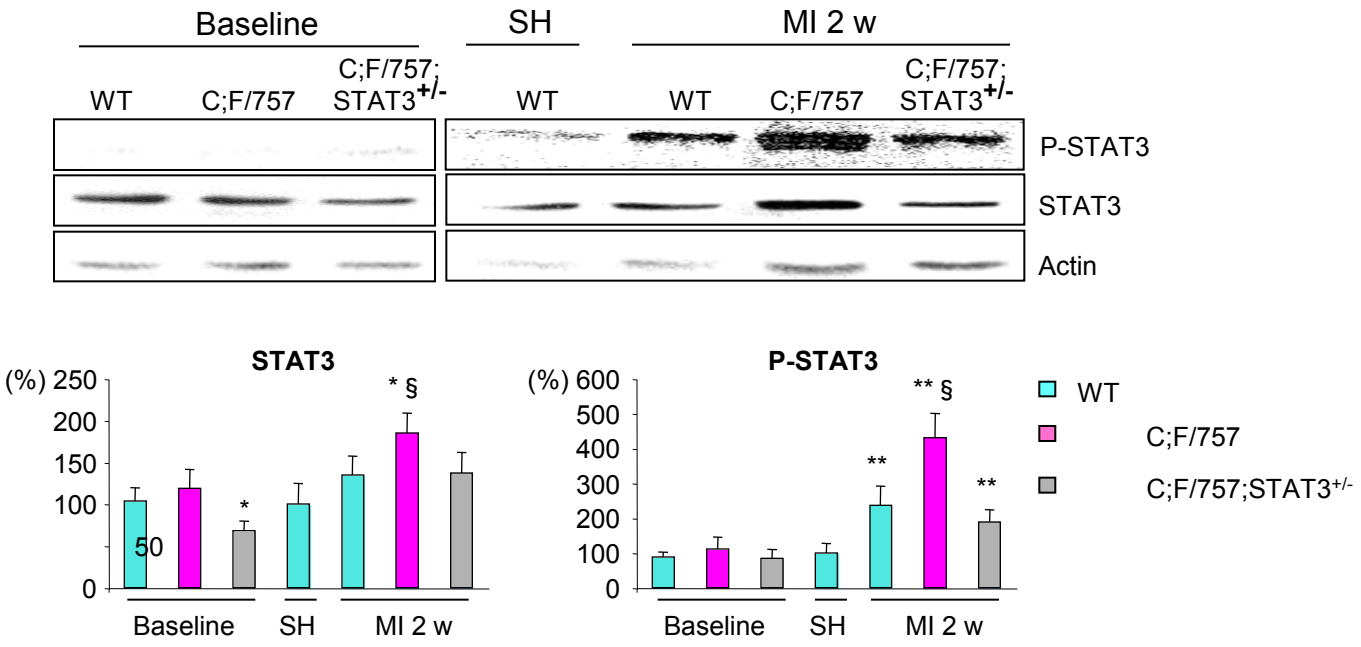


	WT	$Y_{757}F$
EF (%)	$39\pm15^{\S\S}$	$22\pm10^{\S\S **}$
FS (%)	$22\pm11^{\S\S}$	$13\pm6^{\S\S *}$

