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

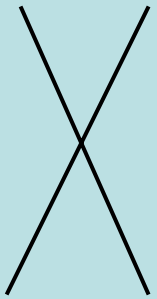
-Would lead to ca 5300x 20 x infinite nb combinations: --> **unmanageable nb of trials to handle**

Malaria Vaccine Development:

Identification of mechanisms mediating protection and/ or Ags inducing protection

« Complex… »

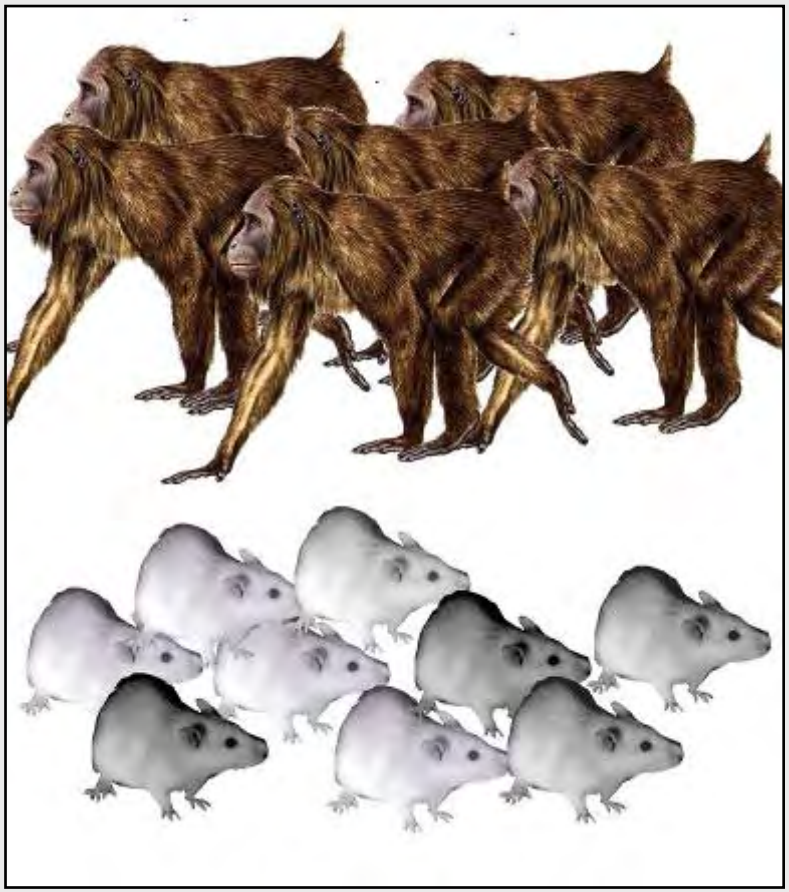
C.a. 5 300 proteins
Each several epitopes
High polymorphism
Human polymorphism



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



Reality

Acute infection	**Chronic infection**
High death rate	**1-3% death rate**
Resistance fast acquired	**Immunity slow to acquire**

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 :	Balb/C	C57Bl6	Thamnomys
Infect (susceptibility)	**> 10.000**	**50**	**5**
Immunize *(by irr-spz)*	**(1X) 1.000**	**3X 30.000**	**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

--> 2 « discriminating » groups
- **Both with high immune responses**
- **One clinically protected, the other not**

-**epidemiological studies** -eg 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

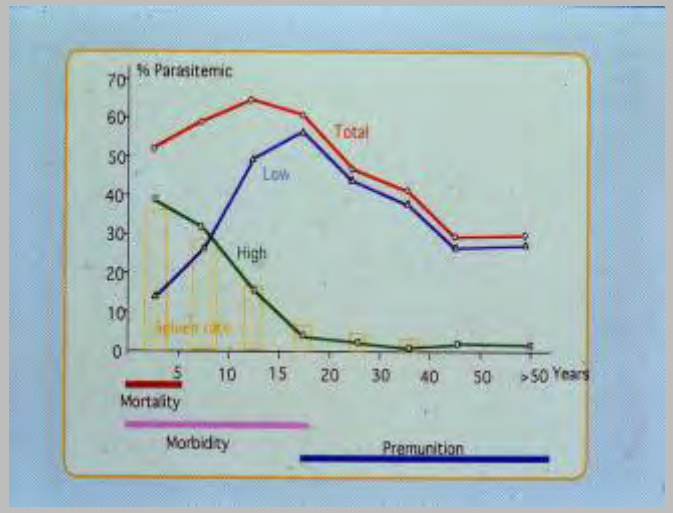
- Acquired immunity to pre-Erythrocytic stage s : eg LSA 3
(under field exposure)

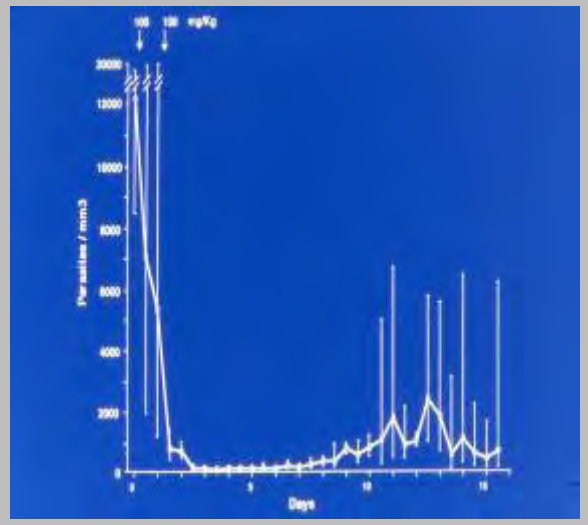
- Cerebral Malaria patients (recovering Vs not --> IgG3) MSP3
SR 11.1

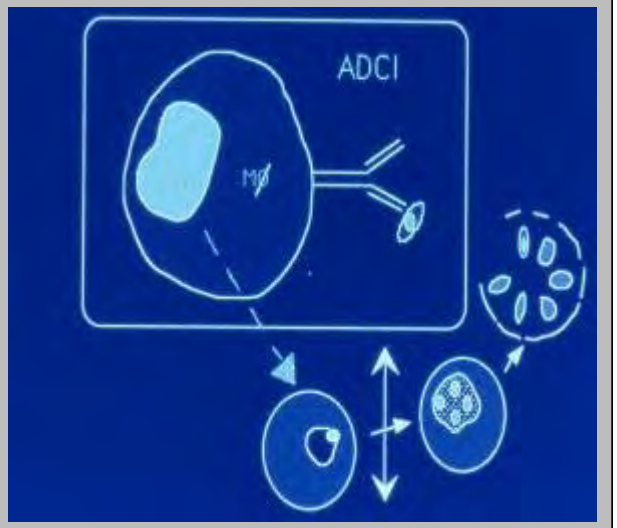
- more to be found

A **clinical** approach to malaria vaccine development

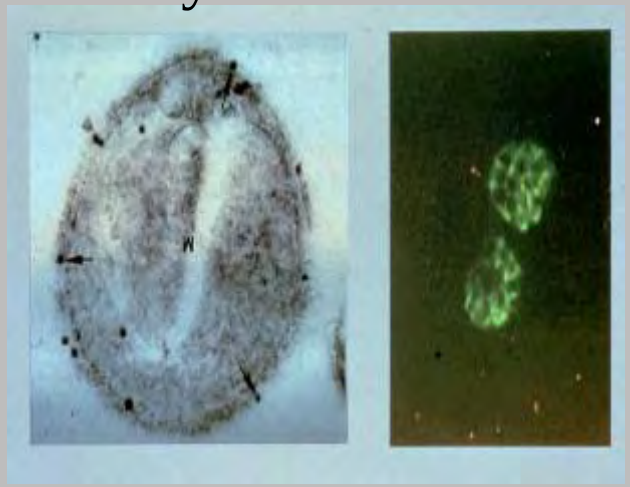
Identification of the ADCI Mechanism (MN-Mediated Ab-Dependant)

