Of mice and men: practical approaches to malaria vaccine discovery and development

--> The consequences of the screen employed to identify a parasite molecule as a « major vaccine candidate «

Or the problem of the « original sin « in malaria vaccine development (which is carried over for long, for ages, with an unlimited number of formulations)
Identification of malaria vaccine candidates

- Little efforts at improving/validating surrogates of protection
- the existing surrogates markers are not sufficient to demonstrate vaccine efficacy

-> Clinical efficacy trials remain today the only means to demonstrate the value of a candidate

However, the path to a vaccine proof of concept combines 3 difficulties:

- The vaccine potential of each candidate is unknown

- the path is long: 6-12 years (vaccine design, pre-clinical evaluation, cGMP production, Phase I, Ib, phase II (and occasionally Phase III trials))
- It requires a delivery platform able to induce the «right» immune response, whereas the characteristics of the latter are ill-defined…

Today 105 clinical trials explore a total of 12 candidates -mainly 3-
The parasite being made of ca 5300 proteins: --> 12 = 0.2% of the total!

Whereas the combination
- of all proteins,
- with diverse delivery platforms,
- diverse Ag combinations

- Would lead to ca 5300x 20 x infinite nb combinations: --> unmanageable nb of trials to handle
Malaria Vaccine Development:
Identification of mechanisms mediating protection
and/or Ags inducing protection

« Complex... »

C.a. 5300 proteins
Each several epitopes
High polymorphism
Human polymorphism

All types of immune effectors
(B,T,CTL…)
Evolving over (long) time
(> 12-20 years)

---› ca 500 fold more difficult than for the most polymorphic viruses, eg flu, HIV
Models

Acute infection
High death rate
Resistance fast acquired

Reality

Chronic infection
1-3% death rate
Immunity slow to acquire

EX-VIVO Bio-Assays
Why vaccines work better in models: 

*conversely often fail in humans...*

Or

« *The consequences of fine molecular tuning of parasites with their host* »
1. Plasmodia are strictly fitted to mostly ONE given host:
   - If introduced in an abnormal host, they die
   - in their normal host they are « adapted »:
     • do not kill their host (or very rarely)
     • are not all killed by their host

   --> Chronic, long lasting, low grade infection is the rule

2. This equilibrium has obviously a molecular basis:
   adaptation = co-evolution over billions of years + random mutations

   → leads to the selection of parasite molecules:
   - That do not induce too much pathology
   - that do not trigger too much defences
3. almost all lab hosts are abnormal host-parasite combinations
   P.y, P.b, P.c, P.v in mice, P.k, P.c, P.f in primates....

4. In an abnormal host: this molecular fitness is lost
   - Infection usually kill all hosts
   - Host kill all parasites
   Reflecting the molecular mismatch
     (eg: loss of self-mimicking molecules)

For vaccine dvlpt, this has important consequences:
→ implies that a larger number of molecules can induce protection
   - either more immunogenic than in normal host
   - or directed to different epitopes
   - or inducing more effective immune responses

- should not be so surprising that the same molecule may fail when vaccinating humans
Minimal number of sporozoites needed to:

**Infect**  
(susceptibility)  

- Balb/C: > 10,000  
- C57BL6: 50  
- Thamnomys: 5

**Immunize**  
(by irr-spz)  

- Balb/C: (1X) 1,000  
- C57BL6: 3X 30,000  
- Thamnomys: uneffective (> 3X 100,000)

*The more abnormal is the host, the easiest it is to protect.... and vice-versa....*
Immunogenicity also depends on the host

New candidates
and new formulations are always first assessed in rodents

which immune response is poorly predictive of that obtained in humans..