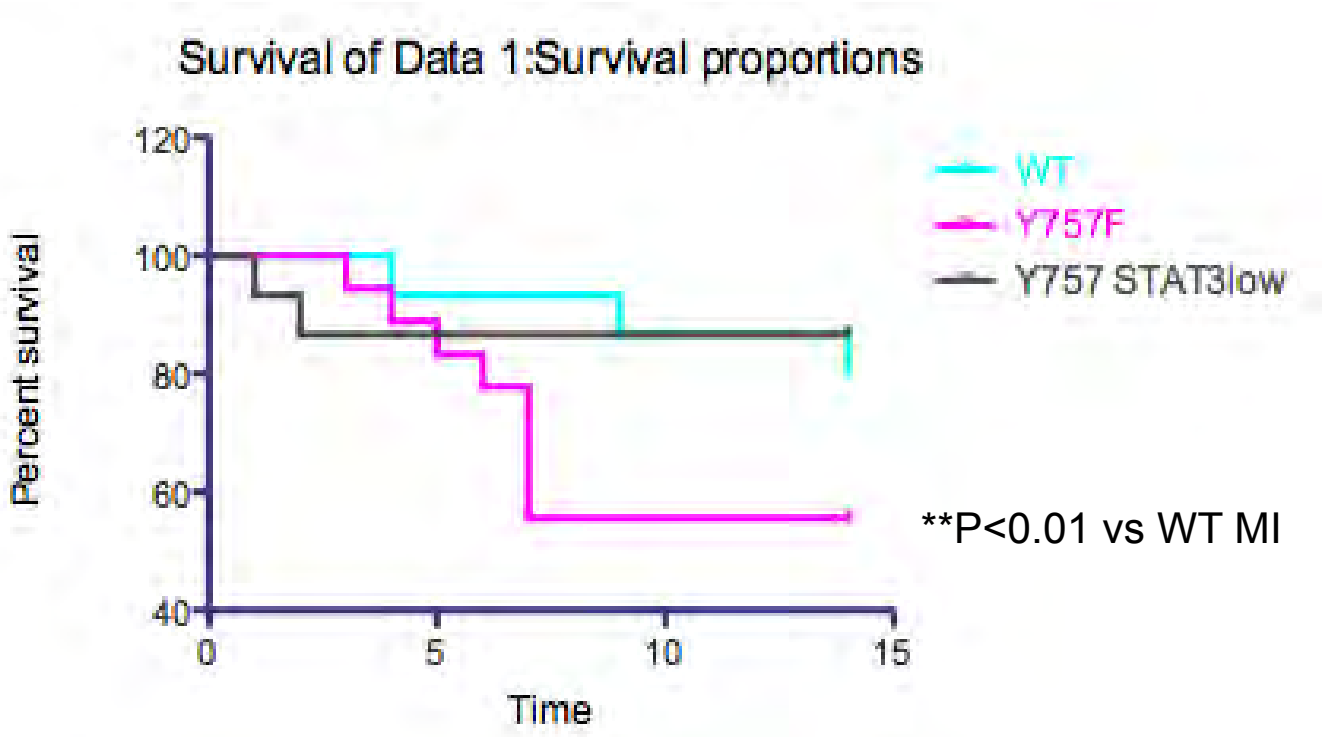
*P<0.05, **P<0.01 vs WT MI

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**



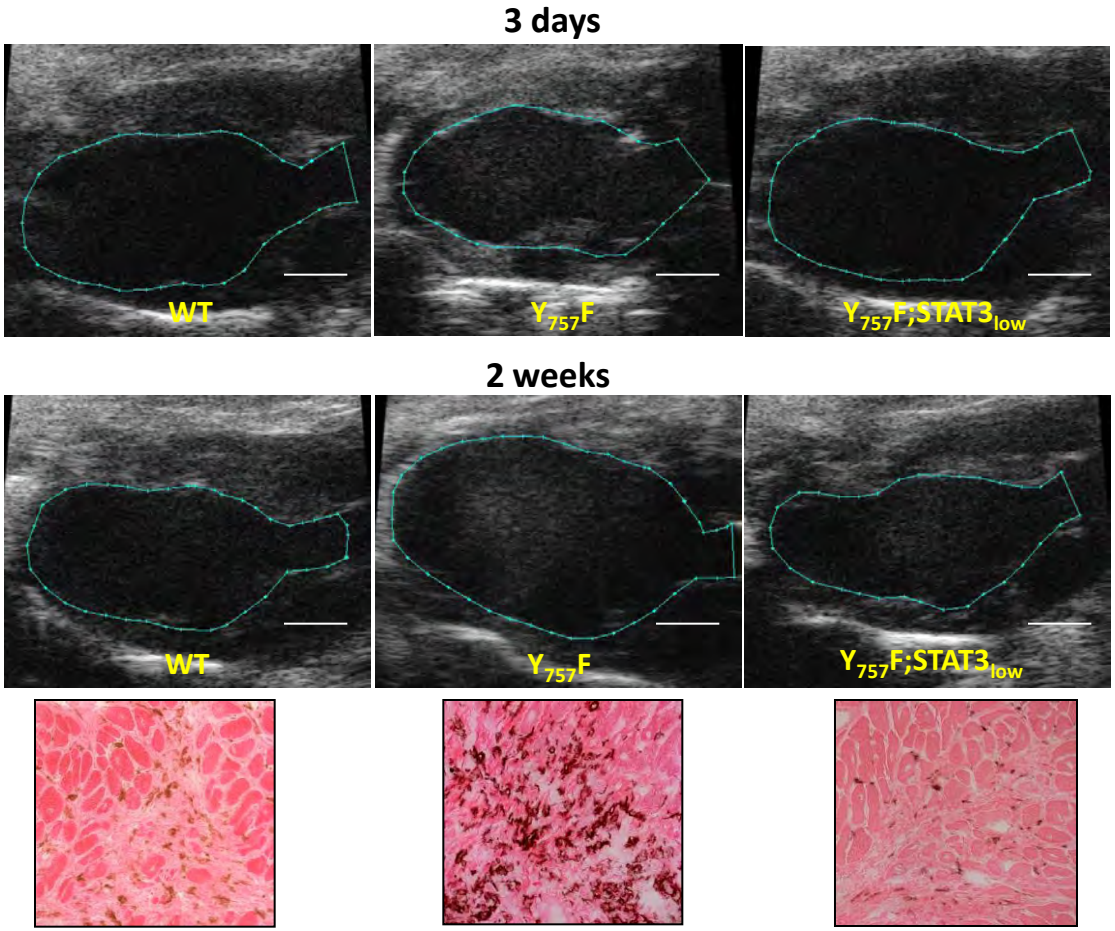
Genetic reduction of STAT3 reduces post MI mortality in $Y_{757}F;STAT3_{low}$ mice compared to $Y_{757}F$ mice

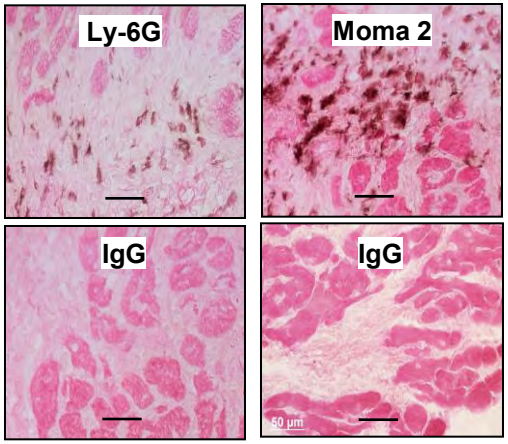


**P<0.01 vs WT MI

Hilfiker-Kleiner et al. Circulation 2010

Inflammation and remodeling of the infarcted heart in dependence of STAT3 levels



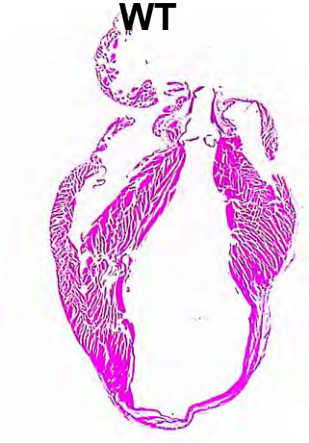


CD45+ macrophages

Hilfiker-Kleiner et al. Circulation 2010

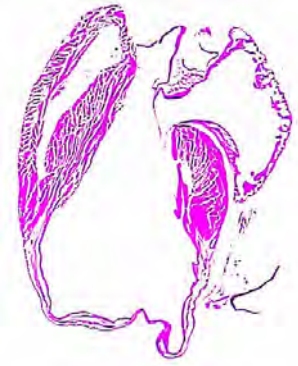
Inflammation and remodeling of the infarcted heart in dependence of STAT3 levels: Collagen content of the infarct scar