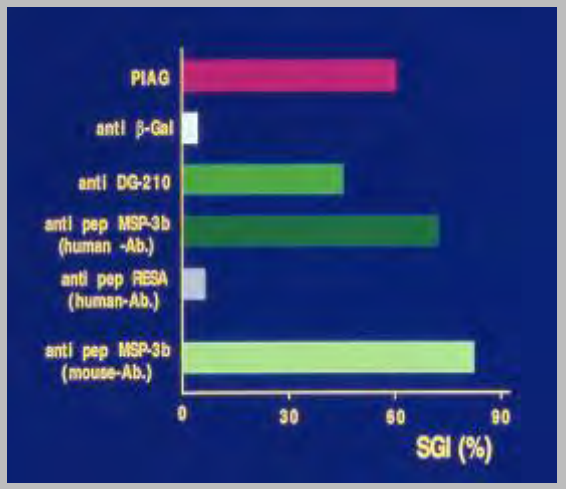




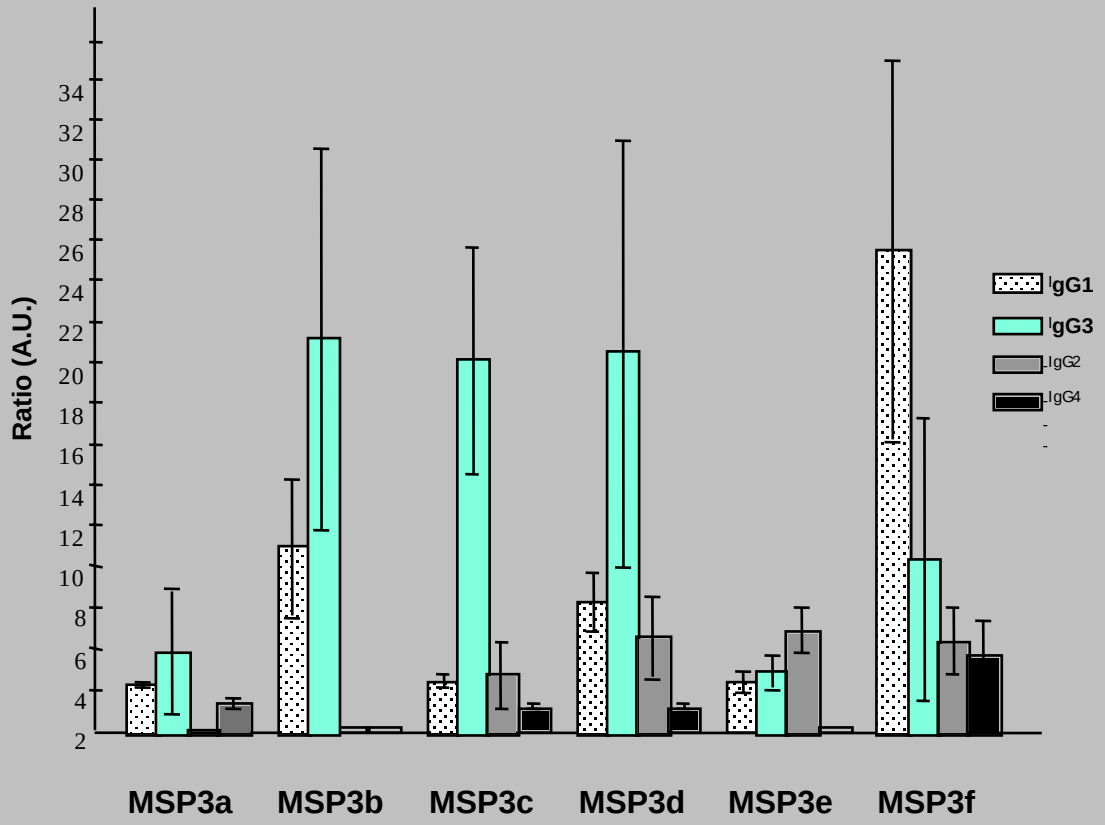


Identification of MSP3 by the ADCI Mechanism



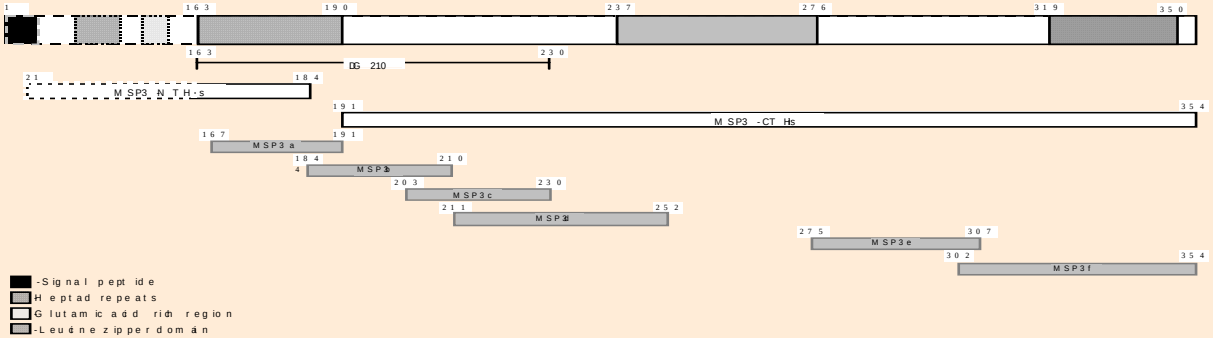


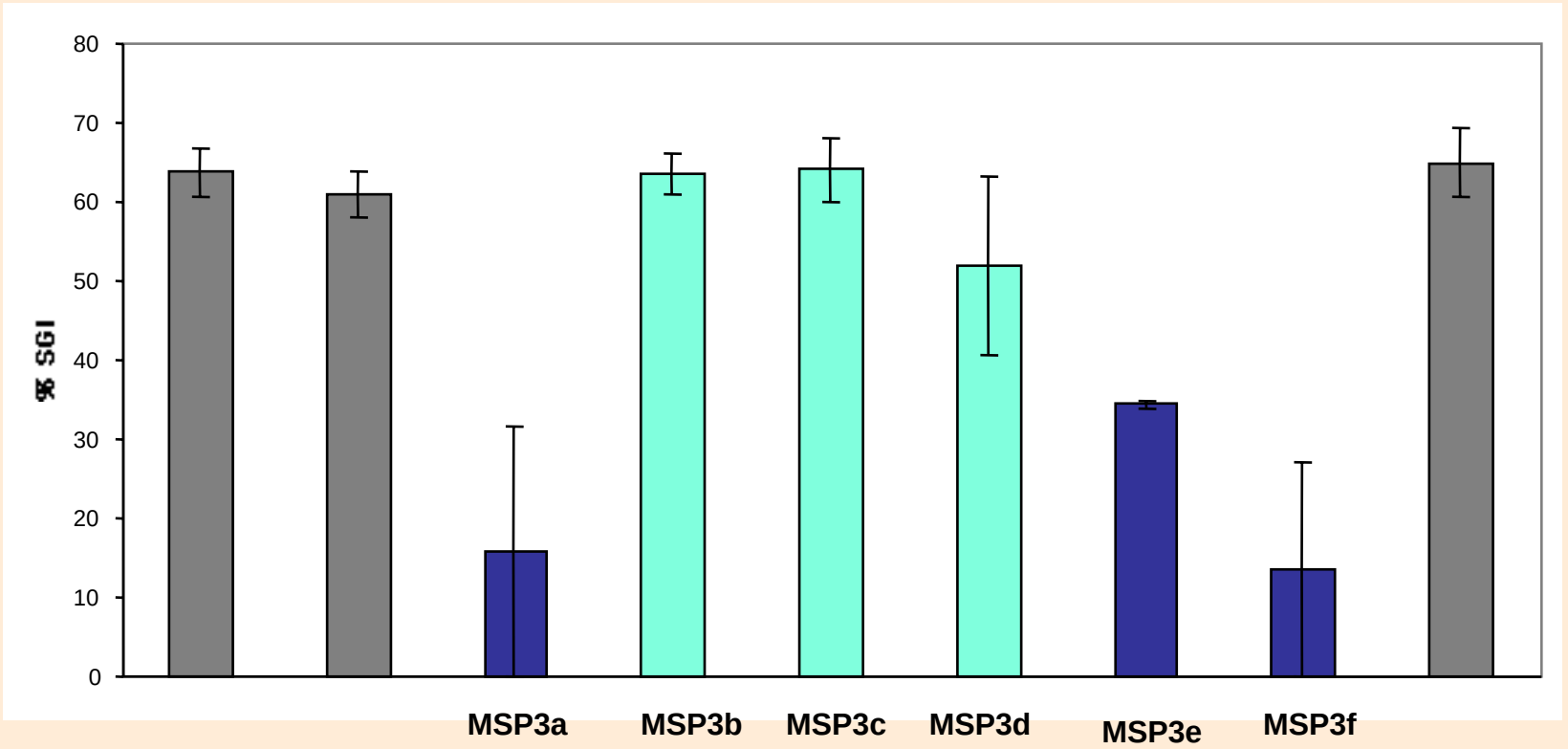
-> neither the mechanism , nor the antigen had been fished out by approaches in models



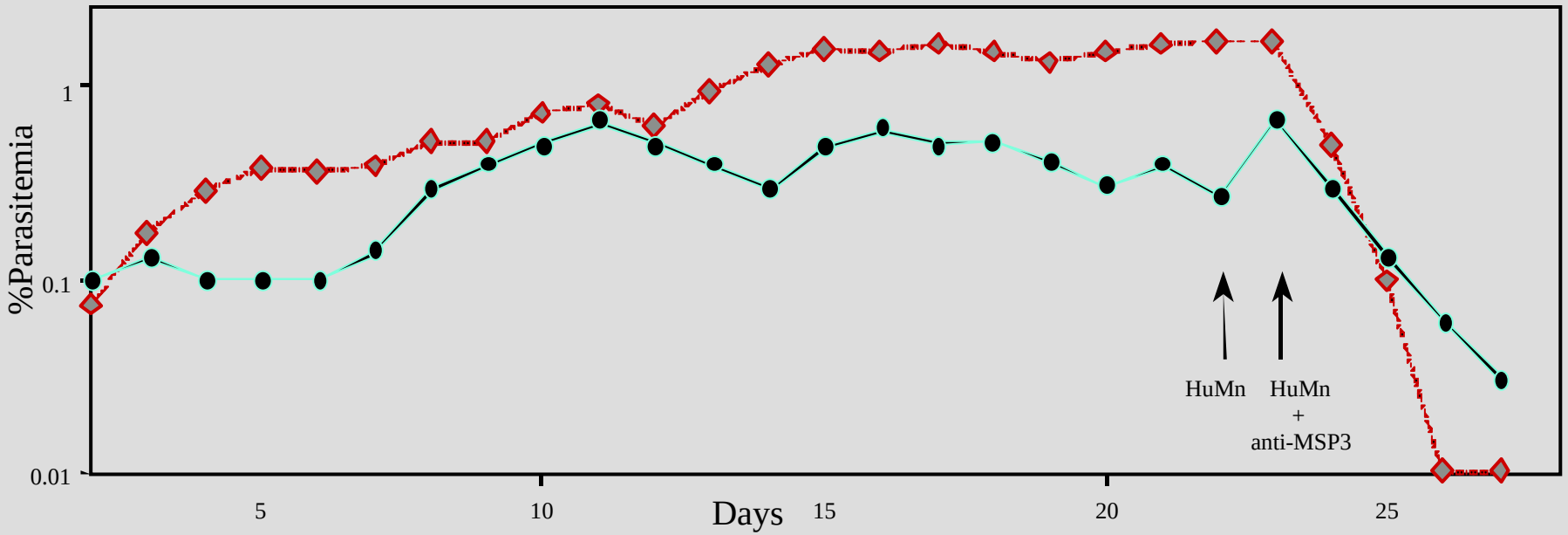
% Prevalence						
IgG1	6.25	14.58	22.91	29.16	8.33	60.41
IgG3	4.16	29.16	47.91	35.41	8.33	6.25
IgG2	0	0	8.33	10.41	4.16	2.08
IgG4	6.25	0	10.41	12.5	0	8.33

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

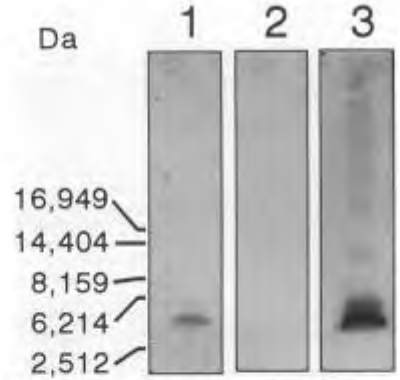


FIG. 3. The titers of IgG specific for MSP-1–EGF1 within two polyclonal antibody preparations were compared by Western blotting. Identical amounts of the 52-residue (6,022 Da) polypeptide representing MSP-1–EGF1, derived from an *E. coli*-expressed fusion protein by site-specific proteolysis, were subjected to Tricine-SDS-PAGE and then transferred to nitrocellulose. Blots were probed with either MAb 111.4 (lane 1), total-IgG (lane 2), or AP-IgG (lane 3). Color development reactions were conducted in parallel and stopped at the same time. Protein molecular mass markers indicated were derived from CNBr-cleaved horse heart myoglobin, at 16,949 Da, 14,404 Da, 8,159 Da, 6,214 Da, and 2,512 Da.

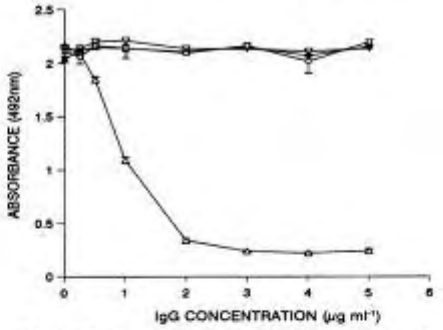


FIG. 4. Affinity-purified antibodies block the binding of an inhibitory MAb. ELISA plates coated in S42ΔA were preincubated with various concentrations of human IgG. Serial dilutions of AP-IgG (△), total-IgG (□), void-IgG (■), and control-IgG (▽) were used in triplicate. An optimal concentration of MAb 12.8 was then added, and the amount bound was determined. The ordinate represents A_{492} as a measure of bound MAb, and the abscissa represents the IgG concentration.

These experiments indicate that compared with the void-IgG sample, a significantly greater proportion (approximately 100-fold by ELISA) of the antibodies within the AP-IgG preparation were directed against determinants present in the first EGF-like module of MSP-1.

Affinity-purified antibodies compete with a protective MAb for antigen binding. The different IgG preparations were assayed for their abilities to block the binding of two inhibitory MAbs to S42ΔA (Fig. 4). The AP-IgG fraction was able to compete with MAb 12.8 at a concentration of 2 μg ml^{-1}, but none of the other IgG was effective in this range. None of the antibodies inhibited the binding of MAb 12.10 at 10 μg ml^{-1} (data not shown).

Parasite in vitro invasion assay. Highly synchronous microcultures of *P. falciparum* containing predominantly schizonts were incubated in the presence of various IgG preparations. After 24 h, encompassing schizont rupture-merozoite release and reinvasion of new erythrocytes, parasitemias were determined. The final parasitemia in cultures supplemented with African IgG preparations did not differ significantly from that of the control (Fig. 5), although EGTA was very effective at blocking invasion. The morphology of the parasites was normal on Giemsa-stained smears for all cultures, incubated with or without antibodies, and no agglutinated clusters of merozoites were observed in any sample.

DISCUSSION

We have investigated whether antibodies to the first EGF-like module of MSP-1 induced by natural infection are inhibitory to parasite growth in vitro. This biological property is manifested by some but not all MAbs specific for the C-terminal cysteine-rich region of MSP-1. Four murine MAbs which are specific for MSP-1 and which inhibit the growth of *P. falciparum* in vitro (2, 15, 42) have been described previously. Two of these antibodies, 12.8 (2) and 5B1 (42), bind to the first of two EGF-like modules in MSP-1 (12); another, 12.10, binds only if the two EGF-like modules are expressed together (9, 12). The present study was aimed at investigating whether or not naturally occurring antibodies with similar specificities

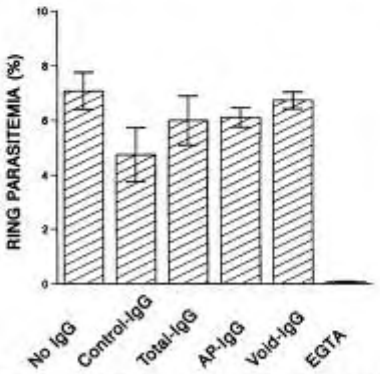
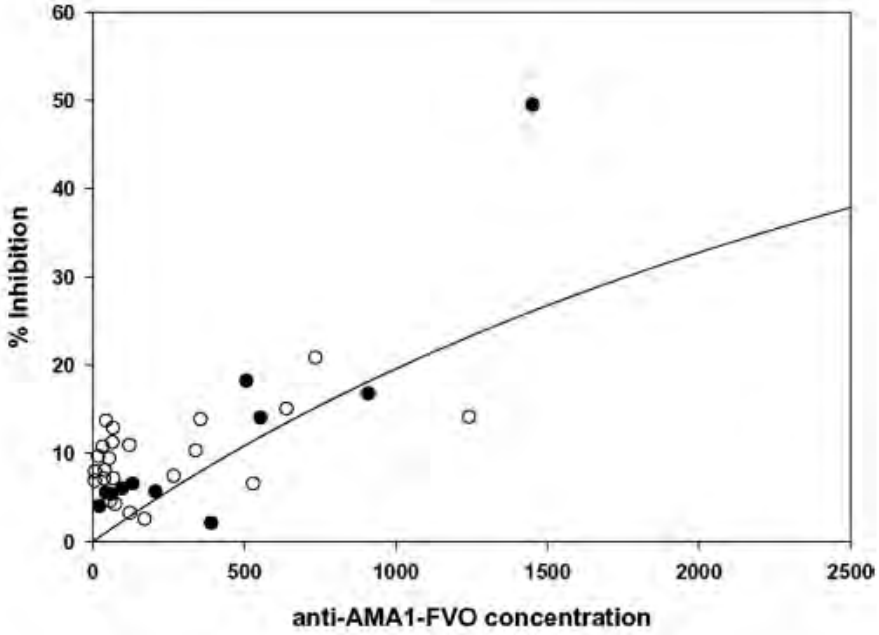
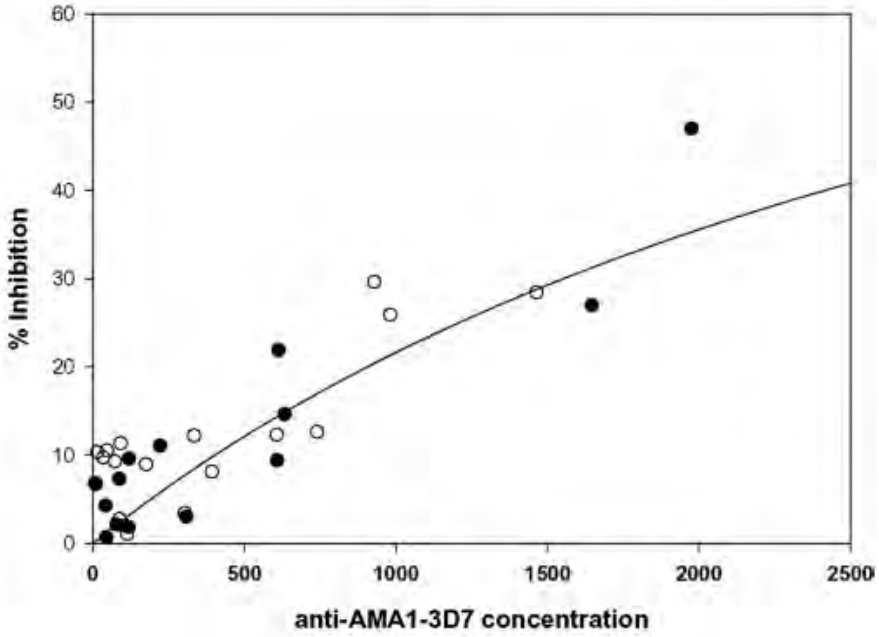


FIG. 5. Invasion inhibition assay. Various IgG preparations were added to individual microcultures of *P. falciparum* to test for their abilities to inhibit parasite invasion of erythrocytes. Growth was also monitored in cultures containing either no added IgG or 5 mM EGTA, previously shown to effectively inhibit merozoite entry into erythrocytes. Bars on the chart represent mean final percentage parasitemias; error bars indicate standard distribution within each sample.

