Liver-Resident Macrophage Necroptosis Orchestrates Type 1 Microbicidal Inflammation and Type-2-Mediated Tissue Repair during Bacterial Infection

Camille Blériot, Théo Dupuis, Grégory Jouvion, Gérard Eberl, Olivier Disson, and Marc Lecuit

Dominika Lukovic
PhD Student at the Department of Internal Medicine II
Introduction

Liver innate immune effectors against bacterial infection

Tissue-resident macrophages

• Known as Kupffer cells
• Local self-renewal activity
• Embryonic derived
• Type-2-like anti-inflammatory phenotype (M2-like)

Monocyte-derived macrophages

• Bone-marrow derived
• Type-1-like pro-inflammatory phenotype (M1-like)
M1-like vs. M2-like phenotype

M1 macrophages (Monocyte-derived M.):
- Activated by LPS and IFN gamma
- Secret high levels of IL-12, low levels of IL-10
- Inhibit cells proliferation and promote tissue damage
- Pro-inflammatory response
- “Fight” program
- Arginine -> nitric oxide

M2 macrophages (Kupffer cells):
- Activated by IL-4
- Secret high levels of IL-10, TGF-beta and low level of IL-12
- Promote cell proliferation and tissue repair
- Anti-inflammatory response
- “Fix” program
- Arginine -> ornithine
Elimination of microorganisms by liver

1. LM enters intestinal barrier and reaches the liver
2. LM is engulfed by KCs
3. Recruitment of monocytes
4. Micro-abscesses formation
5. pro-inflammatory response

- Listeria monocytogenes (Lm)
- Bone-marrow derived monocyte
- Basophil
- Monocyte-derived macrophage (M1)
- Kupffer cell (M2)
- Dying Lm-infected Kupffer cell
- Hepatocyte
Lm Induces Local Proliferation of Liver Macrophages

- Macrophages (F4/80+)
- Liver cells (E-cadherin +)
- Neutrophils (Ly/6G+)

- After the infection increased total liver macrophages
- Macrophages proliferation was detectable in 24 hours, peaked at 3 dpi
- Bacteria were totally cleared before 10 dpi
*Lm* Induces Local Proliferation of Liver Macrophages

**A**- FACS analysis of liver cells (Percentage of Ki67+ cells out of CD45+F4/80+ cells)

**B+C**- Confocal imaging of frozen liver sections (F4/80, Ki67, Listeria, Ly-6G)

**D**- Quantification over time of F4/80+Ki67+ cells in frozen liver sections

**E**- Kinetics of liver bacterial load from WT mice
Lm-induced Liver macrophage Proliferation Requires M-CSF and Basophil-Derived IL-4

- GW2580 (inhibitor of M-CSFR)
- Total liver macrophages decreased in infected and uninfected mouse
- In IL4\(^{-/-}\) mice was decreased liver macrophage proliferation, whereas level of bone marrow monocyte was stable
- Basophils as a source of IL4 (CD49b\(^{\text{int}}\)Fc\(\varepsilon\)R1\(^{\text{int}}\)CD117\(^{-}\))
Lm-induced Liver macrophage Proliferation Requires M-CSF and IL-4

A- Quantification of F4/80+Ki67+ cells in liver sections (G2580 is M-CSFR inhibitor)

B- FACS analysis of gated CD45+F4/80+ liver cells, percentages of Ki67+ cells

C- Quantification of Ki67+ cells out of F4/80+ cells on frozen sections of the liver

D- FACS analysis of liver CD45+ cells (Basophils CD49b^{int}FceR1^{int}CD117^{-})
Lm-induced Liver macrophage Proliferation Requires M-CSF and Basophil-Derived IL-4

- GW2580 (inhibitor of M-CSFR)
- Total liver macrophages decreased in infected and uninfected mouse
- In IL4⁻/⁻ mice was decreased liver macrophage proliferation, whereas level of bone marrow monocyte was stable
- Basophils as a source of IL4 (CD49b^{int}FcεR1^{int}CD117⁻)
Lm-induced Liver macrophage Proliferation Requires M-CSF and Basophil-Derived IL-4

E- Confocal imaging on frozen liver sections (CD200R3 basophils)

F- ELISA of IL-4 in supernatants of the homogenized liver of the LM-infected mice

G- relative expression of IL-4 in sorted liver basophils obtained in uninfected and infected mice

H- LM bacterial burden in the liver of LM-infected mice
Proliferating Liver Macrophages Derive from Recruited Monocytes

A-FACS analysis of gated CD45+/F4/80+ liver cells
- KCs (F4/80^{hi}CD11b^{lo}Ly6C^{lo})
- Inflammatory monocytes (F4/80^{lo}CD11b^{int}Ly6C^{hi})
- Monocyte-derived macrophages (F4/80^{int}CD11b^{hi}Ly6C^{int})
Proliferating Liver Macrophages Derive from Recruited Monocytes

B- FACS analysis of liver KCs from WT mice.

C- Quantification of F4/80+ and F4/80+Ki67+ cells

G- FACS analysis of liver KCs obtained from GFP+ monocyte-transferred WT
Lm induces Kupffer Cell Necroptosis

10^8 CFU

4hpi

Monocyte recruitment starts

d1/d2
Lm induces Kupffer Cell Necroptosis
Hepatocytes-derived IL-33 induces monocyte-derived macrophage proliferation

A: IL-33 production peaks at 24h (expression & ELISA)

B: blocking cell death by necrostatin-1s >> decreased IL-33 production

C: necrostatin-1s >> decreased proliferation of macrophages

D: IL-33-receptor deficient mice shows decreased MoMs proliferation. Same with Ab inhibition of IL33 receptor.
The Type 1 inflammatory liver response to Lm is counterbalanced by type 2 response.
The Type 1 inflammatory liver response to Lm is counterbalanced by type 2 response.
Lm-induced macrophage proliferation dampens inflammation allowing the liver to return to homeostasis.

uninf.

Lm-inf. 21dpi.
Lm hepatic infection

- KCs death
- Recruitment & differentiation
- (M1) MoMs
- Phagocyt. Protective responses
- Replace KCs

Type 1 inflammation
- KCs
- MOMs
- Neutrophils

Type 2 inflammation
- Basophils

Resolution

MoMs (M1)

M2
Summary

• Phagocytized bacteria induce necroptosis of liver-resident macrophages

• Macrophages necroptosis triggers both type 1 and type 2 responses

• Monocyte-derived macrophages replace dead tissue resident macrophages

• Sequential type 1 and type 2 responses orchestrate liver return to homeostasis
Thank you for attention