Tracking dynamic innate immune responses in experimental malaria infection

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April 10, 2014 - MBI - CTW (Ohio State University)
Outline

1- Background
   Immunity to malaria
   MaHPIC Innate Immune Profiling Core

2- Results
   EX04: Experimental *P. cynomolgi* infection in *M. mulatta*
   Multiparametric analysis of dynamic innate responses

3- Next stages
   Integration with other Cores and modeling
   Extension to natural infection (NHP, humans)
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Host / parasite interactions
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- Danger sensing
- Invasion of primary tissue

HOST ⇌ PARASITE
Host / parasite interactions

- Danger sensing
- Innate immune response (from d0, on)

Parasite

- Invasion of primary tissue
- Modulation of innate immune system (active = virulence factors)
Host / parasite interactions

- **Danger sensing**
- **Innate immune response**
  (from d0, on)
- **Adaptive immune response**
  (from d10-14, on in primo-infection, from d0, on in recurrence)

- **Invasion of primary tissue**
- **Modulation of innate immune system**
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- **Modulation of adaptive immune system**
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- **Escape to other host**
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(sick host: fever, cachexia)
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- Disease (none/acute/chronic/latent), Immunity (sterilizing/non-sterilizing)
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**MODIFIERS**
- Host genetics
- Other microbes (commensals / parasites)
- Treatment

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Innate and adaptive immunity: overview
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ADAPTIVE IMMUNITY:
- requires time,
- provides specificity,
- can provide memory,
- tissues >> blood
Innate and adaptive immunity: overview

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Innate and adaptive immunity: overview

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Nature Reviews | Cancer
Innate and adaptive immunity: overview

ADAPTIVE IMMUNITY: requires time, provides specificity, can provide memory, tissues >> blood
Innate and adaptive immunity: overview

INNATE IMMUNITY:
- immediate
- "hardwired"
- common systemic effects,
tissues and blood

ADAPTIVE IMMUNITY:
- requires time,
- provides specificity,
can provide memory,
tissues >> blood

Nature Reviews | Cancer

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Malaria pathogenesis is associated with altered innate immune processes:

(i) **neutrophil** and **monocyte** modulation, altering phagocytosis / killing and increasing host susceptibility to bacteria;
(ii) **platelet** activation, leading to the release of inflammatory mediators;
(iii) **eosinophil** and **NK cell** activation during blood stages;
(iv) the presence of hemozoin in **monocytes / macrophages** and **neutrophils**, which may be a viable biomarker for disease severity.

**Mission of the Innate Immunity Core:**
To characterize the impact of innate immune processes in malaria-infected hosts, using small aliquots of peripheral blood (PB) and bone marrow (BM) collected as part of the MaHPIC study.
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**Mission of the Innate Immunity Core:**
To characterize the impact of innate immune processes in malaria-infected hosts, using small aliquots of peripheral blood (PB) and bone marrow (BM) collected as part of the MaHPIC study.
Custom protocol

100 µl Whole Blood

Unstimulated → PMA → Bacteria → Stored

Wash

Cells → Platelets → Lyse RBCs

Future Experiments and Validations

100 µl Bone Marrow

Unstimulated → Stored

Wash

Cells → Platelets → Lyse RBCs

Future Experiments and Validations
Cytometry probes: a wide array to pick from

- Several 1000s of antibodies, each available in various colors
- Non-antibody probes for enzymes active caspase-1 (FLICA), ROS (CellRox), phagocytosis (pHrodo), carbohydrates (lectins), lipids, ions (Ca, Cl, Zn), etc.

>> CD markers
CD11b / 14 / 16 / 45 / 63 / 66b, etc.

>> Redox
PDI, Trx

>> Enzymes
Caspase-1 (FLICA)

>> Phosphoprotein profile
Ph MAPKs
Ph Akt
Ph Syk

Total Ph Y
Ph NFkB
Ph STAT5
Ph S6rp

Activated (Phosphorylated) Kinases
Activated (Phosphorylated) Effectors

Phosphatases

Maleimide
Lectins
Annexin V
Cholera Toxin B

Ca
DHR
DAF

Cell Rox
Thiol Tracker
DAF2DA

pHrodo

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Custom assays

<table>
<thead>
<tr>
<th>Condition</th>
<th>Color</th>
<th>Pacific Blue</th>
<th>Pacific Orange</th>
<th>FITC / Alexa 488</th>
<th>Per-CP Cy5.5</th>
<th>PE / Alexa 555</th>
<th>PE-Cy7</th>
<th>APC / Alexa 647</th>
<th>Alexa 700</th>
<th>APC-Cy7</th>
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</thead>
<tbody>
<tr>
<td>PB-Baseline</td>
<td>CD63</td>
<td>Live/Dead</td>
<td>FLICA</td>
<td>CD16</td>
<td>CD66b</td>
<td>CD41a</td>
<td>CellRoxDR</td>
<td>CD45</td>
<td>CD69</td>
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</tr>
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<td>PB-Stim PMA</td>
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<td>PB-Stim Bacteria</td>
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<td>Live/Dead</td>
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<td>CD66b</td>
<td>CD41a</td>
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<td></td>
</tr>
<tr>
<td>BM-Baseline</td>
<td>CD34</td>
<td>Live/Dead</td>
<td>FLICA</td>
<td>CD16</td>
<td>CD66b</td>
<td>CD41a</td>
<td>CellRoxDR</td>
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</tr>
</tbody>
</table>