No inhibition by human anti-MSP1 Abs at 1 mg/ml (that target the same epitope as An inhibitory Mab)





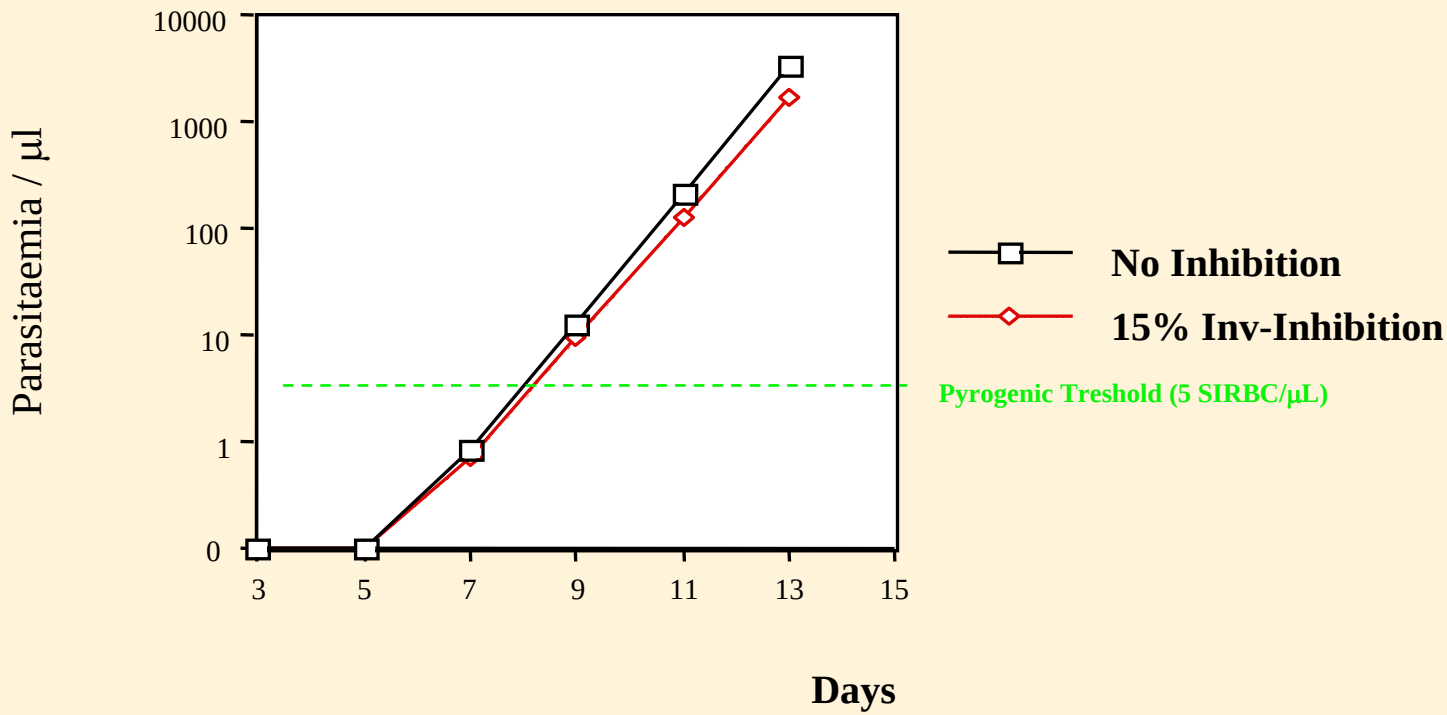
Anti-AMA1 Abs in volunteers

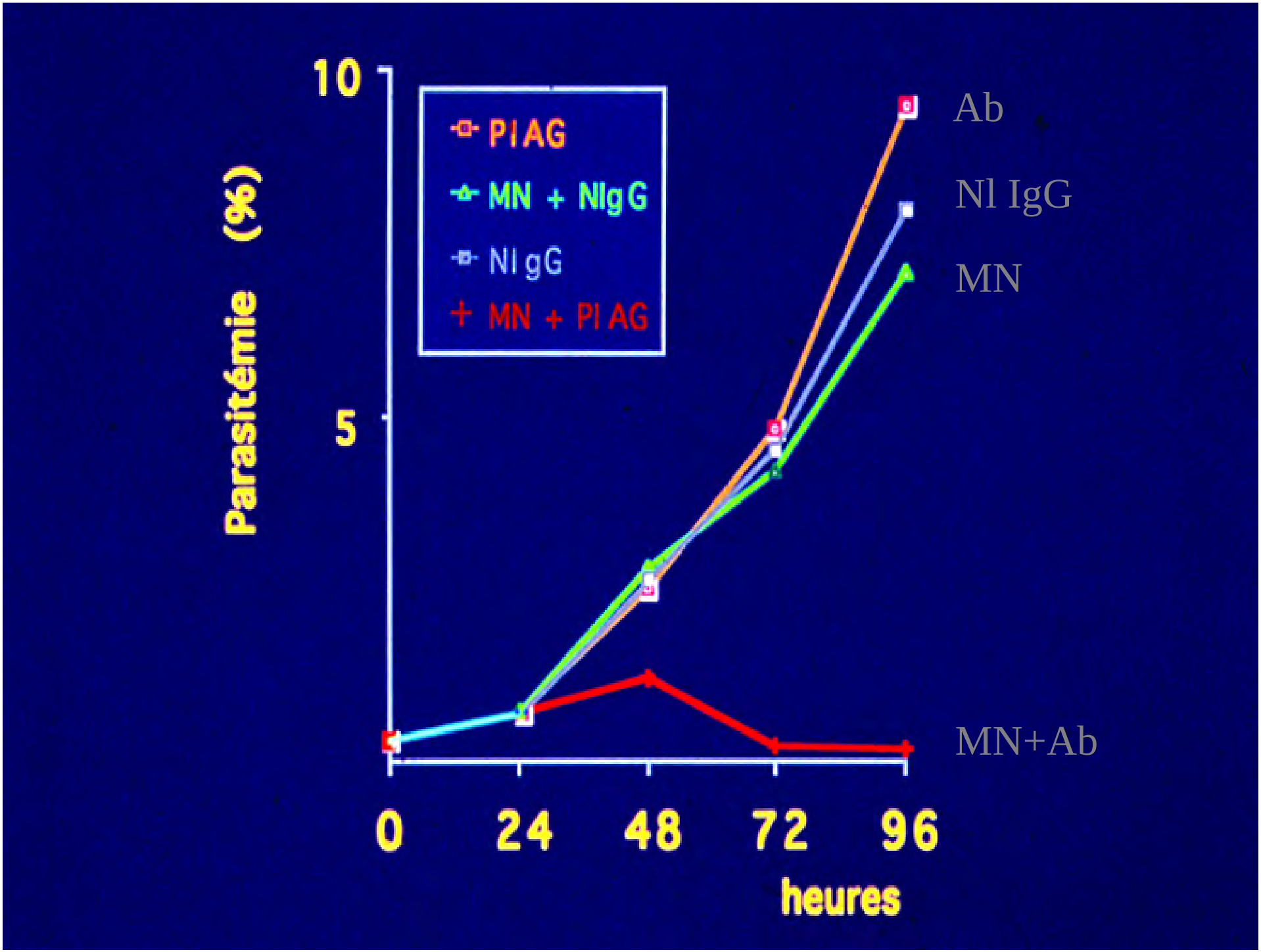
5-10 mg / ml (30-60% of all IgG)

→ 15% reduction of invasion

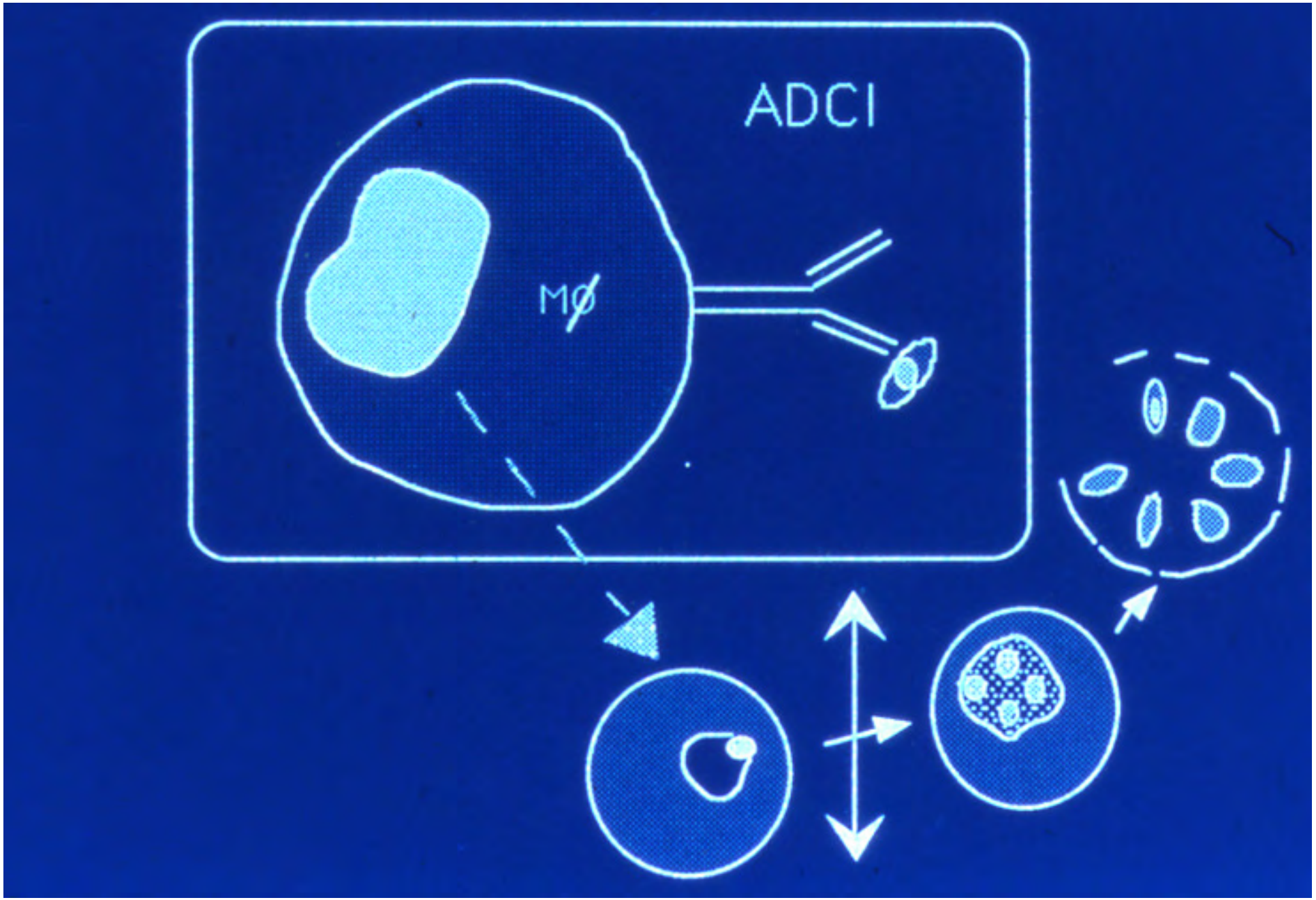
NB: 5 sporozoites, 1/2 entering an hepatocyte, 1/2 liver mero entering a RBC (actual data from chimp challenges)

