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Lessons from the Field: Parasite DNA Drives the Innate Immune Response to Malaria (it's not just the caiparinhas)

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Lessons from the field: parasite DNA drives the innate immune response to malaria (it's not just the caiparinhas)
Drosophila melanogaster larvae
Christiane Nüsslein-Volhard, Nobel Laureate (Medicine, 1995)
mutant *Drosophila melanogaster* larvae

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TOLL
Amazing
Bodacious
Cool
Corky
Crazy
Frantic
Furious
Great
Like blazes
Mad
Madcrap
Screaming
Super
Wild
Wicked
Wow
Cloning of *Toll*: homology to the human IL-1 receptor
Aspergillus fumigatus

Mutations in *Toll* predispose to fungal infections


*Aspergillus fumigatus*
Mammalian Toll-like receptors

- 10 human TLRs
- 4 adapter TLRs
There are 4 TLR adapter molecules

- TLR4
- TLR3
- TLR7, 9
- TLR5

TLR1/2
TLR2/6

MyD88-dependent

MyD88
IRAKs

TLR4

MyD88-independent

TRAM
TRIF

TLR3
There are two broad classes of TLRs: endosomal and surface TLRs.
TLR9 is the prototypical endosomal TLR.
TLR9 must move from the ER to the endosome to encounter its ligand (CpG DNA).
Malaria: the facts in 2010

35% of the world's population is at risk

http://www.map.ox.ac.uk
Epidemiology

• Malaria is generally considered to be a tropical disease.

• Four major species of plasmodium, but *P. falciparum* and *P. vivax* are by far the most important.

• *P. falciparum* is worldwide; *P. vivax* is not frequent in Africa but frequent in South America and Asia.
The paroxysm of malaria
The paroxysm of malaria represents an inflammatory cytokine storm

- Fever (up to 104°F or 40°C)
- Rigor
- Headache
- Myalgies
  - During the paroxysm, extremely high levels of TNFα and IL1β have been measured
- The paroxysm is often followed by a period of extreme fatigue
What is the origin of the cytokine storm in malaria?
Our initial hypothesis

• The fever in malaria is caused by the activation of a TLR.
The Malarial Parasite is Coated with a Glycosylphosphatidyl Inositol Anchor (GPI)
The concentration of GPI on the surface of merozoites is too low to account for the ability of parasite extracts to stimulate cytokine production.
GPI is not likely to be an important cause of cytokine stimulation in malaria.
Hemozoin

- *P. falciparum* metabolizes hemoglobin into hemin, which is subsequently detoxified by forming the inert crystal, hemozoin.
Shizuo Akira
Akira et al.

Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin.

Scooped by Akira...again!
Natural Hemozoin Activates Cytokine Production via TLR9 and its adapter protein, MyD88

Mouse dendritic cells
The stimulatory activity of hemozoin was destroyed by Dnase.
Thus, the cytokine inducing component of hemozoin is (plasmodial) DNA
Current hypothesis:

Hemozoin functions to traffic DNA into an intracellular compartment to which TLR9 can be recruited
Parisa Kalantari, Ph.D.
Hemozoin/DNA complexes are rapidly internalized into a lysosomal compartment in macrophages.

100 ug of sHz and 30ug of CpG-Alexa 642-

CpG DNA is internalized into **lysosomes** when complexed to Hemozoin.
Free CpG DNA (unlike hemozoin-bound DNA) has delayed internalization into a lysosomal compartment.

Hz and CpG added to macrophages - No prebinding

Lysotracker: Ex:380

30 minutes
The concept that plasmodial DNA is introduced into cells by hemozoin to activate TLR9 via CpG DNA motifs was compelling, except for the fact that plasmodial DNA is highly AT-rich!
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- The *P. falciparum* genome contains a stem loop motif: ATTTTTTAC over 6000 times!
The AT-r stem-loop motif is found in a variety of other organisms

<table>
<thead>
<tr>
<th>Genome (size)</th>
<th>AT content (%)</th>
<th>Number of putative AT-ODN motifs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmodium falciparum (22.8Mb)</td>
<td>82%</td>
<td>6130</td>
</tr>
<tr>
<td>Variola (371.1kb)</td>
<td>68%</td>
<td>24</td>
</tr>
<tr>
<td>Vaccinia (389.4kb)</td>
<td>67%</td>
<td>25</td>
</tr>
<tr>
<td>Listeria monocytogenes (5.8Mb)</td>
<td>61%</td>
<td>480</td>
</tr>
<tr>
<td>Homo sapiens (3.08 Gb)</td>
<td>56%</td>
<td>268828</td>
</tr>
<tr>
<td>Human adenovirus 5 (35.9kb)</td>
<td>45%</td>
<td>5</td>
</tr>
<tr>
<td>Leishmania major (32.8Mb)</td>
<td>38%</td>
<td>184</td>
</tr>
<tr>
<td>Human herpesvirus 2 (154.7kb)</td>
<td>30%</td>
<td>2</td>
</tr>
</tbody>
</table>
Shruti Sharma
One can study the AT-r stem-loop motif using synthetic ODN