WT

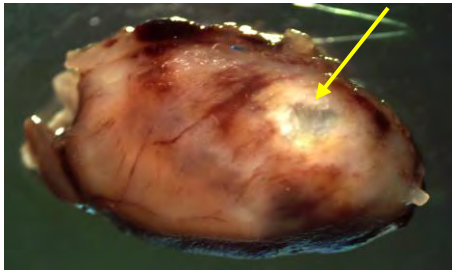


Rupture: 7%

CF/757



22%

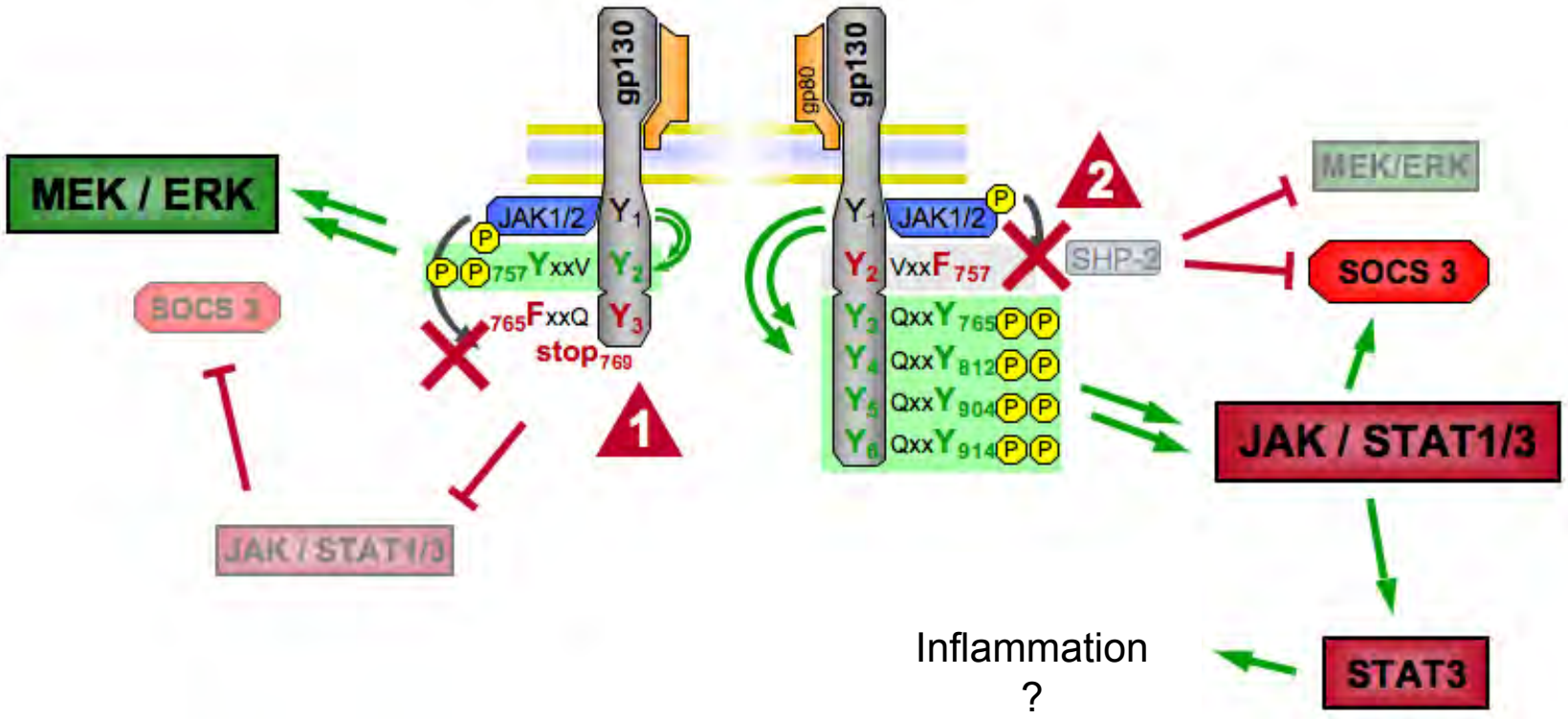


CF/757;STAT3+/-

0%

Hilfiker-Kleiner et al. Circulation 2010

What is the link between unrestricted and up-regulated STAT3 activation and adverse outcome after MI?

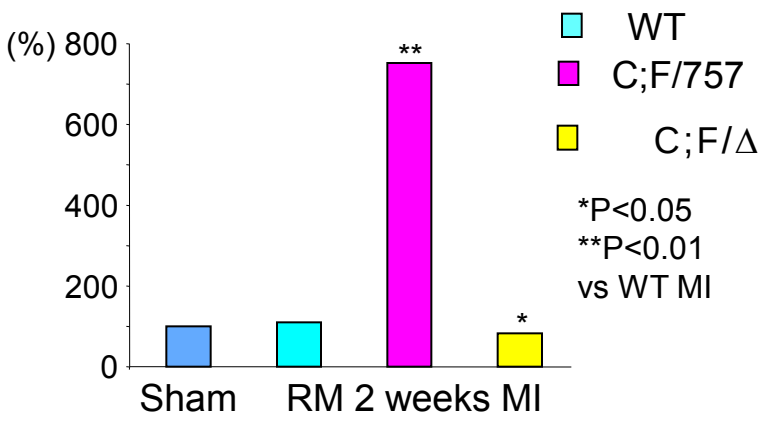


Fischer and Hilfiker-Kleiner BJP 2008

Overlay of micro array data STAT3 “gain-of-function” CF/757 versus “hypomorphic” CF/Δ reveals up-regulation of the MBL-C/complement system

Es1	**-13,71**	esterase 1
Es1	**8,34**	
Kng1	**-12,37**	kininogen 1
Kng1	**13,86**	
Fgb	**-8,00**	fibrinogen
Fgb	**9,49**	
H2-Q10	**-2,18**	histocompatibility 2
H2-Q10	**4,65**	
C8a	**-7,99**	complement component 8
C8a	**3,95**	
F10	**-2,27**	coagulation factor X
F10	**5,56**	
Mbl-C	**-4,98**	mannose binding lectin
Mbl-C	**6,03**	