3	0	0
5	0	0
7	0,8	0,67
9	12	9
11	204	122
13	3276	1648
15		

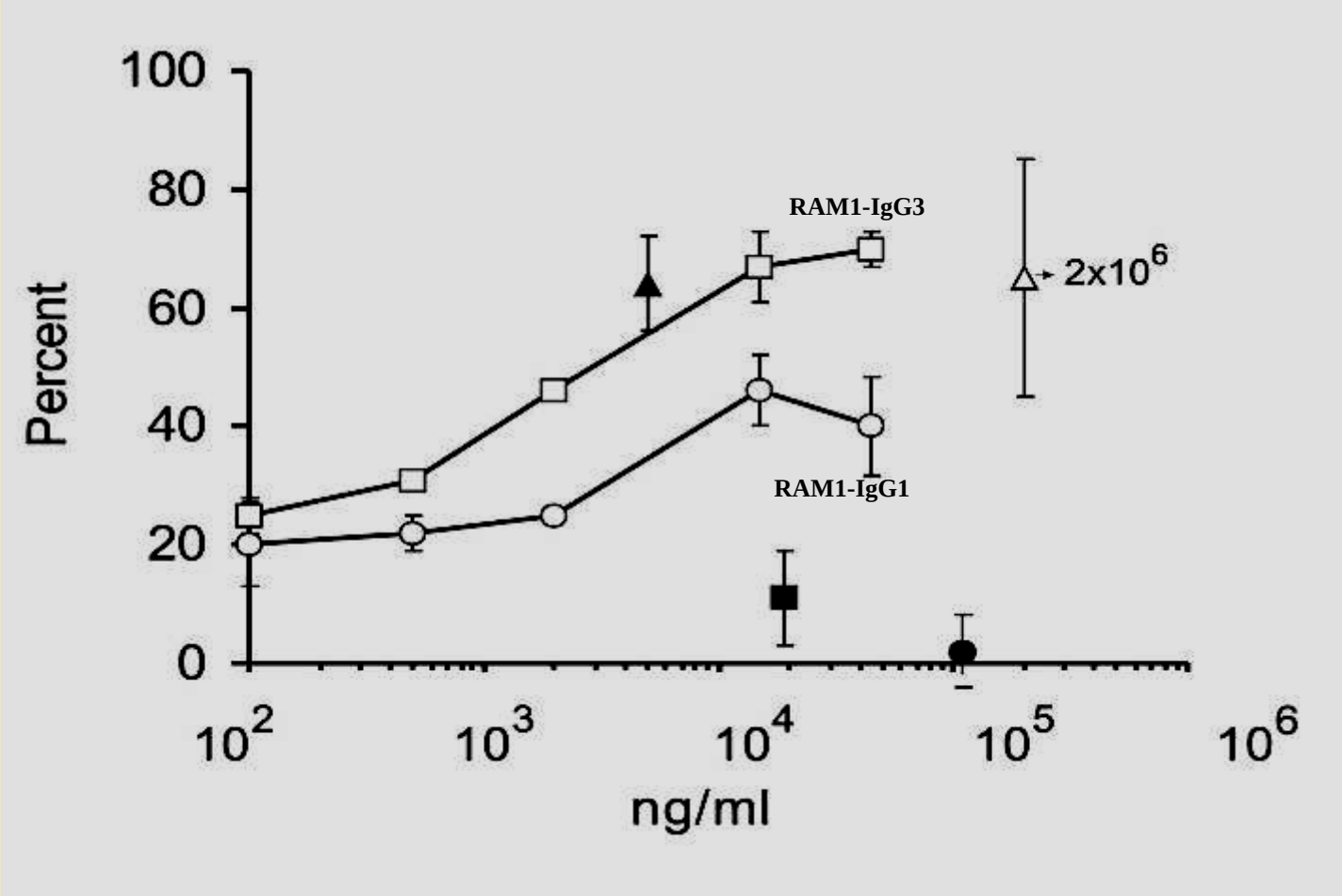



10
5
Parasitémie (%)
PI AG
MN + NIg G
NI gG
MN + PI AG
Ab
Nl IgG
MN
MN+Ab
0
24
48
72
96
heures

Abs act by an hormonal effect : trigger the release of parasitostatic substances by MN



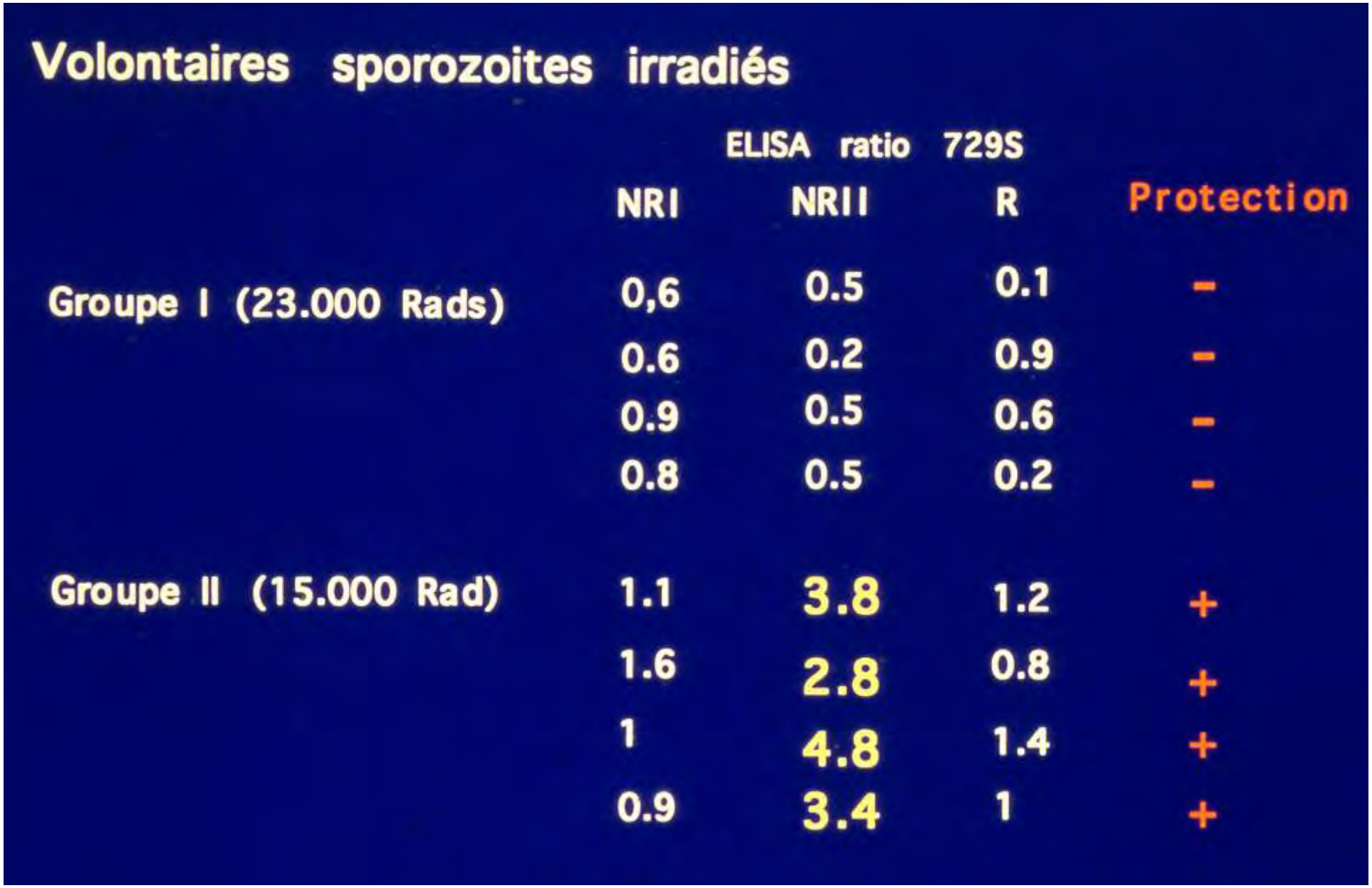
Dose-dependant effect in ADCI of Human Antibodies



---> 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



High B and T-cell Antigenicity Of various regions of LSA3 In humans

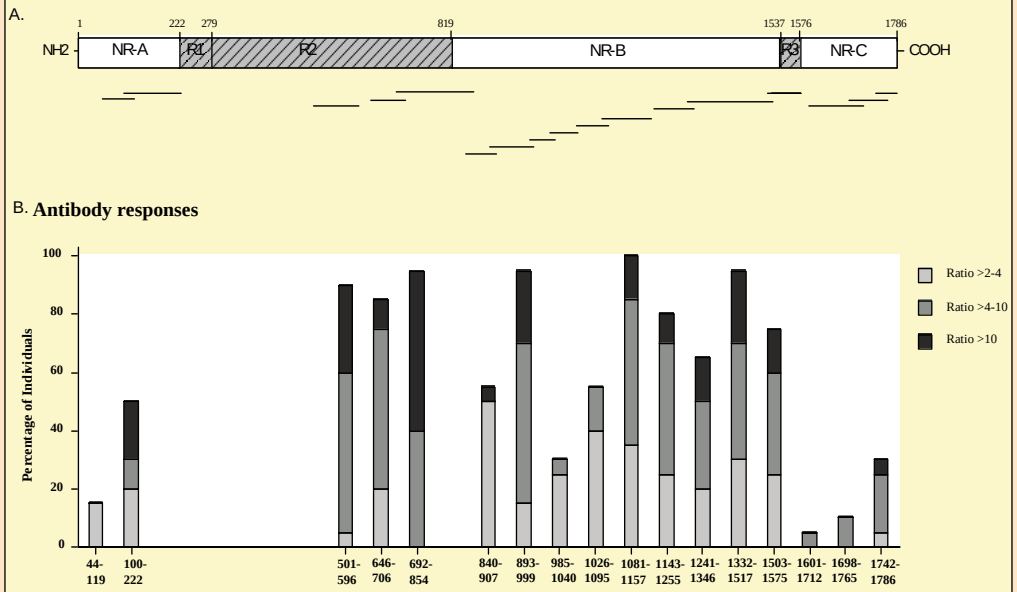
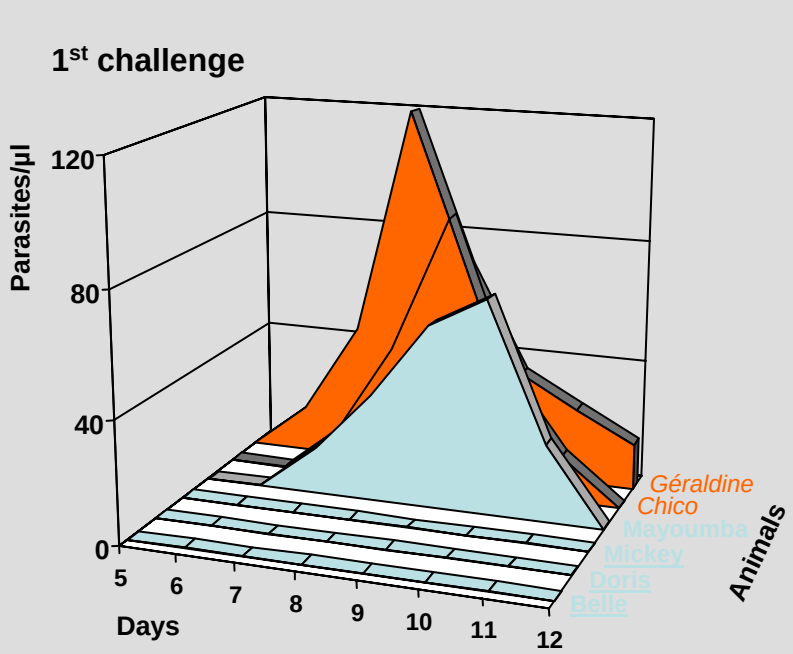
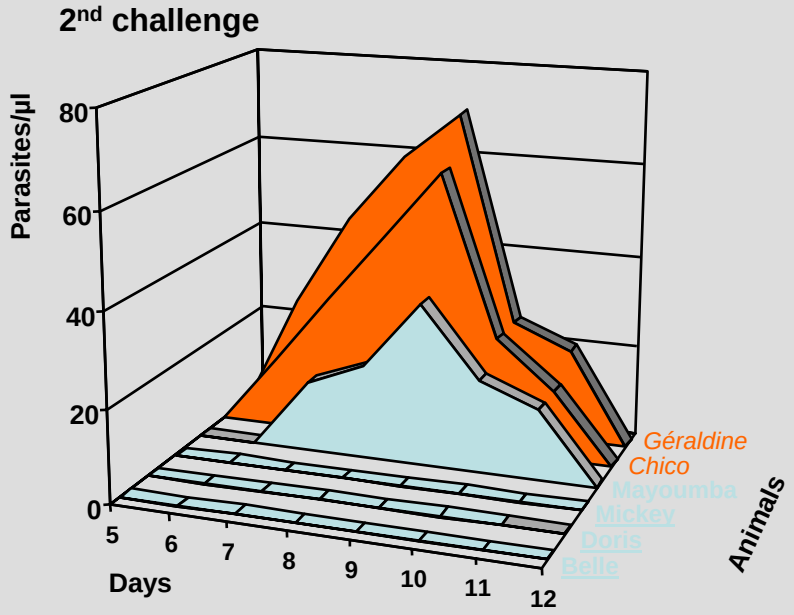


Figure 62: A. Schematic representation of *P. falciparum* LSA3 antigen, clone K1, showing the non-repeat (NR-A, NR-B, and NR-C) and repeat (R1, R2 and R3) fragments and the relative position of the 17 Long Synthetic Peptides (LSP). Prevalence of antibody (B) and proliferati responses (C) to the 17 LSA3-LSP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

Protection induced in chimpanzee





98 days post -immunisation

238 days post -immunisation

Heterologous strain Challenge

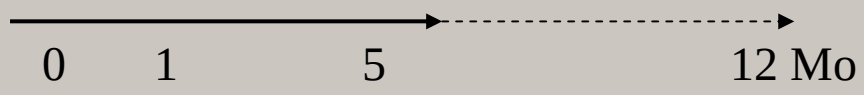
- Production, Quality Assurance, Quality Controls, Pharmacotoxicity