- AT-2: GCACAC\textbf{ATTTTTTAC}CTAAAAC
The stem-loop structure is *essential* for innate immune activity.
Porto Velho is the capital city of Rondonia State; it is the most rapidly growing major city in Brazil and currently has a population of about 400,000 persons.
The ideal caiparinha comes from caipira ("hillbilly") - an example of T3 research

Ingredients

* 1/2 lime, quartered
* 1 teaspoon white sugar
* 2 1/2 fluid ounces cachaca
* 1 cup ice cubes

Directions

1. In a large rocks glass squeeze and drop in 2 eighths of lime. Add sugar, crush and mix with a spoon. Pour in the cachaca and plenty of ice. Stir well.
Studies of febrile malaria patients (*P. falciparum*) demonstrated that IFN-stimulated genes (ISG) are up-regulated during malaria.
IFN alpha/beta receptor (IFNAR) knockouts are highly resistant to mouse cerebral malaria.
Hence, type I IFNs appear to be an essential part of the inflammation seen in malaria.
There two major TLR signaling pathways

**MyD88-dependent**
- TLR1/2
- TLR2/6
- TLR3
- TLR7, 9
- TLR5
- MyD88
- IRAKs

**MyD88-independent / TRIF dependent**
- TLR4
- TLR3
- Mal
- MyD88
- IRAKs
- TRAM
- TRIF
*P. falciparum* infected rbc, hemozoin/DNA and purified malaria DNA activate a type I interferon response
TRIF/MyD88 DKO have no TLR function

MyD88-dependent

TLR1/2
TLR2/6

MyD88
Mal
IRAKs

MyD88
TLR5

MyD88
TLR7, 9

MyD88
Mal
IRAKs
TRAM

TRIF

MyD88-independent

TLR4

MyD88
Mal
TRAM

TRIF

TLR3

IRAKs

IRAKs

IRAKs

IRAKs

X
Neither AT-r ODN nor plasmodial genomic DNA induce IFN production via TLRs!
AT-r activation of type I interferons is not due to activation of known nucleotide sensors

- DAI
- RNA helicases
- RNA Polymerase III
STING/TBK1 and IRF3/7 are critical components of AT-r DNA sensing pathway.
Like the IFNAR KOs, IRF3/7 double knockouts are similarly resistant to cerebral malaria.
Both STING KOs and TBK1 hypomorphic mice appear to be resistant to cerebral malaria.
AT-rich DNA must be in the cytosol of cells to activate IFN or pro-inflammatory cytokines.
How does the DNA on the surface of hemozoin move from the phagosome to the cytosol?
Hemozoin traffics into the phagolysosome and then into the cytosol
The phagocytosis of inert particles results in phagolysosomal leakage.

- Silicic acid
- Urate
- Asbestos
The phagocytosis of inert particles results in phagolysosomal leakage.

- Silicic acid
- Urate
- Asbestos
- Hemozoin
Hemozoin crystals lead to lysosomal rupture and leakage of lysosomal content into the cystosol.
Genomic DNA dissociates from the surface of hemozoin and is released into the cytosol.
Recap

◆ DNA recognition appears to be a major cause of inflammation in malaria.

◆ There appear to be several ways that the human host responds to plasmodial DNA:
  ◆ As DNA traffics through the lysosomal compartment, it engages TLR9 via CpG motifs.
  ◆ When DNA gains access to the cytosol, an AT-rich motif activates an as yet unknown receptor via a TBK-1, IRF3/7 and STING dependent mechanism to produce type I interferons.
  ◆ (Direct activation of inflammasomes)

◆ Hemozoin promotes innate immune activation.
  ◆ By carrying DNA into the phagolysosomal compartment, where it engages TLR9, and then by allowing the DNA to escape to the cytosol.
  ◆ (By activating the NLRP3 inflammasome activation)
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University of Massachusetts Medical School

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- IRF ko mice

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- STING ko mice

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