Reatime PCR of MBL-C mRNA in RM

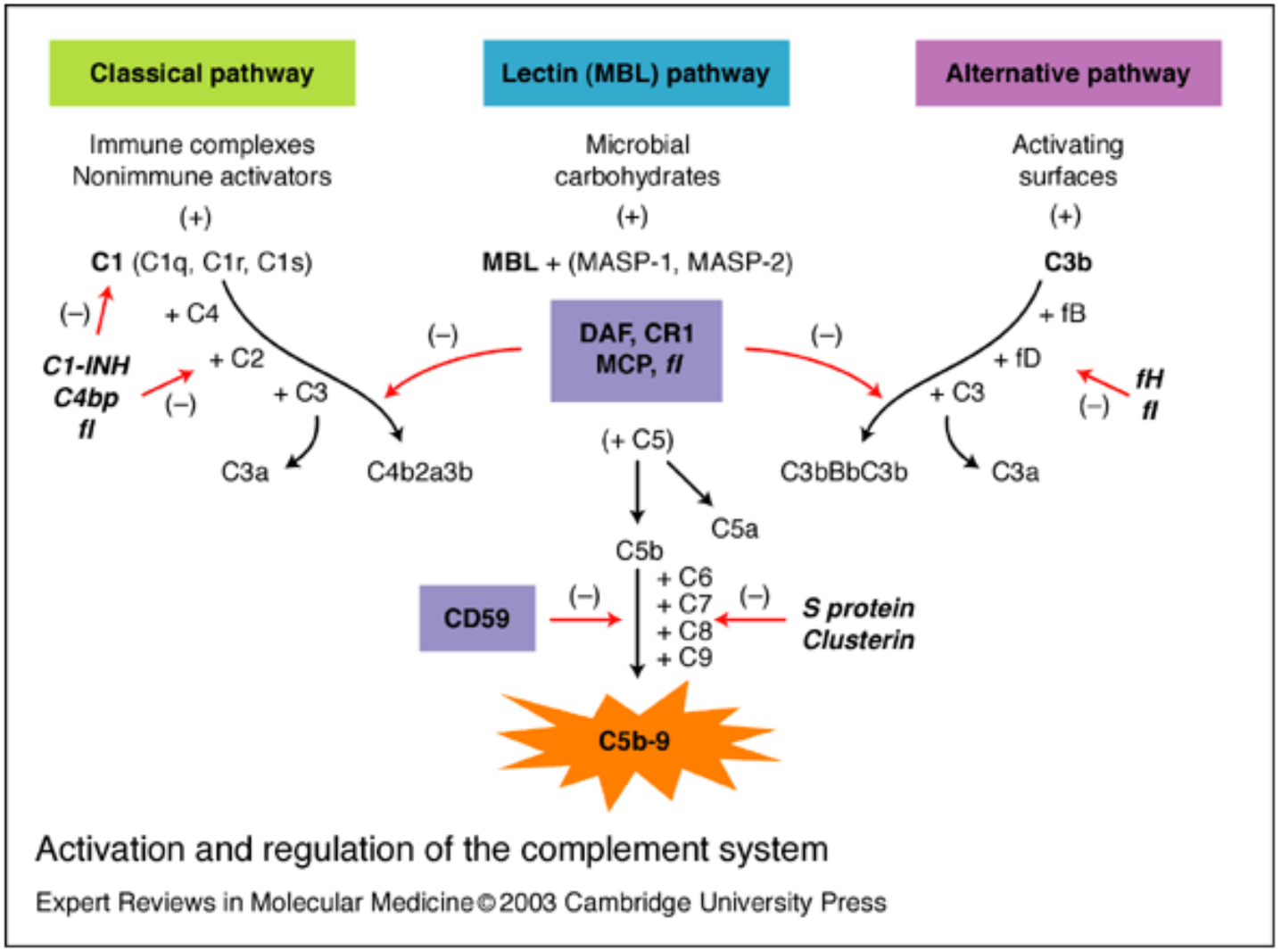


CF/757 up

CF/Delta down

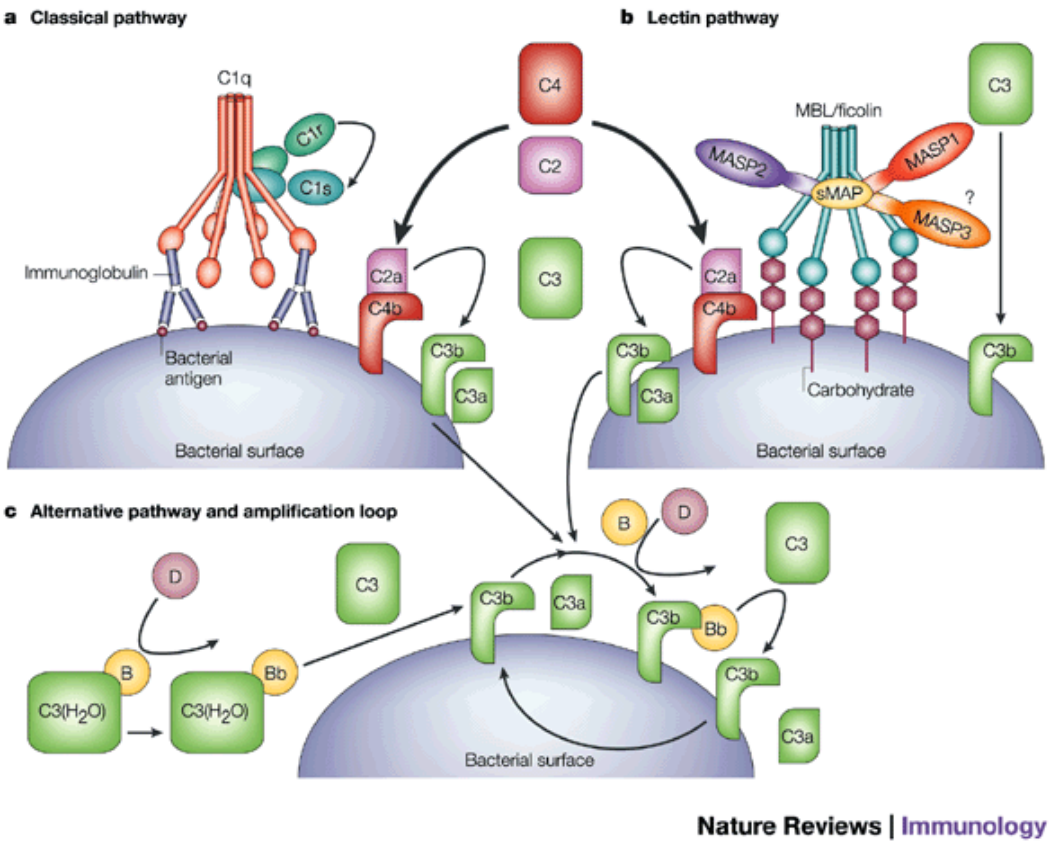
Hilfiker-Kleiner et al. Circulation 2010