(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

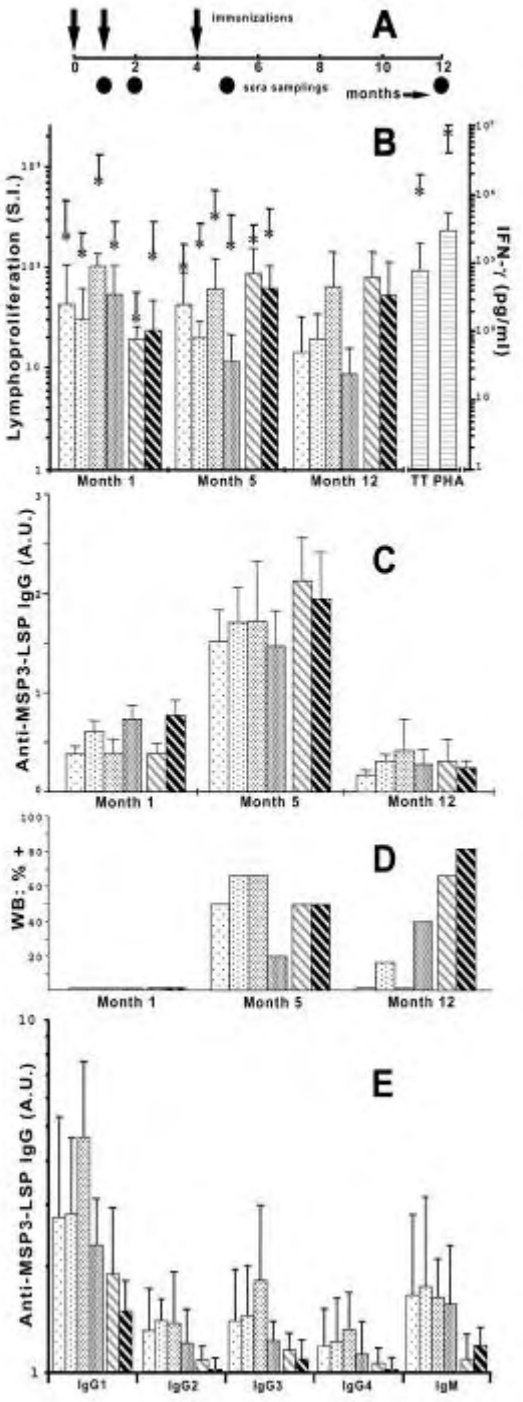


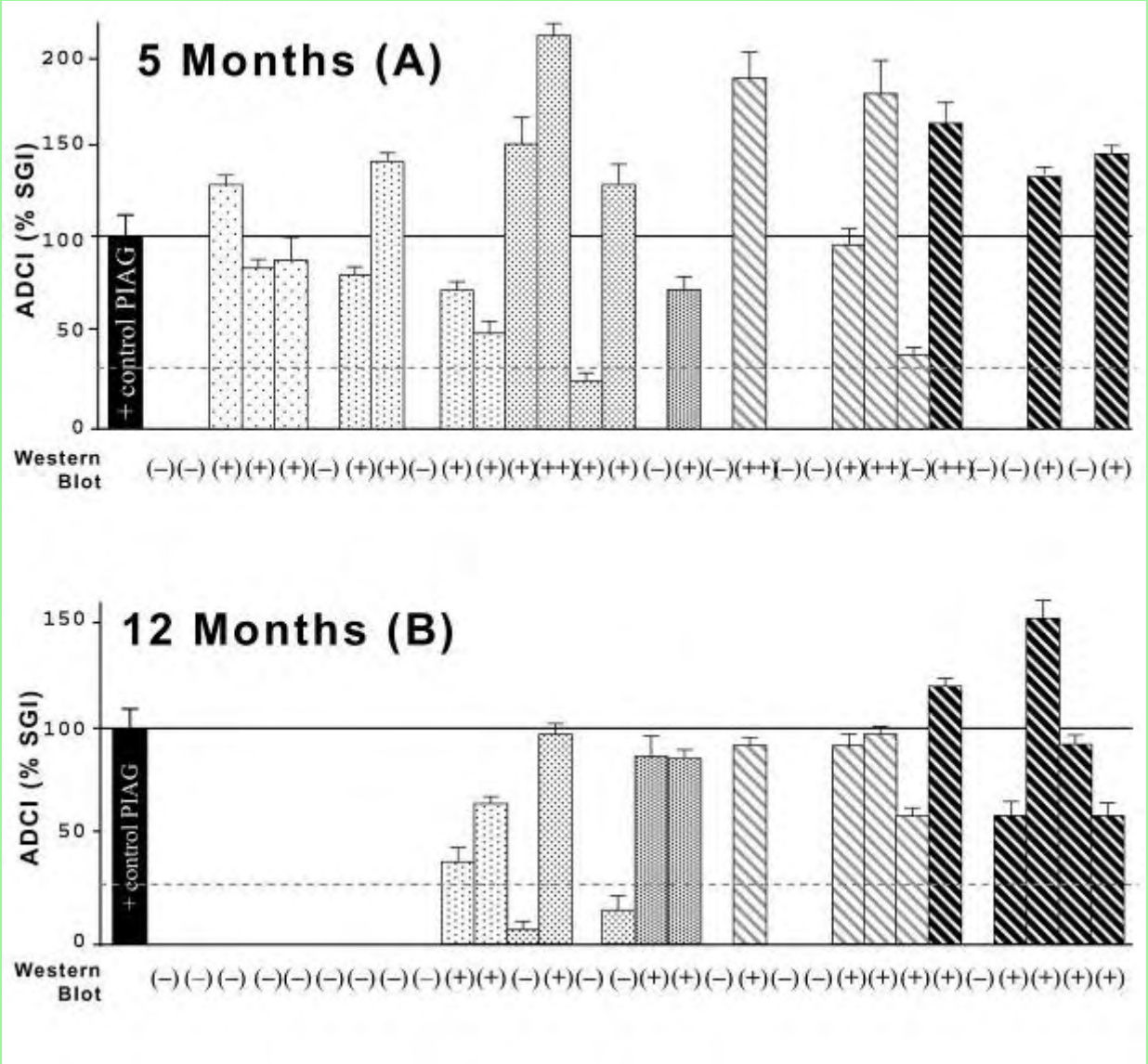
Figure: 1

ADCI activity of

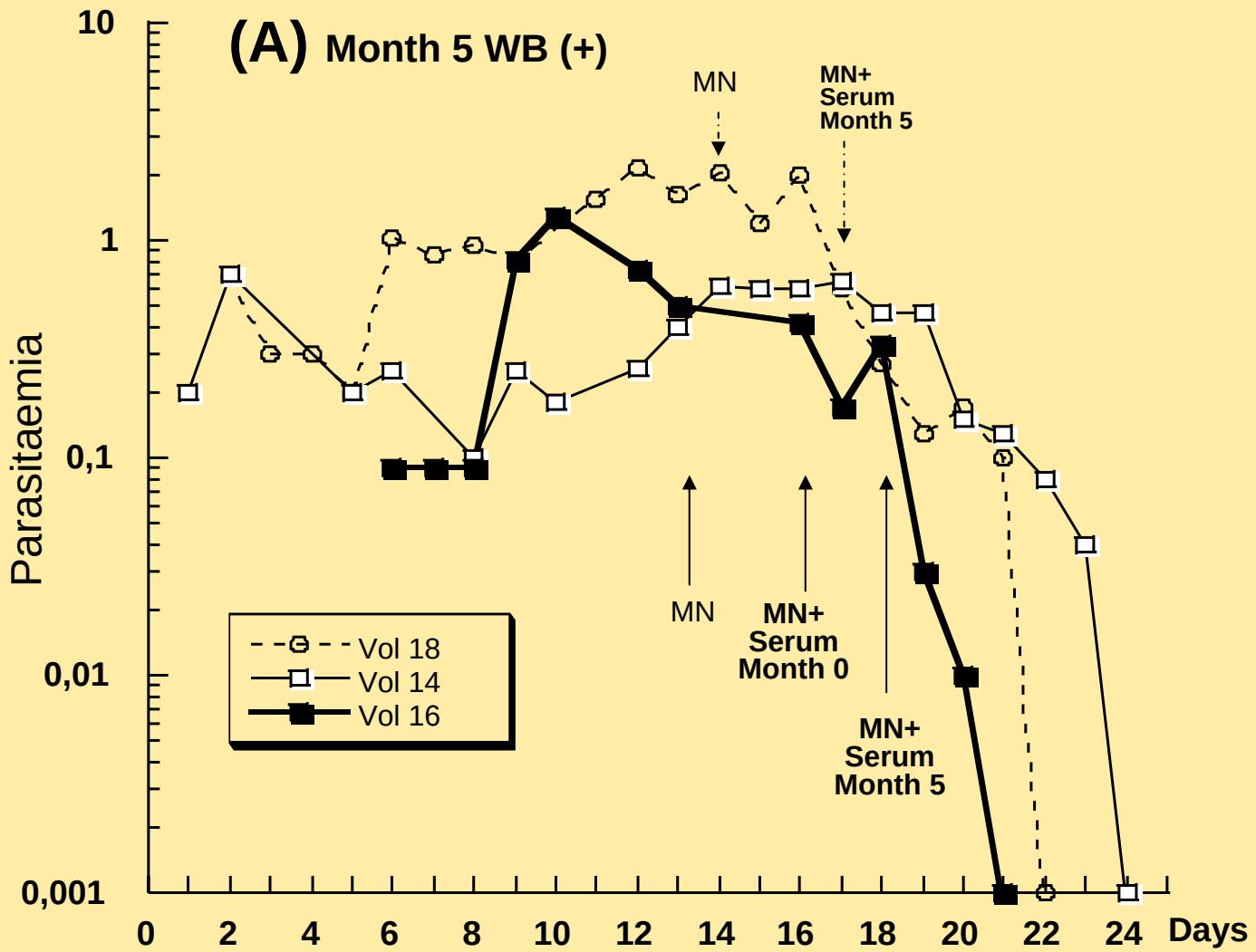
vaccine induced Abs

: 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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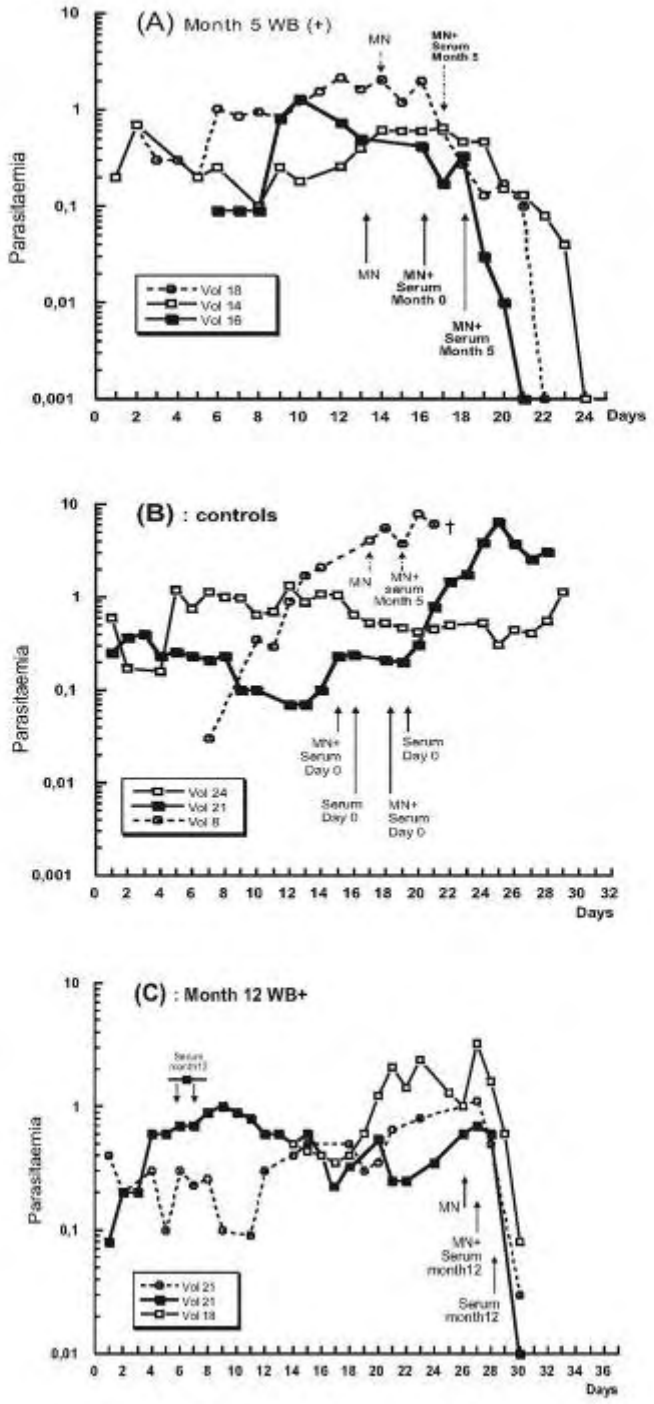