May differ qualitatively, or quantitatively
eg in mice: - rubella (human+, mouse -)
    - ASO2 (CTL-Vs-Abs)
    - Alum (MSP3)

eg. In Aotus: montanide, ASO2, alum / FCA

eg. CS and MSP1 essentially selected by Balb/C immune system

→ there is also a need for new models in which HUMAN lympho responses could be assessed
→ New transgenic mice could be developped (matter of will..)
Some **Clinical situations**
which can be employed for Ag selection

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**- epidemiological studies**
- formerly: Children: "non-protected"
  - Vs Adults: "protected"
- more recently "P" Vs "NP" within all age groups

**- IgG transfer** (induced protection)
- close in vivo/ in vitro correlations
  - eg: MSP3

**- Irr-Spz immunized Volunteers**
- (protected Vs non protected)
  - eg LSA3

**- Acquired immunity to pre-Erythrocytic stage s**
- (under field exposure)
  - eg LSA 3

**- Cerebral Malaria patients** (recovering Vs not -> IgG3)
  - MSP3
  - SR 11.1

- more to be found
A clinical approach to malaria vaccine development
Identification of the ADCI Mechanism (MN-Mediated Ab-Dependent)

Identification of MSP3 by the ADCI Mechanism

-> neither the mechanism, nor the antigen had been fished out by approaches in models
mapping: different Ig subclass distribution to each epitope
Anti-parasite ADCI activity of Abs directed to each MSP3 epitopic peptide
Passive transfer in P. falciparum infected Scid mice of Anti-MSP3-b and MSP 3-d Abs
No inhibition by human anti-MSP1 Abs at 1 mg/ml (that target the same epitope as An inhibitory Mab)
Anti-AMA1 Abs in volunteers

5-10 mg / ml (30-60% of all IgG) → 15% reduction of invasion
NB: 5 sporozoites, 1/2 entering an hepatocyte, 1/2 liver mero entering a RBC (actual data from chimp challenges)

<table>
<thead>
<tr>
<th>Days</th>
<th>Parasitaemia / µL</th>
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<tbody>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
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<tr>
<td>7</td>
<td>0,8</td>
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<td>13</td>
<td>3276</td>
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<tr>
<td>15</td>
<td>1648</td>
</tr>
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Parasitaemia / µL vs Days

- **No Inhibition**
- **15% Inv-Inhibition**

Pyrogenic Treshold (5 SIRBC/µL)
Parasitémie (%)

- PI AG
- MN + Nl IgG
- Nl IgG
- MN + PI AG

0 24 48 72 96

heures

Ab
Nl IgG
MN
MN+Ab
Abstract by an hormonal effect: trigger the release of parasitostatic substances by MN
Dose-dependant effect in ADCI of Human Antibodies

--- > anti-MSP3 Ab are effective at very low concentrations (70 picoMoles) 
(ca 1000 fold less than for GIA activity) = similar to that of hormones
Identification of LSA3, by immune responses from irr-spz immunized volunteers

<table>
<thead>
<tr>
<th>Groupe I (23,000 Rads)</th>
<th>ELISA ratio</th>
<th>729S</th>
<th>Protection</th>
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<tr>
<td>0.6</td>
<td>0.5</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>0.6</td>
<td>0.2</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>0.9</td>
<td>0.5</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>0.8</td>
<td>0.5</td>
<td>0.2</td>
<td>-</td>
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<table>
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<th>ELISA ratio</th>
<th>729S</th>
<th>Protection</th>
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<td>3.8</td>
<td>1.2</td>
<td>+</td>
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<tr>
<td>1.6</td>
<td>2.8</td>
<td>0.8</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>4.8</td>
<td>1.4</td>
<td>+</td>
</tr>
<tr>
<td>0.9</td>
<td>3.4</td>
<td>1</td>
<td>+</td>
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</table>
High B and T-cell Antigenicity Of various regions of LSA3 In humans

Figure 62: A. Schematic representation of *P. falciparum* LSA3 antigen, clone K1, showing the non-repeat (NR-A, NR-B, and NR-C) and repeat (R1, R2 and R3) fragments and the relative position of the 17 Long Synthetic Peptides (LSP). Prevalence of antibody (B) and proliferative responses (C) to the 17 LSA3-LSP in 20 individuals from Dielmo. As indicated in the legends, the responses were classified in 3 ranges according with the ratio of antibodies or the stimulation Index (S.I) of proliferative response.
Protection induced in chimpanzee

