The dual role of macrophages in mycobacterial- and HIV disease: host cell and generator of protective mediators with therapeutic potential
World population | 6.2 billion
---|---
Total deaths from all causes | 57 million | 100%

<table>
<thead>
<tr>
<th>Rank</th>
<th>Cause of death</th>
<th>Number</th>
<th>Percentage of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lower respiratory infections</td>
<td>3.9 million</td>
<td>6.8%</td>
</tr>
<tr>
<td>2</td>
<td>HIV/AIDS</td>
<td>2.8 million</td>
<td>4.9%</td>
</tr>
<tr>
<td>3</td>
<td>Diarrheal diseases</td>
<td>1.8 million</td>
<td>3.2%</td>
</tr>
<tr>
<td>4</td>
<td>Tuberculosis (TB)</td>
<td>1.6 million</td>
<td>2.7%</td>
</tr>
<tr>
<td>5</td>
<td>Malaria</td>
<td>1.3 million</td>
<td>2.2%</td>
</tr>
<tr>
<td>6</td>
<td>Measles</td>
<td>0.6 million</td>
<td>1.1%</td>
</tr>
<tr>
<td>7</td>
<td>Pertussis</td>
<td>0.30 million</td>
<td>0.5%</td>
</tr>
<tr>
<td>8</td>
<td>Tetanus</td>
<td>0.21 million</td>
<td>0.4%</td>
</tr>
<tr>
<td>9</td>
<td>Meningitis</td>
<td>0.17 million</td>
<td>0.3%</td>
</tr>
<tr>
<td>10</td>
<td>Syphilis</td>
<td>0.16 million</td>
<td>0.3%</td>
</tr>
<tr>
<td>11</td>
<td>Hepatitis B</td>
<td>0.10 million</td>
<td>0.2%</td>
</tr>
<tr>
<td>12</td>
<td>Tropical diseases (6)</td>
<td>0.13 million</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

The dual role of macrophages in mycobacterial- and HIV disease: host cell and generator of protective mediators with therapeutic potential

- With 2-3 million deaths in 2000, HIV and Mycobacterium tuberculosis top the World Health Organization (WHO) list of deaths caused by infectious agents.
- Mycobacteria and HIV do not operate independently; a secondary epidemic of tuberculosis is accompanying the rise in the number of HIV-infected persons.
- MAC-disease (Mycobacterium avium complex) is not normally acquired by healthy individuals, but cause opportunistic infections in AIDS patients
- Increasing resistance to antibiotics is observed in all pathogenic mycobacteria, in particular in the developing countries
The dual role of macrophages in mycobacterial- and HIV disease: host cell and generator of protective mediators with therapeutic potential

The AIM of the project is to characterize the role of effector responses (cytokines, chemokines, reactive oxygen species (ROS), antimicrobial proteins (lipocalin 2)) resulting from Toll-like receptor (TLR) activation of macrophages in mycobacterial- and HIV disease. The project will contribute to a better understanding of the pathogenesis of mycobacteria in HIV-infected individuals, as well as to discovery of new therapeutic modalities.

"Innate immunity in the pathogenesis of HIV and mycobacterial disease"
Partner 1: IKM, Medical faculty, NTNU Trondheim

Trude Helen Flo, Terje Espevik, Øyvind Halaas, (Magnus Steigedal)

Expertise & contribution

Innate immunity: pathogen recognition receptors, effectors (lipocalin2)

Antibody production

Imaging: confocal microscopy
  Uptake, transport, localization and signaling inside cells

In vivo infection models in mice, in vitro cell assays
Partner 2: Medical department, Rikshospitalet, Oslo

Stig Frøland, Pål Aukrust, Linn Landrø

Expertise & contribution

Clinical disease: HIV/AIDS, opportunistic infections (MAC)

HIV/AIDS patients, blood samples, tissue samples

In vitro cell assays
The Macrophage: host cell and generator of effector responses

Macrophages are "janitor" cells of the innate immune system
- Phagocytosis (eating)
- Intracellular killing
- Antigen presentation (T-cell activation $\rightarrow$ IFN$\gamma$)
- Effector molecule production: Toll-like receptors

*Mycobacteria* evade the potent antibacterial program normally mounted inside the macrophage and make them their host

Macrophages also function as a *HIV* reservoir and contribute to a persistent and inappropriate immune activation in infected individuals
The Toll-like receptor family: pathogen recognition

Mycobacteria

Lipoproteins
Lipopeptides
Peptidoglycan
Zymosan (yeast)

LPS
HSP70
Flagellin
dsRNA
CpG
HIV ssRNA

Antiviral compounds

TLR2, TLR1
TLR4
TLR5
TLR6
TLR7
TLR8
TLR9
TLR10

CD14
MD-2
MyD88
TIR

Inflammatory response
Lipocalin 2

www.ntnu.no
Activation of immune cell TLRs: Effector proteins

Anti-microbial effector proteins/peptides
Signalling molecules

Flagellin
LPS
MD2
TLR4, TLR5
Interrelationship between HIV, mycobacteria and TLRs

Reduced CD4+ T-cells, cytokine dysregulation and enhanced oxidative stress may contribute to an increased susceptibility of HIV-infected patients to mycobacterial infections.