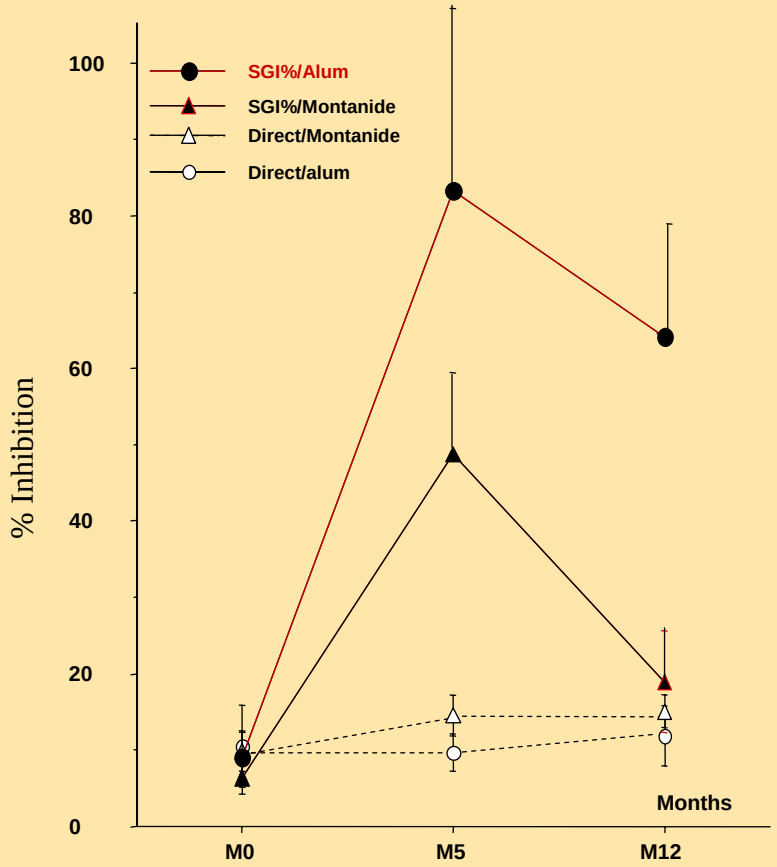

100
80
60
40
20
0
% Inhibition
SGI%/Alum
SGI%/Montanide
Direct/Montanide
Direct/alum
Months
M0
M5
M12

Figure 4

---> THE MSP3-LSP IS A MALARIA VACCINE THAT IS SAFE

WELL TOLERATED WHEN ADJUVATED BY ALUM

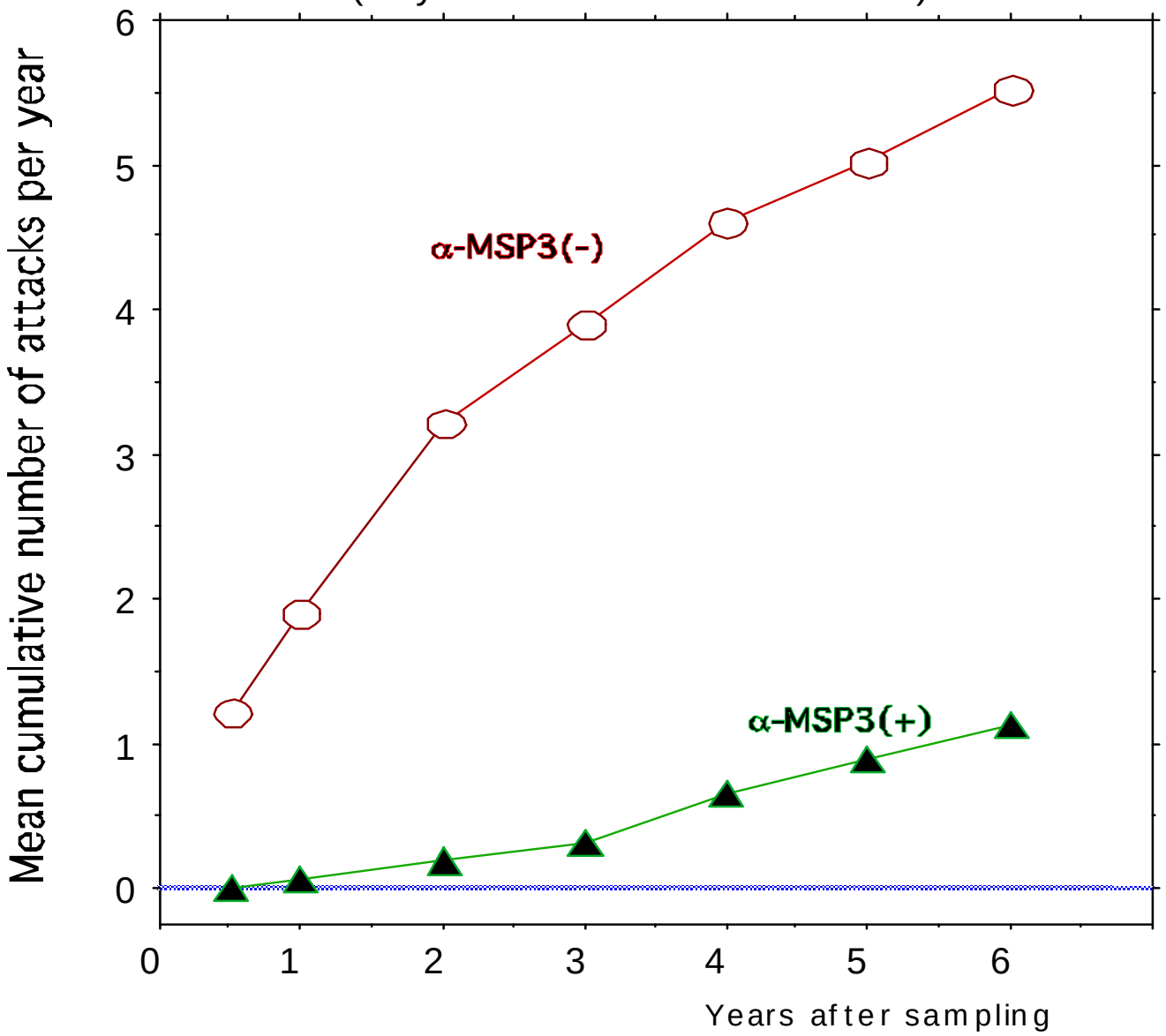
AND ELICITS IN HUMANS Abs ABLE TO KILL P.falciparum

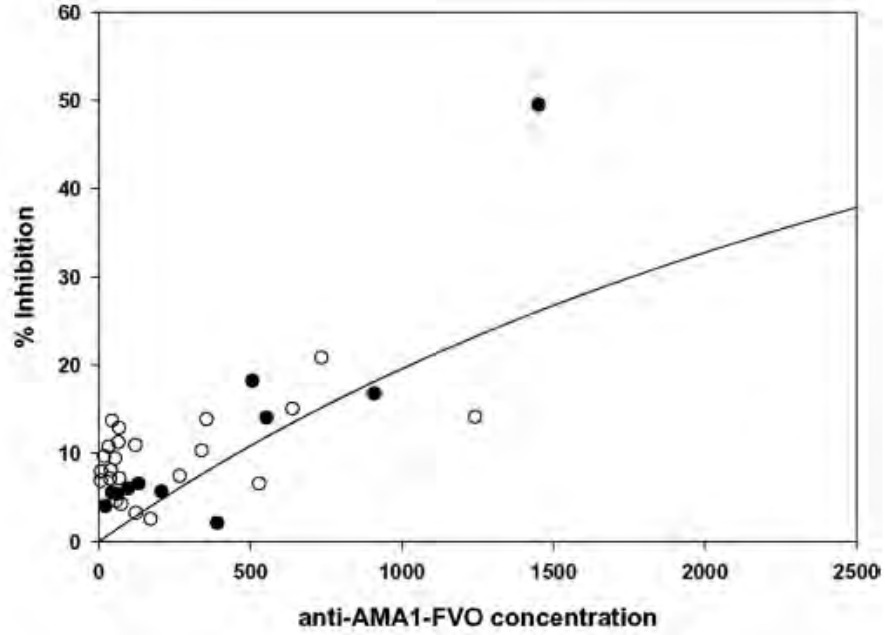
Even low doses of MSP3,
injected with simple adjuvants,
readily induced Abs of cytophilic classes,
long-lasting,
directed to fully conserved epitopes,
with strong biological effect against P.falciparum

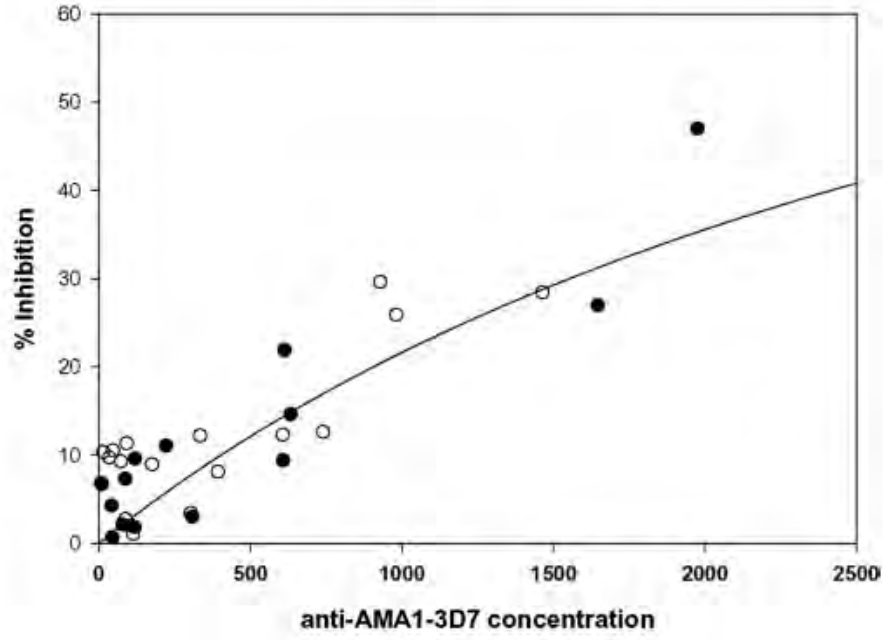
Cytophilic Abs have been consistently found associated with protection

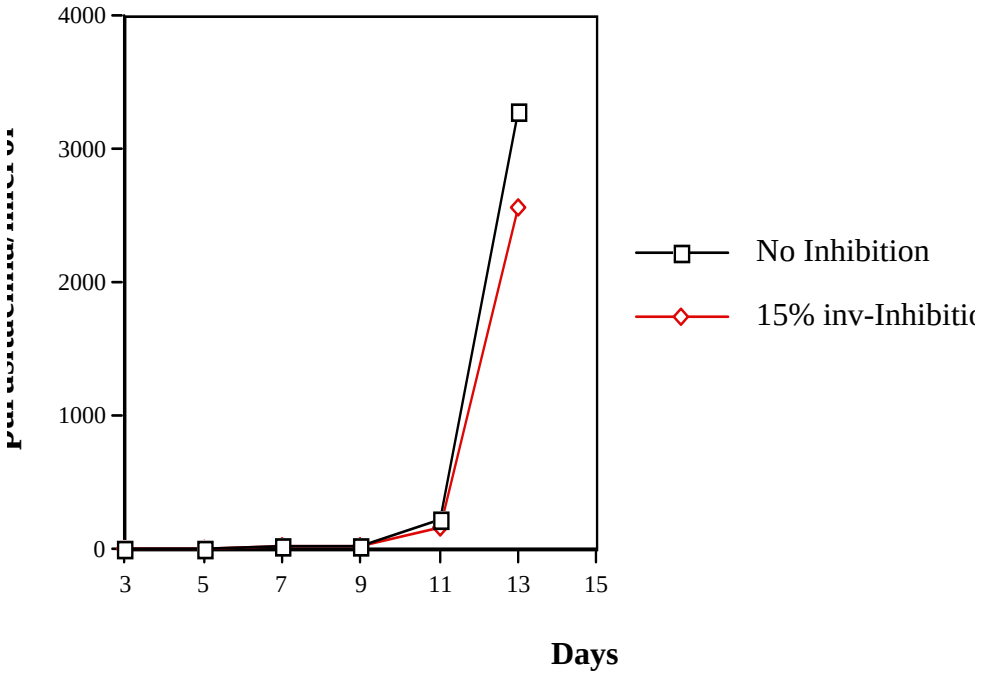
Clinical Prediction resulting from a single Ab determination

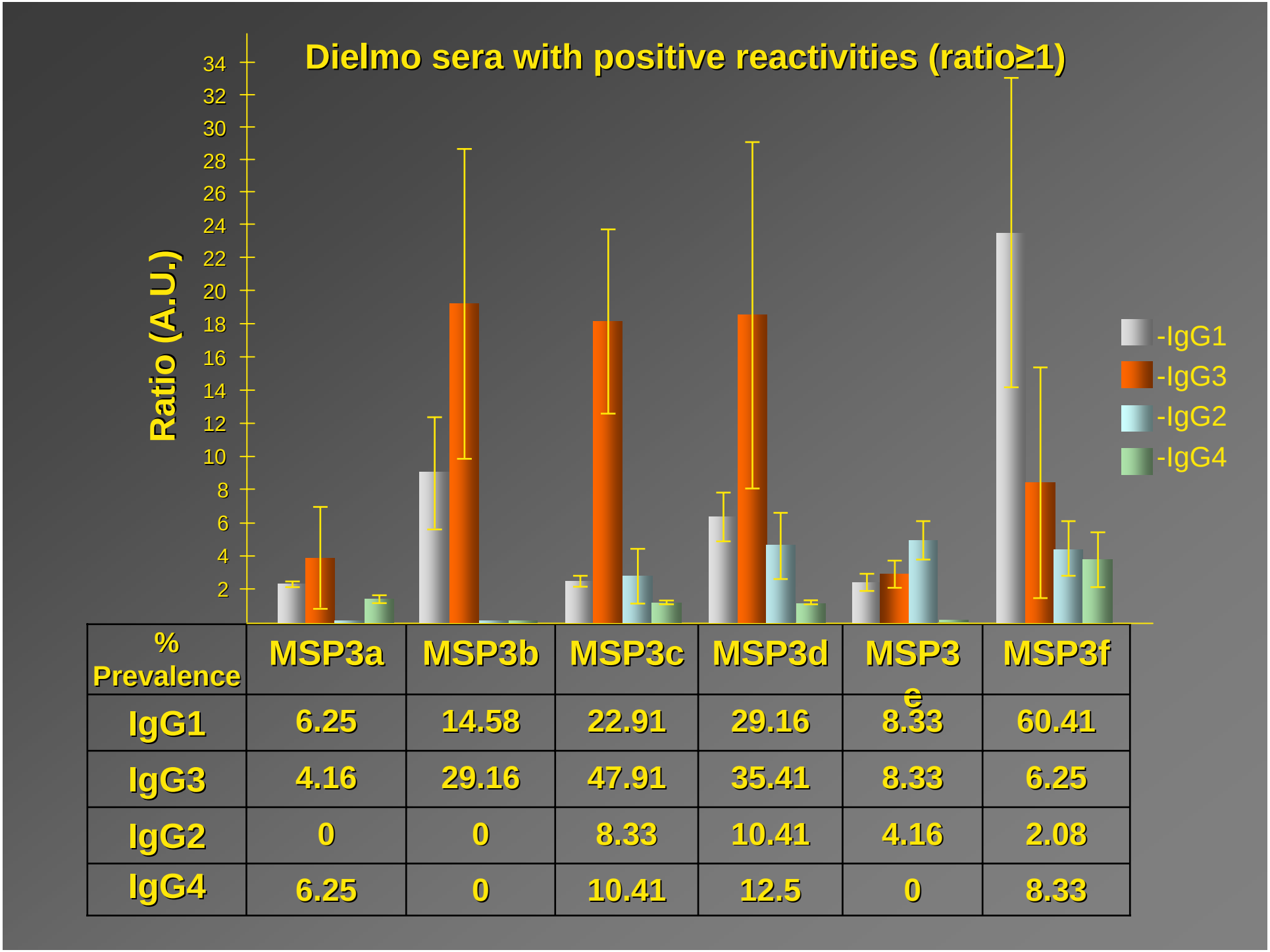
(on year 0 in children from Dielmo)