98 days post -immunisation

Heterologous strain Challenge

238 days post -immunisation

Heterologous strain Challenge
• Production, Quality Assurance, Quality Controls, Pharmacotoxicity
  (Sedac Therapeutics)

• Single-site, open, randomized, dose escalating Phase I study
  (Univ Hospital, Lausanne)

• 36 volunteers in 6 groups

• 2 adjuvants:

  Montanide
  10-10-10 --> 10-10-10
  30-30-30 --> 30-30-10
  100-100-100--> 100-10-10
  300-300-300--> 20-20-20

  Alum
  30-30-30 --> 30-30-30
  100-100-100--> 100-10-10

  ----> Safety, Tolerance, Immunogenicity, Bio-activity
High Immunogenicity of MSP3 Adjuvated by Alum In human volunteers
ADCI activity of vaccine induced Abs: as high or higher as that of individuals with full acquired protection.
Passive transfer of the volunteers sera (diluted 1/40) clears a *P. falciparum* parasitaemia
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Figure 4
The MSP3-LSP is a malaria vaccine that is safe, well tolerated when adjuvated by alum, and elicits in humans Abs able to kill P. falciparum.

Even low doses of MSP3, injected with simple adjuvants, readily induced Abs of cytophilic classes, long-lasting, directed to fully conserved epitopes, with strong biological effect against P. falciparum.
Clinical Prediction resulting from a single Ab determination

(on year 0 in children from Dielmo)

Cytophilic Abs have been consistently found associated with protection
### Prevalence of Anti-MSP3 Antibodies

<table>
<thead>
<tr>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
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<tbody>
<tr>
<td>6.25</td>
<td>0</td>
<td>4.16</td>
<td>6.25</td>
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### Ratio of Anti-MSP3 Antibodies

<table>
<thead>
<tr>
<th>% Prevalence</th>
<th>MSP3a</th>
<th>MSP3b</th>
<th>MSP3c</th>
<th>MSP3d</th>
<th>MSP3e</th>
<th>MSP3f</th>
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</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>6.25</td>
<td>14.58</td>
<td>22.91</td>
<td>29.16</td>
<td>8.33</td>
<td>60.41</td>
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<tr>
<td>IgG3</td>
<td>4.16</td>
<td>29.16</td>
<td>47.91</td>
<td>35.41</td>
<td>8.33</td>
<td>6.25</td>
</tr>
<tr>
<td>IgG2</td>
<td>0</td>
<td>0</td>
<td>8.33</td>
<td>10.41</td>
<td>4.16</td>
<td>2.08</td>
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<tr>
<td>IgG4</td>
<td>6.25</td>
<td>0</td>
<td>10.41</td>
<td>12.5</td>
<td>0</td>
<td>8.33</td>
</tr>
</tbody>
</table>

**Dielmo sera with positive reactivities (ratio ≥ 1)**
Functional analysis of naturally occurring antibodies

All antibodies were used at equal effective concentration adjusted by IFA against parasite protein.

The diagram shows the percentage specific growth inhibition and parasitemia over days, with different antibodies tested against MSP3a through MSP3f and PIAG proteins. The bars indicate the specific growth inhibition, and the line graphs below depict the parasitemia over time, with additional notes for HuMn and anti-MSP3f or anti-MSP3.
Figure: 4
Parasitaemia vs Days

(B): controls

Vol 24
Vol 21
Vol 8

MN
MN+ Serum Month 5

Serum Day 0
MN+ Serum Day 0

Vol 24
Vol 21
Vol 8

Days

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
(C) : Month 12 WB+

Parasitaemia

Days

Vol 21
Vol 21
Vol 18

Serum month12
MN
MN+
Serum month12

0,01
0,1
1
10

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

Days