Singlets: FSc-A vs FSc-H diagonal
- Live: L/D low
  - Platelets: CD41a+CD45-
  - Leukocytes: CD45+ (CD41a- vs. CD41a+, with or without aggregated platelets, respectively)
    - Neutrophils: Side scatter high, CD66b+, CD45 low
    - Eosinophils: Side scatter high, CD66b+, CD45 high
  - NK cells: Side scatter low, CD45 high, CD16+
  - M1 monocytes / macrophages: Side scatter intermediate, CD45 high, CD16-
  - M2 monocytes / macrophages: Side scatter intermediate, CD45 high, CD16+

This strategy simultaneously enables measurement of differentiation and activation status, as follows:
- BM precursors (all above subsets): CD34+
- Degranulation / surface activation (P, N, E, M1, M2): CD63, CD69 levels
- Oxidant production: CellRoxDR levels
- Caspase-1 activation: FLICA levels
- Phagocytosis: pHrodo levels
Assessing specific subsets in blood (25 µl)

SEQUENTIAL GATING: BLOOD NEUTROPHILS
EX13: Caspase-1 in blood neutrophils

PROFILE OF ACTIVATION: ACTIVE CASPASE-1 IN BLOOD NEUTROPHILS

EX13: CONTROL INJECTION (NON-INFECTED SALIVARY GLAND)

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EX04: Caspase-1 in blood neutrophils

PROFILE OF ACTIVATION: ACTIVE CASPASE-1 IN BLOOD NEUTROPHILS

EX04: EXPERIMENTAL MALARIA (P. CYNOMOLGI)

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EX04: neutrophil function (baseline)

Log10 Parasitemia (Ct/µl)

Neutrophils: Caspase-1 (MFI)

Neutrophils: ROS (MFI)

Days post-infection
EX04: neutrophil function (baseline)

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Neutrophils:
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TP1 TP2 TP3 TP4 TP5 TP6 TP7

Days post-infection

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TP1  TP2  TP3  TP4  TP5  TP6  TP7

Saturday, April 11, 14
EX04: leukocyte subset function (baseline)
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Log10 Parasitemia (Ct/µl)

Neutrophils: Caspase-1 (MFI)

Neutrophils: ROS (MFI)

Timepoint

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Log10 Parasitemia (Ct/µl)

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Neutrophils: ROS (MFI)

Timepoint

Friday, April 11, 14
EX04: neutrophil function (added bacteria)

- Neutrophils: ROS with bacteria (MFI)
- Neutrophils: Uptake of bacteria (MFI)
- Log10 Parasitemia (Ct/µl)

Days post-infection

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EX04: neutrophil function (added bacteria)
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Days post-infection

Log10 Parasitemia (Ct/µl)

Neutrophils: ROS w/ bacteria (MFI)

Neutrophils: Uptake of bacteria (MFI)

TP1  TP2  TP3  TP4  TP5  TP6  TP7

Monkey ID
RFa14  RFv13  Rtc14  RMe14  RSb14

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Data output and sharing

**Data Output** (Flow cytometry ~ 500 files, 10,000 datapoints so far)

- **EX13** [mock infection]: completed
  
  5 animals x 4 main timepoints x 8 samples x 20 outcomes
  5 animals x 4 early timepoints x 2 samples x 20 outcomes
  
  = 4,000 datapoints

- **EX04** [*P. cynomolgi*]: completed
  
  4 animals x 7 main timepoints x 8 samples x 20 outcomes
  1 animal x 3 main timepoints x 8 samples x 20 outcomes
  5 animals x 4 early timepoints x 2 samples x 20 outcomes
  
  = 5,760 datapoints

- **Exp #18** [*P. coatneyi*]: underway

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Data Sharing
Several iterations for analysis
Several iterations for metadata
Feedback from Informatics / Genomics

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## Omics integration

<table>
<thead>
<tr>
<th></th>
<th>Sharing of primary analytical scheme</th>
<th>Data will benefit InnImm Core</th>
<th>InnImm Core data will be used</th>
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</thead>
<tbody>
<tr>
<td>Adaptive Immunity</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td></td>
<td>X</td>
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<td>Lipidomics</td>
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<tr>
<td>Mathematical modeling</td>
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MaHPIC: onward and beyond

Summary

✴ Framework for quantitative, dynamic analysis of innate subsets in small volumes
✴ Responses in leukocyte, platelet, leukocyte-platelet aggregates, as envisioned
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NHP Lab Projects - In preparation

- Role of spleen in malaria - (Galinski)
Host / parasite interactions

MODIFIERS
- Host genetics
- Other microbes (commensals / parasites)
- Treatment

HOST

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Acknowledgments

MaHPIC Team

✴ Chet Joyner
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Q & A

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