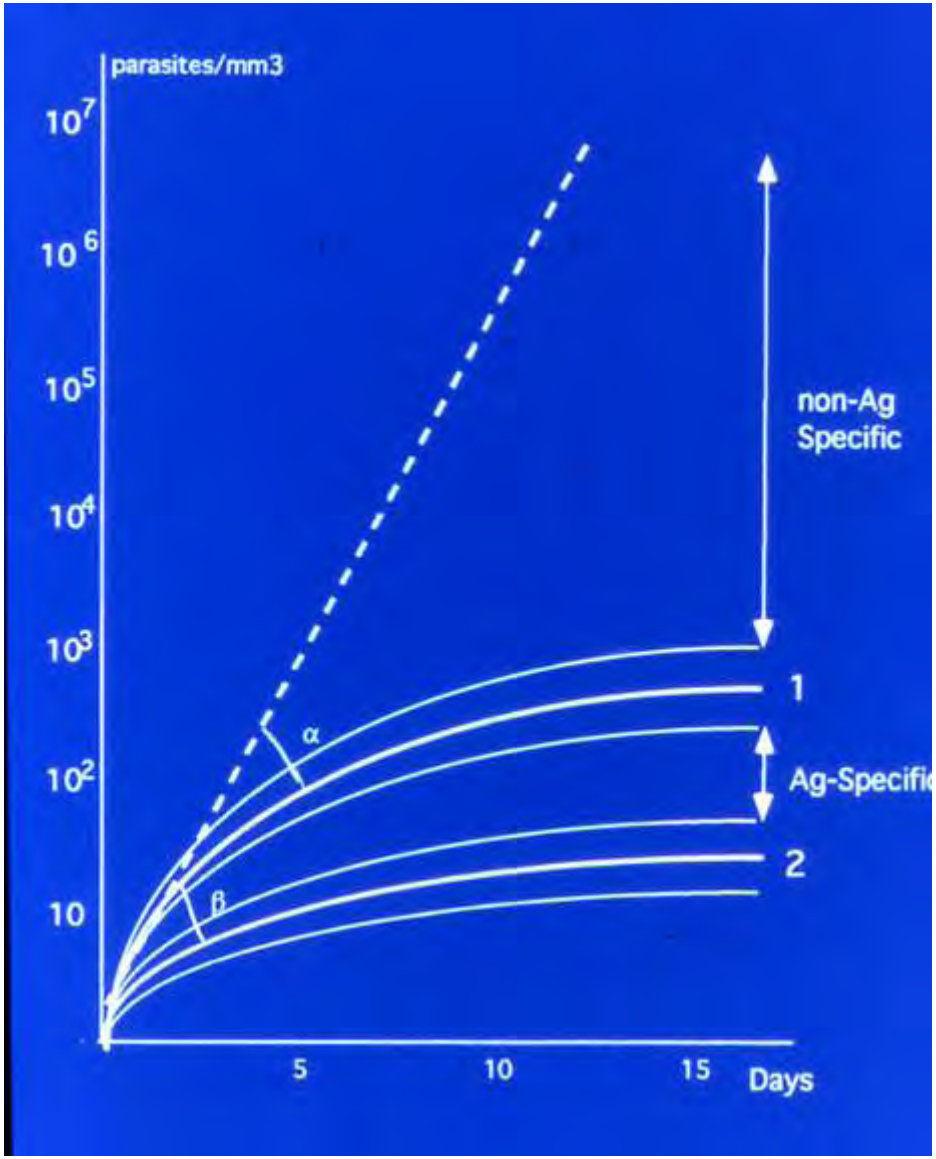

% Inhibition
anti-AMA1-FVO concentration
0
10
20
30
40
50
60
0
500
1000
1500
2000
2500


% Inhibition
anti-AMA1-3D7 concentration
0
10
20
30
40
50
60
0
500
1000
1500
2000
2500

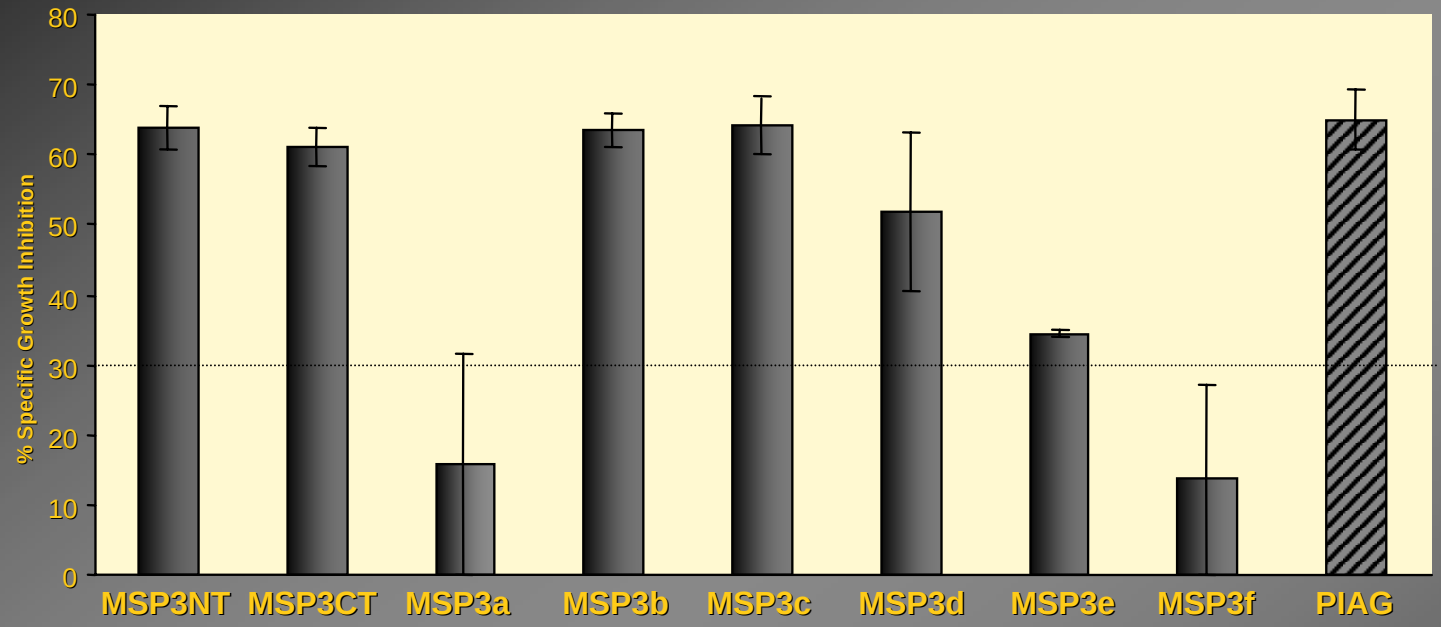

4000
3000
2000
1000
0
3
5
7
9
11
13
15
Days
No Inhibition
15% inv-Inhibitic

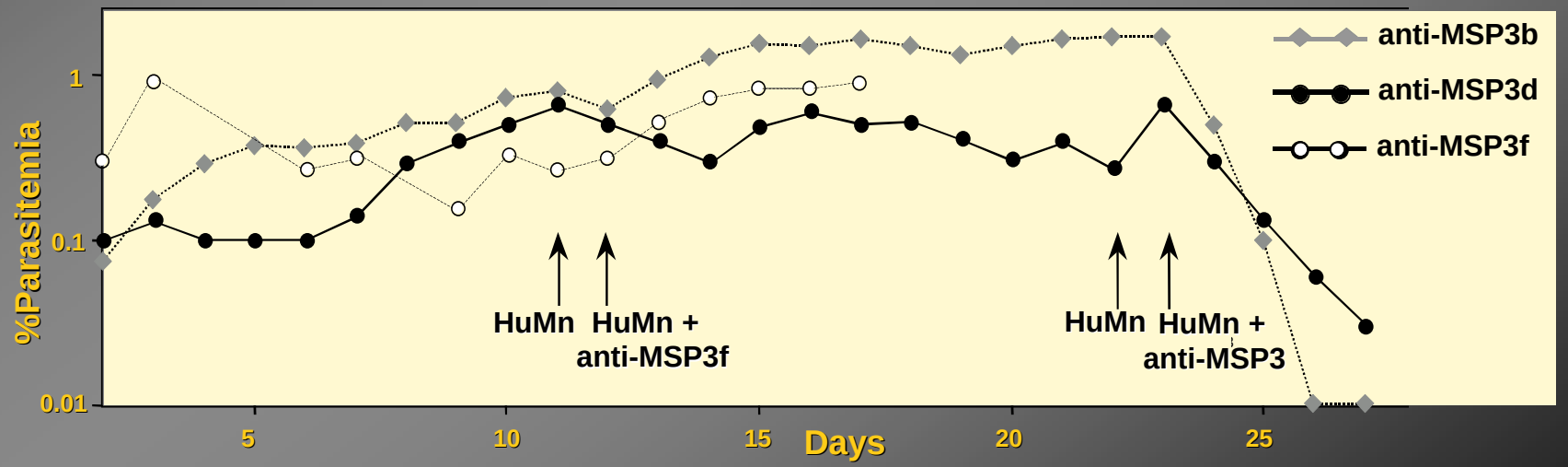


% Prevalence	MSP3a	MSP3b	MSP3c	MSP3d	MSP3e	MSP3f
IgG1	6.25	14.58	22.91	29.16	8.33	60.41
IgG3	4.16	29.16	47.91	35.41	8.33	6.25
IgG2	0	0	8.33	10.41	4.16	2.08
IgG4	6.25	0	10.41	12.5	0	8.33


parasites/mm3
10^7
10^6
10^5
10^4
10^3
10^2
10
non-Ag
Specific
1
Ag-Specific
2
α
β
5
10
15
Days