TLR-activation and/or mycobacterial infection supports HIV-replication and genotypic conversion.

**Objective 1**

Compare TLR-initiated effector responses in healthy individuals with those in HIV-infected individuals with or without accompanying mycobacterial (MAC) disease.
Lipocalin 2

TLR-initiated effector protein: induced and secreted as a result of TLR-activation

Anti-bacterial: Works by starving bacteria for iron
Systemic iron homeostasis

Hentze MW et al., Cell, 2004:17:285
Regulation of systemic iron homeostasis

"Iron withholding strategy of host defense"

Hentze MW et al., Cell, 2004:17:285
Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron

TRUDE H. FLO\textsuperscript{1,2,*}, KELLY D. SMITH\textsuperscript{1,3,*}, SHINTARO SATO\textsuperscript{4}, DAVID J. RODRIGUEZ\textsuperscript{1}, MARGARET A. HOLMES\textsuperscript{5}, ROLAND K. STRONG\textsuperscript{5}, SHIZUO AKIRA\textsuperscript{4} & ALAN ADEREM\textsuperscript{1}
Lipocalin 2 binds to bacterial siderophores

Molecular Cell, Vol 10, 1033-1043, November 2002

The Neutrophil Lipocalin NGAL Is a Bacteriostatic Agent that Interferes with Siderophore-Mediated Iron Acquisition

David H. Goetz\textsuperscript{1,2}, Margaret A. Holmes\textsuperscript{2}, Niels Borregaard\textsuperscript{3}, Martin E. Bluhm\textsuperscript{4}, Kenneth N. Raymond\textsuperscript{4}, and Roland K. Strong\textsuperscript{2}

![Diagram showing the interaction of Enterochelin with Pocket #1]
Lipocalin 2 is bacteriostatic by starving bacteria for iron
Lipocalin 2 is bacteriostatic by starving bacteria for iron.
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Lipocalin 2 is bacteriostatic by starving bacteria for iron.
E. Coli H9049
i.p. 1-2x10^8 CFU
E. Coli H9049 makes E (recognized by Lcn2) → low virulence
E. Coli H9049 does not make F (not recognized by Lcn2)

**Hypothesis:** If E. coli H9049 could access F it would overcome the growth inhibition mediated by lipocalin 2
Lipocalin 2 is siderophore-specific
**Science. 2003 Feb 7;299(5608):906-9**

**Passage of heme-iron across the envelope of Staphylococcus aureus.**


Committee on Microbiology, Department of Molecular Genetics and Cell Biology, University of Chicago, 920 East 58th Street, Chicago, IL 60637, USA.

**Science. 2004 Sep 10;305(5690):1626-8.**

**Iron-source preference of Staphylococcus aureus infections.**

Skaar EP, Humayun M, Bae T, DeBord KL, Schneewind O.

Committee on Microbiology, 920 East 58th Street, University of Chicago, Chicago, IL 60637, USA.
Lipocalin 2 is siderophore-specific
Staphylococcus aureus is not affected by lipocalin 2
Mycobacterium tuberculosis mutant in mycobactin show impaired growth inside THP-1 macrophages


Siderocalin (Lcn 2) Also Binds Carboxymycobactins, Potentially Defending against Mycobacterial Infections through Iron Sequestration.

Holmes MA, Paulsene W, Jide X, Ratledge C, Strong RK.

Objective 2
Establish the role of lipocalin 2 in protection against mycobacteria
Objective 3

Studying the binding and intracellular transport of lipocalin 2 in macrophages
Uptake of lipocalin 2 in mouse macrophages

0 min

120 min

A488-Lcn2

MFI (Lcn2A488)

Time (min)

0 15 30 60 120
Mycobacteria arrest phagosome maturation

Access of Tf-Fe

Transferrin

Lipocalin 2

Merge
Both mycobacteria and the HIV-virus activate innate immune cells through TLRs. We hypothesise that distortions in the TLR signalling pathways may contribute to immunodeficiency, HIV replication and increased susceptibility of HIV-infected individuals to mycobacterial diseases.

**Objective 1**
Compare TLR-initiated effector responses in healthy individuals with those in HIV-infected individuals with or without accompanying mycobacterial (MAC) disease

We further hypothesise that lipocalin 2 is mycobacteriostatic by starving the bacteria for iron

**Objective 2**
Establish the role of lipocalin 2 in protection against mycobacteria

**Objective 3**
Studying the binding and intracellular transport of lipocalin 2 in macrophages
Collaborators / Acknowledgements:

**NTNU, Trondheim**
- Trude Helen Flo
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- Shintaro Sato

**Rikshospitalet, Oslo**
- Stig Frøland
- Pål Aukrust
- Linn Landrø