The lectin (MBL) pathway of complement activation



Activation and regulation of the complement system

The Mannose-binding Lectin and complement activation: Host defense, innate immunity, cell damage



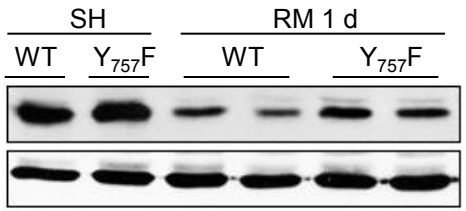
Carbohydrates on microorganims

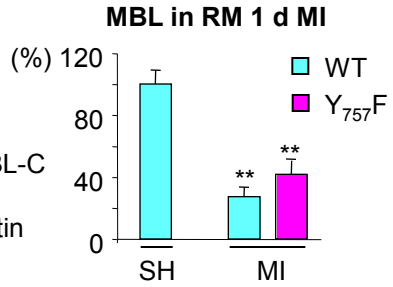
Endogenous ligands

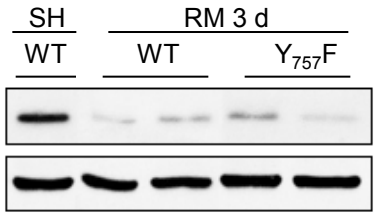
- Necrosis and apoptotic cells)
- Transformed (tumor) cells
- Anoxic endothelial cells

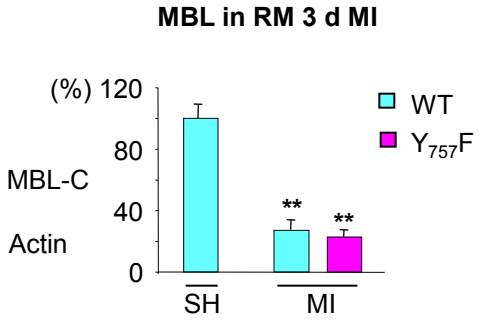
Additional genes up-regulated in infarcted LVs from CF/757 mice: MBL-A (3.3-fold), MASP-2 (3.3-fold), C3 (2.5-fold), C3ar1 (3.3-fold), C4bp (8-fold), C6 (3.8-fold), C8b (7.7-fold) and C8g (2.5-fold)

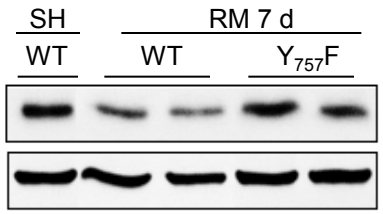
MBL-C is down-regulated in infarcted WT mice. Activated STAT3 re-induces expression on MBL-C in the sub-acute phase of MI in Y757F