Functional analysis of naturally occurring antibodies





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

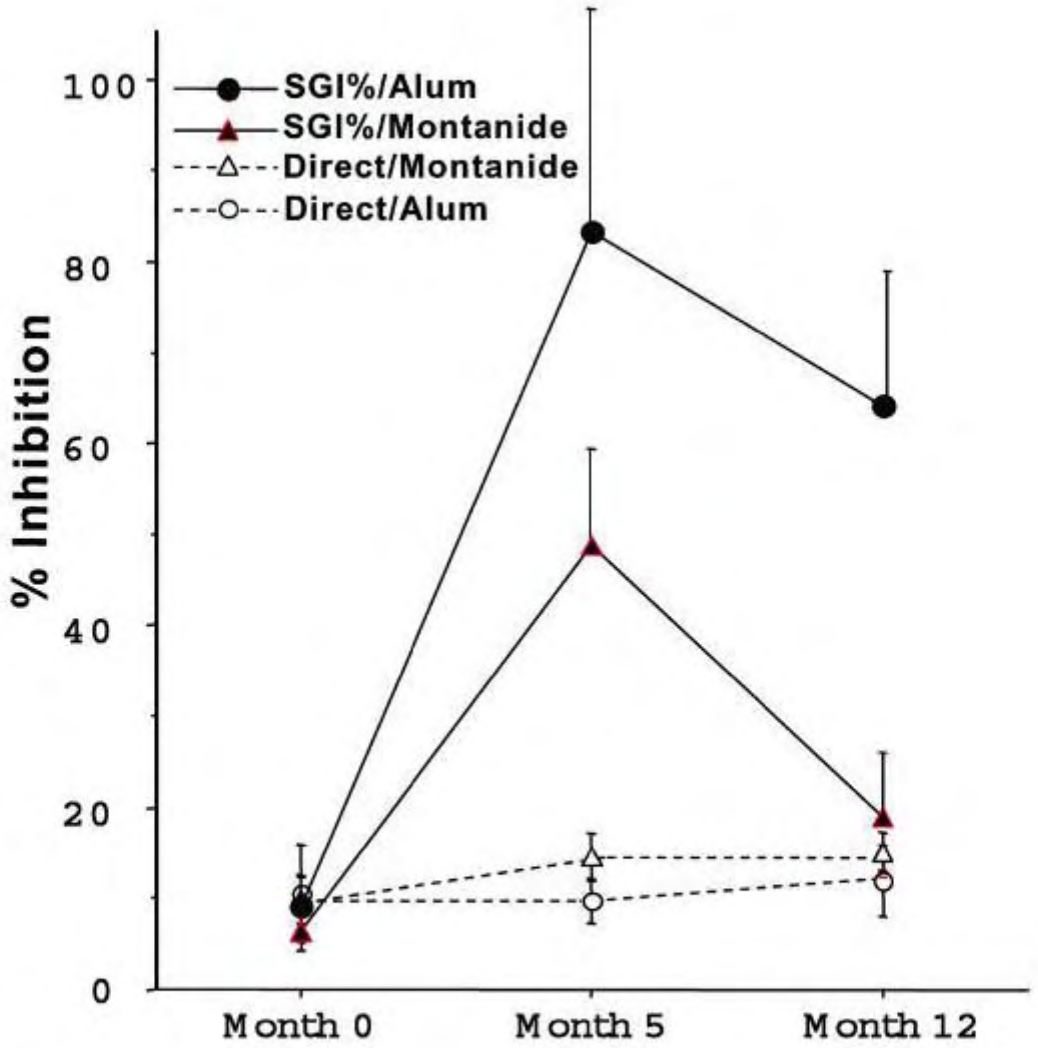
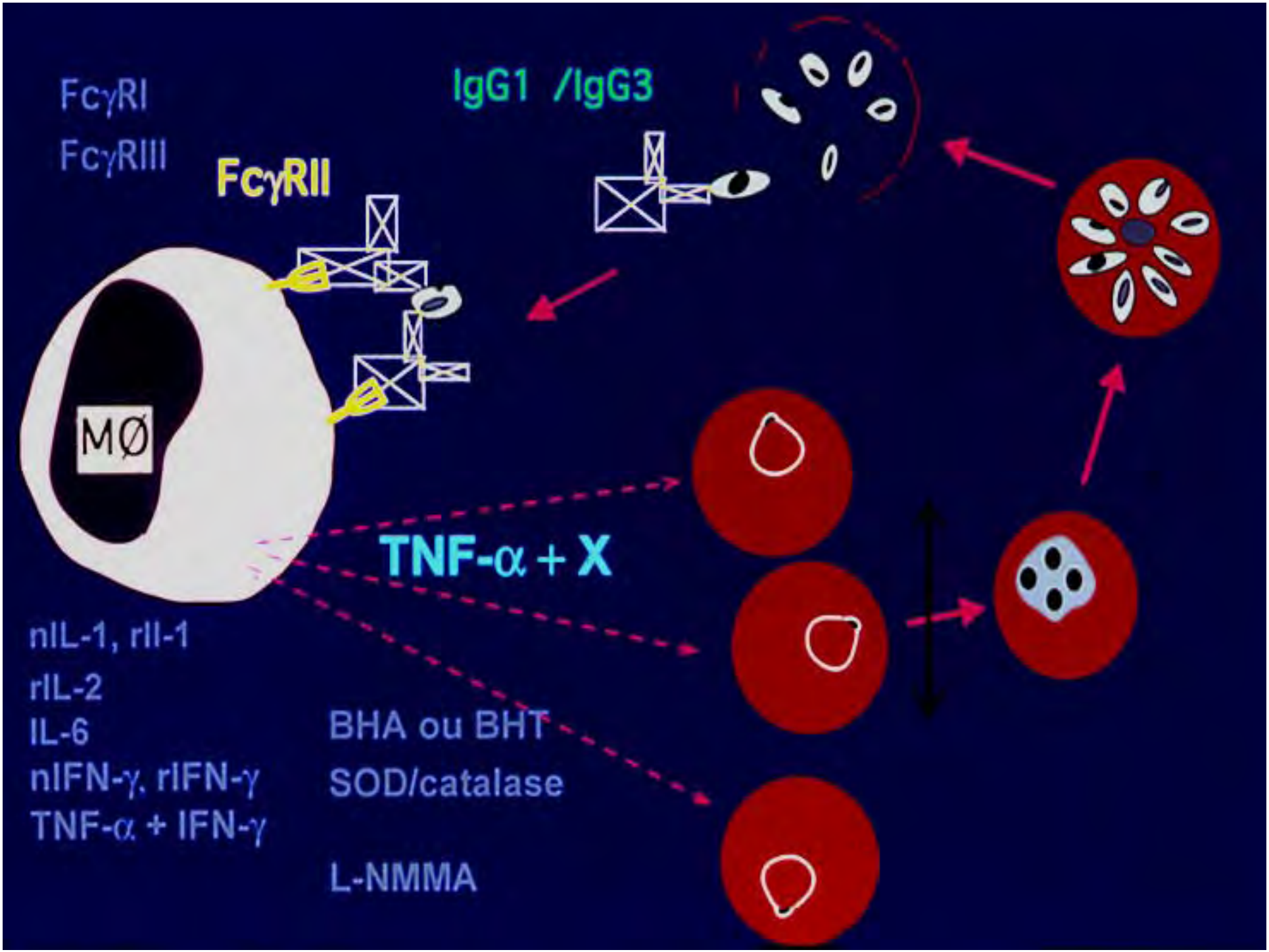
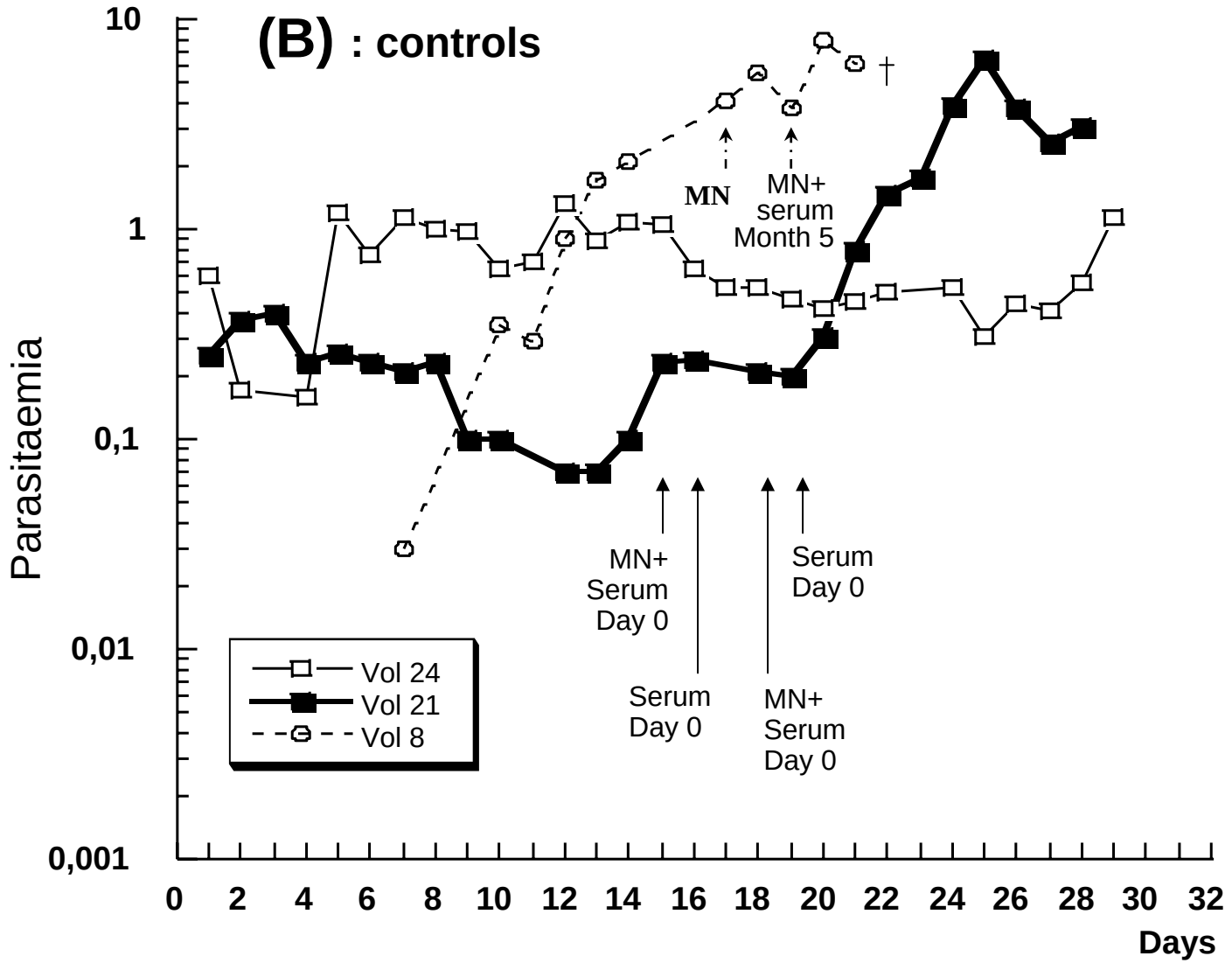
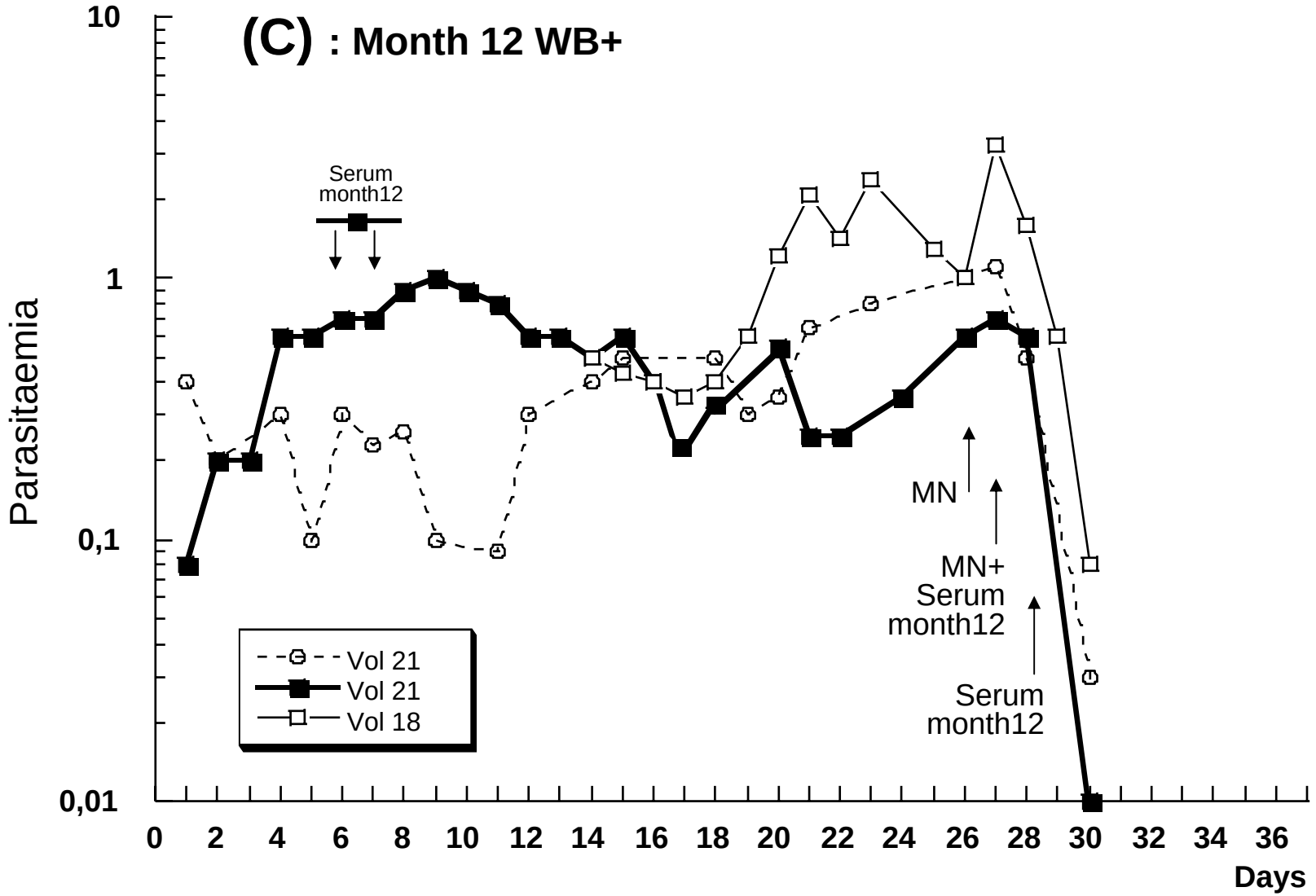

SGI%/Alum
SGI%/Montanide
Direct/Montanide
Direct/Alum
% Inhibition
100
80
60
40
20
0
Month 0
Month 5
Month 12

Figure: 4


FcγRI
FcγRIII
FcγRII
IgG1 /IgG3
MØ
TNF-α + X
nIL-1, rIl-1
rIL-2
IL-6
nIFN-γ, rIFN-γ
TNF-α + IFN-γ
BHA ou BHT
SOD/catalase
L-NMMA


(B) : controls
10
1
0,1
0,01
0,001
Parasitaemia
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Days
†
MN
MN+
serum
Month 5
MN+
Serum
Day 0
Serum
Day 0
Serum
Day 0
MN+
Serum
Day 0
Vol 24
Vol 21
Vol 8


(C) : Month 12 WB+
Parasitaemia
10
1
0,1
0,01
Serum
month12
MN
MN+
Serum
month12
Serum
month12
Vol 21
Vol 21
Vol 18
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
Days