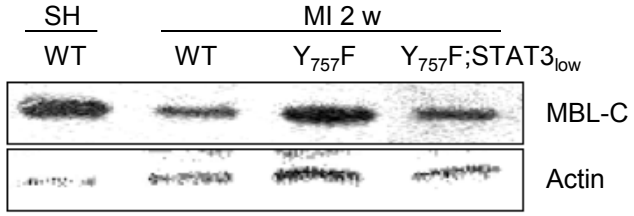


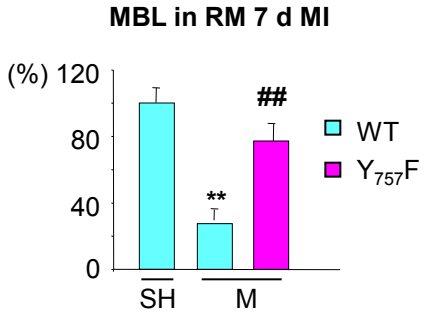


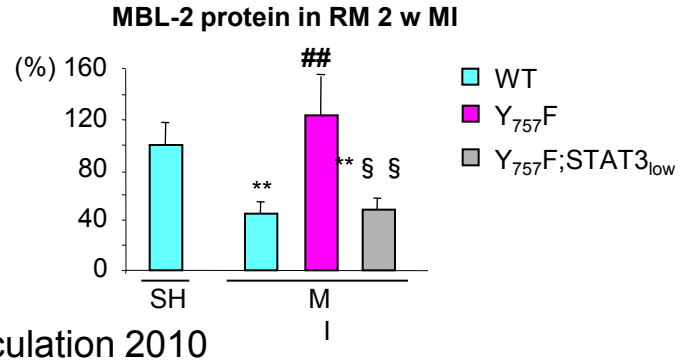






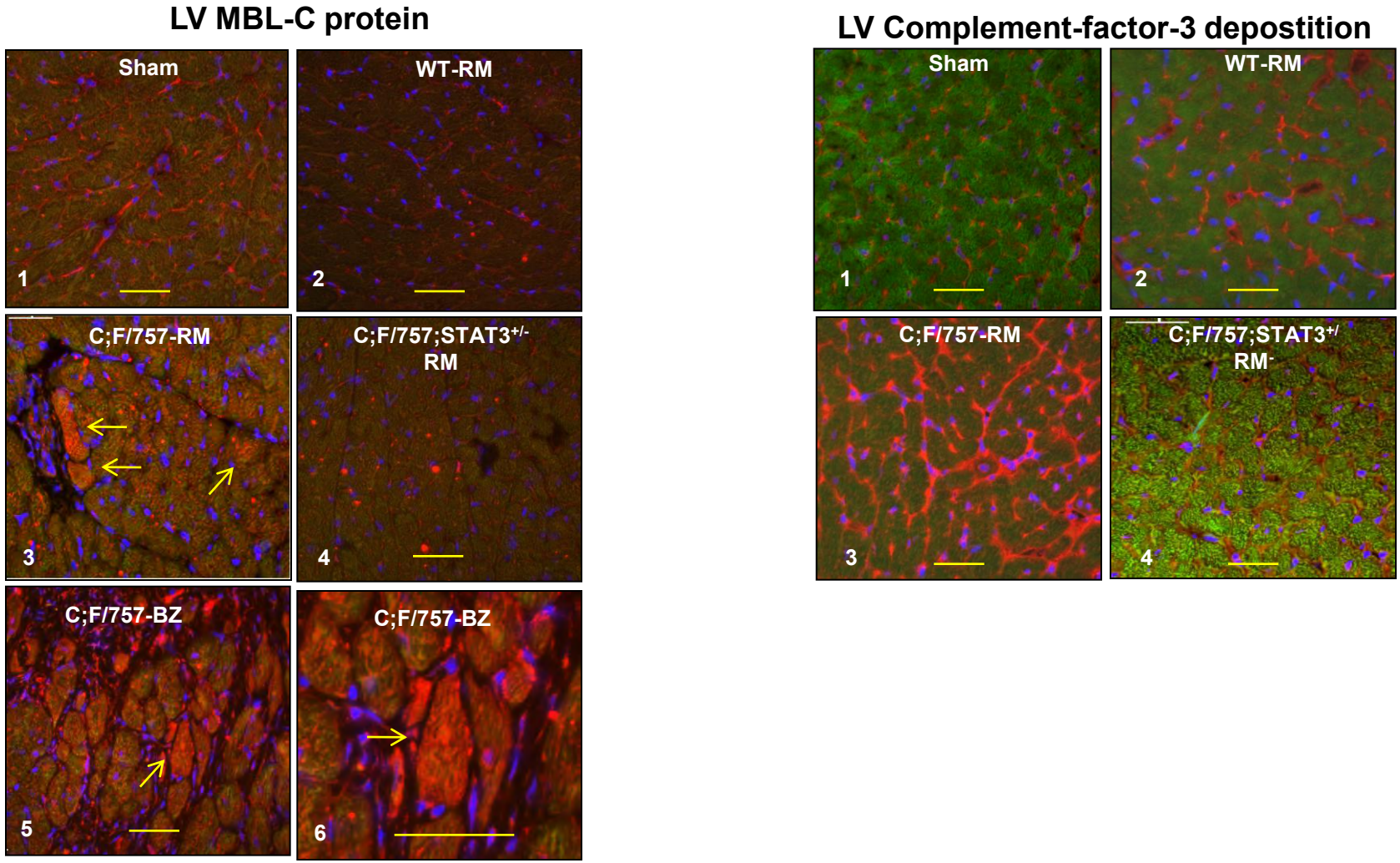






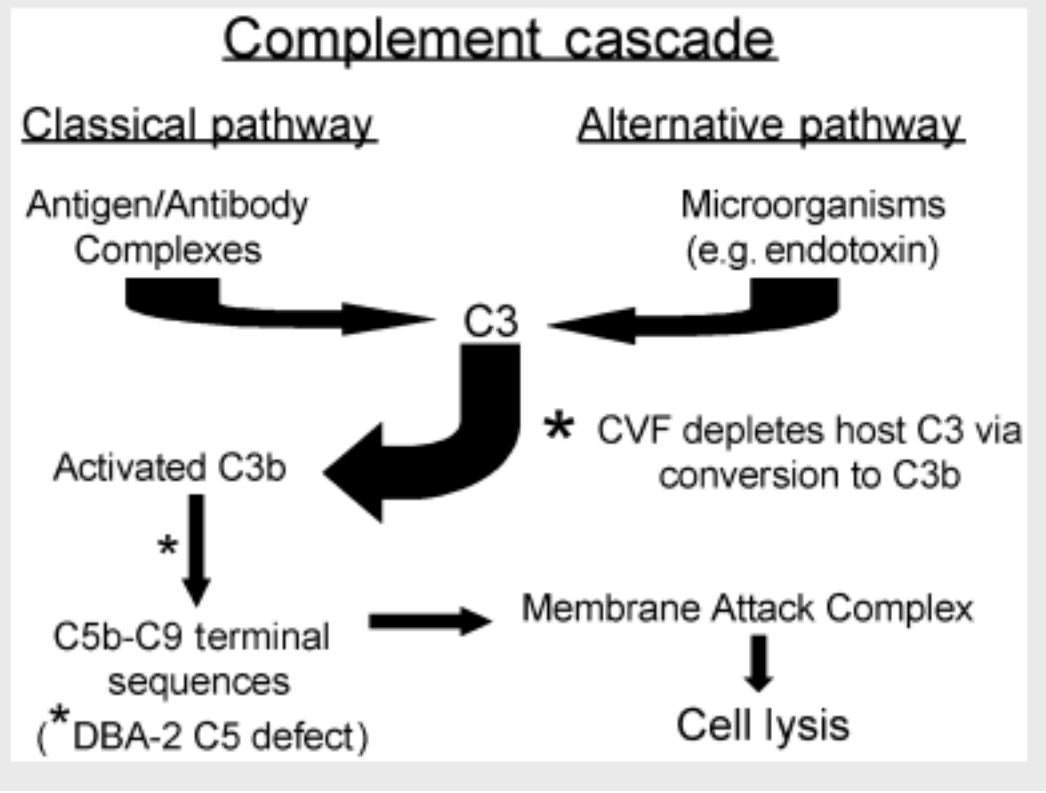
Hilfiker-Kleiner et al. Circulation 2010

Reduction of STAT3 attenuates MBL-C and Complement-factor-3 deposition after MI in CF/757 mice



Hilfiker-Kleiner et al. Circulation 2010

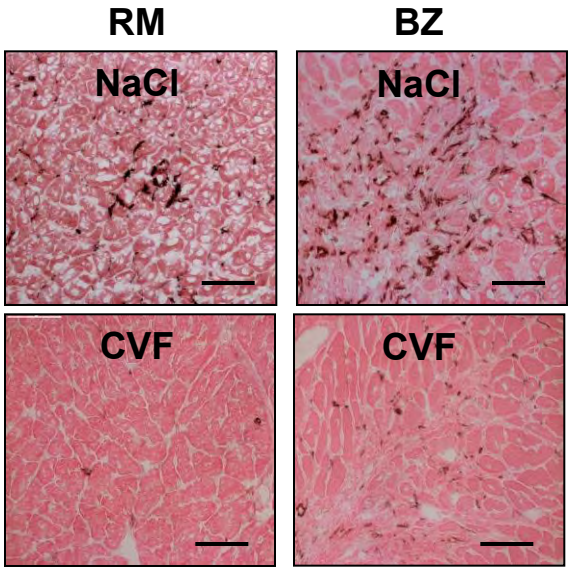
Complement inhibition with cobra venom factor (CVF) starting 3 days post MI in CF/757 mice



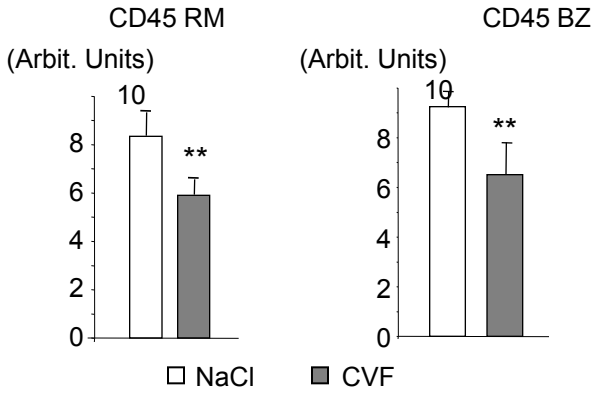
Hodgetts and Grounds, Immunology and Cell Biology (2001)

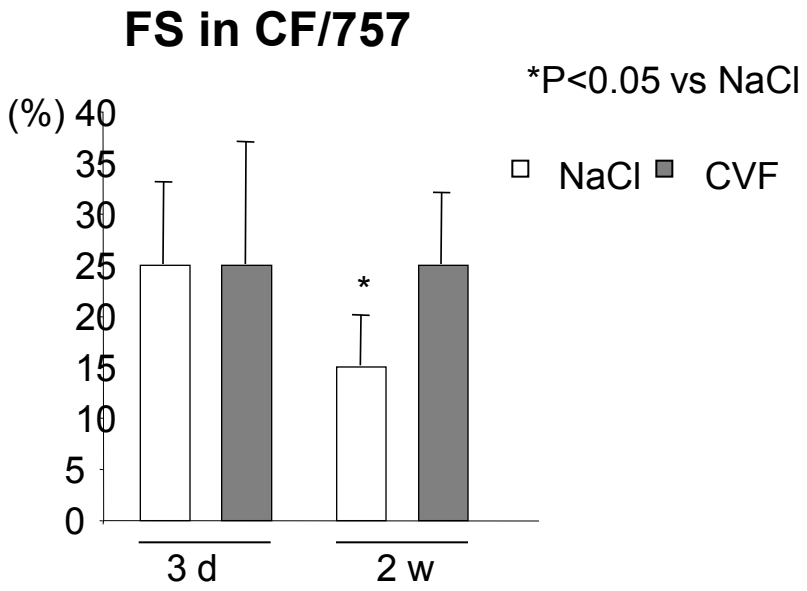
Cobra Venom Factor (CVF) resembles the C3b degradation product C3c which is unable to form the C3/C5 convertase. As a result, the presence of CVF causes depletion of complement components, thereby exhausting the complement response.

Cobra venom factor (CVF) therapy attenuates cardiac inflammation and improves cardiac function and survival in CF/757 mice



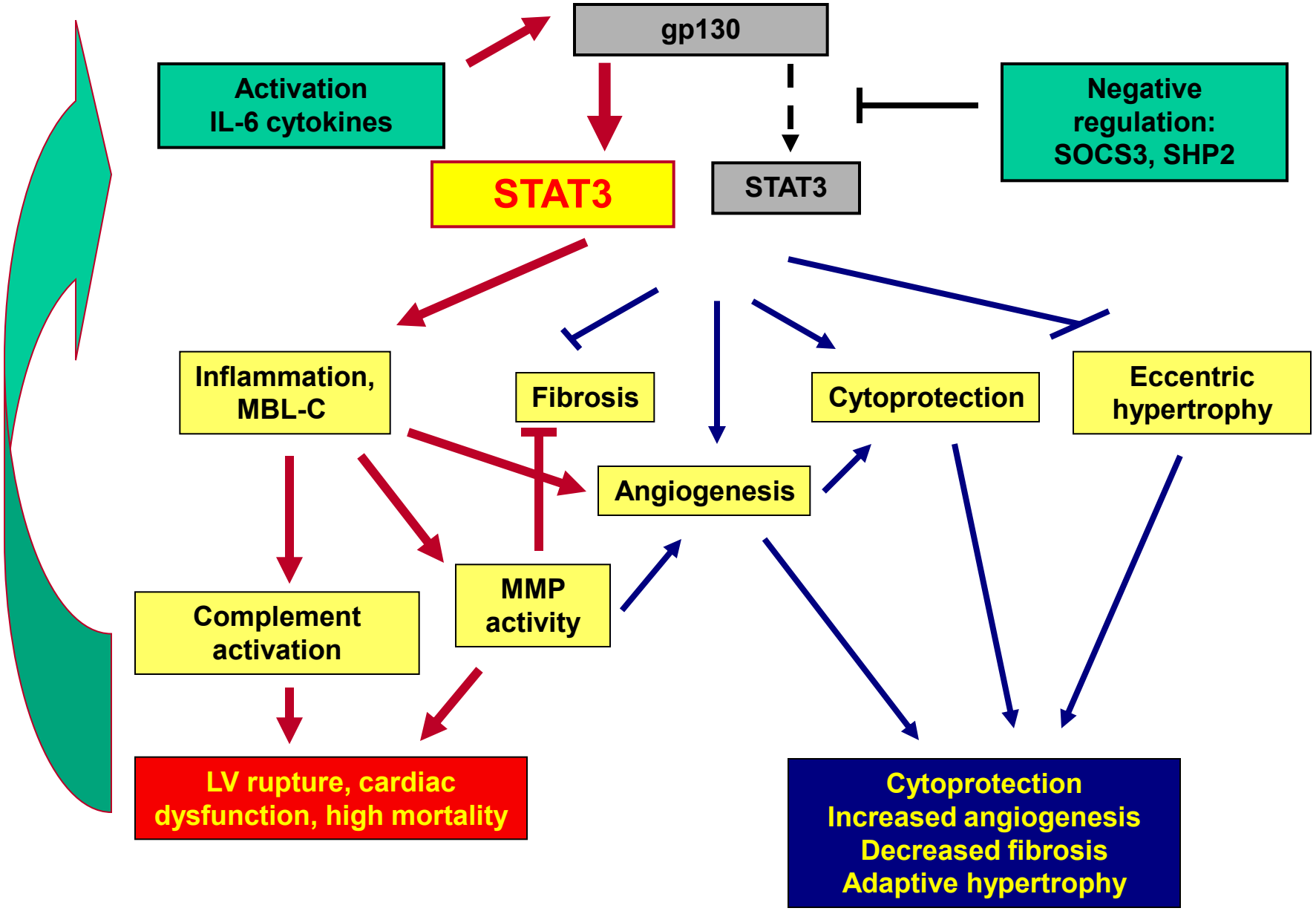
CD45, brown, H&E



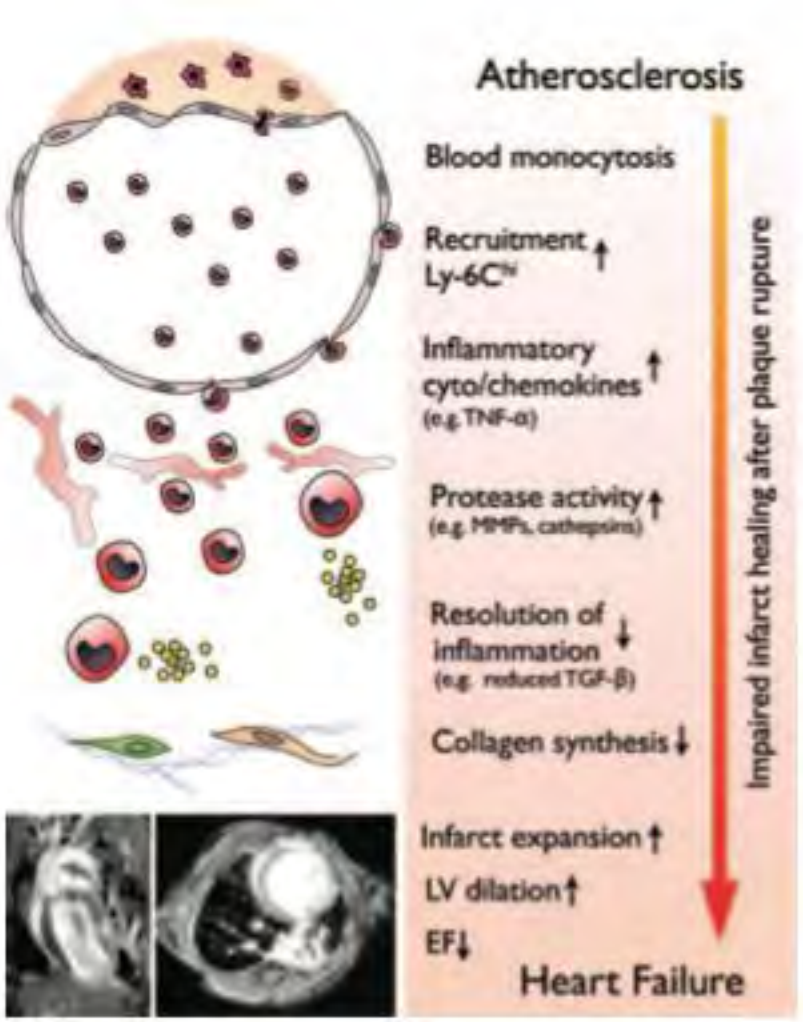


2 weeks survival rate in CF/757
100% in CVF, n=11
64% in NaCl, n=11, P<0.01
LV rupture occurred in 18% of NaCl treated mice.

Hilfiker-Kleiner et al. Circulation 2010


gp130
Activation
IL-6 cytokines
Negative
regulation:
SOCS3, SHP2
STAT3
STAT3
Inflammation,
MBL-C
Fibrosis
Cytoprotection
Eccentric
hypertrophy
Angiogenesis
Complement
activation
MMP
activity
LV rupture, cardiac
dysfunction, high mortality
Cytoprotection
Increased angiogenesis
Decreased fibrosis
Adaptive hypertrophy

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 modulatse the activity of marcrophages at the site of inflammation.

Tregs seem to play are 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.

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