ANTIBODIES AGAINST ERYTHROCYTES ENHANCE INVASION OF MOUSE RETICULOCYTES

By *Plasmodium berghei*
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SUMMARY

Twenty mice were injected i.p. with $1x10^5$ *P. berghei* infected red cells . Blood smears of these mice were prepared daily till the mice were died . Mice were categorized in three groups depending on similar type of course of parasitaemia after inoculation . Seven mice of Group A ($A_1 – A_7$) showed similar type of behavior and died due to low parasite infection . Another group of seven mice (B_1 to B_7) showed a gradual increase in parasitaemia till mice died due to high infection . Group C (C_1 to C_6) smears showed a vary rise and fall in infection and all the mice were cleared of parasite infection .
The antibody titre as detected by IFA , observed in these groups was 1 : 2048 , 1 : 1024 and 1 : 4096 in groups A , B and C respectively . The group C which showed protective immunity against *P . berghei* infection depicted strong fluorescence against normal erythrocyte membrane too . The fluorescence against normal RBC_S membrane was observed very weak in group A , whereas it was negligible in group B .

INTRODUCTION

Malaria continues to cause morbidity and mortality on a large scale in tropical countries . According to the report given by WHO [1] , human malaria parasite causes 300 – 500 million clinical cases which include young children under 5-6 years of age , pregnant women and non – immune travelers . Annual mortality caused by malaria ranges from 0.5 – 2.5 million including over a million death in children aged under five [2] .
Earlier studies have greatly influenced the knowledge about invasion of parasite in red cells [3] and the pioneer work suggested the role of receptors in recognition of susceptible host cells by merozoites for invasion .[4]
In vitro studies of *P . knowlesi* have shown the behavior of merozoites to be even more complex, since the process of invasion involves various sequential changes in shapes of both host and parasite cells [5]
A study was deseribed that the interactions between *P . falciparum* merozoites and human RBC is mediated by specific parasite proteins and sialoglycoproteins (SGPS) on the surface of the host cell.[6]
The first *plasmodium* molecule found to be located in the microneme organelles of the merozoite is the 135 kDa *P . Knowlesi* Duffy binding proteins (pk DBP) [7] . The gene encoding this proteins was subsequently used as a probe to clone the corresponding gene from *P.vivax* (Pv DBP) [8]. It was suggested that the invasion of glycophorin A and B deficient erythrocytes by *P . falciparum* as trypsin or neuraminidase treated glycophorin B deficient cells were refractory to the parasite EBA-175 , a parasite protein appears to bind to glycophorin A . [9,10] suggested the EBA as transmembrane proteins characterized by conserved cysteine rich domain expressed in the micronemes of invasive merozoites .
Strong evidences have implicated EBA – 175 as being a parasite – ligand used in the recognition of human RBC by *p . falciparum* merozoite [11] .
Merozoite surface protein 2 (MSP-2) a highly polymeric in nature , generates

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antiparasite humoral response in mice [12]. Vaccination of animals against sporozoute leads to formation of antibodies that inhibit sporozoute motility and block the invasion of blood vessels by sporozoute [13]. A study found that plasmodium – infected erythrocyte release a low molecular weight soluble factor that inhibits the activation of macrophages and dedritic cells [14]. The present work is an attempt to understand the preferential invasion of reticulocytes by the parasite in *p . berghei* infection .

MATERIAL & METHODS

Parasite Normal mice were injected intraperitoneally (i.p.) with parasitized red cell in citrate saline and the course of parasitaemia was monitored .

Accordingly the mice were grouped in different categories . NK 65 , a lethal strain of *P . berghei* was maintained in Balb / C mice swiss white mice . 20 mice each of 20-25 gms . were inoculated with $1x10^5$ parasitized cells and daily course of infection was monitored by Giemsa stained blood smears . The percentage of total number of red blood cells :

Collection of Blood

The serum was collected in two ways :

1- **A few drops of blood were collected from each mouse separately . It was allowed to clot at room temperature for one hour . The clot was broken and serum was isolated .**
2- **Mice were anesthetized by diethyl ether and bled by jugular vein incision . The blood from broken clot was then centrifuged at 1,000 g for 15 minutes and the serum was aspirated and collected .**

Statistics analysis :

Chi (X^2) test was used for statically analysis in present study .

Indirect Fluorescent Antibody (IFA) test

IFA test was done according to slightly modified method (1972) [13] . The slides were counter stained with Evan's blue and studied under UV . Fluorescence microscope (Leica DMILS , Germany) .

RESULTS

Group A :

The group contained seven mice (A_1 to A_7) . All the mice of this group died due to infection and maximum parasitaemia remained between (24% - 60%) in most of mice (Fig . 1) . One of mice had only 7% maximum parasitaemia . The parasitaemia ranged between (3 - 15%) till day 10 and both reticulocytes and erythrocytes were infected . One mice died on day 12 , other two on day 14 , and one on day 16 . In the remaining three mice the parasitaemia further increased and reached about 48% to 60% followed by increase in reticulocytosis and decrease in parasitaemia . These mice were found dead due to infection on day 24 , 26 and 28 .

Group B :

This group also contained seven mice (B_1 to B_7) but on the contrary to group A the animals died to high parasitaemia (Fig . 2) . Maximum parasitaemia ranged between 58% to 82% . Both erythrocytes and reticulocytes were infected but with gradual increase in parasitaemia and appearance of reticulocytes . In two of the mice of this group more than 80% cell were infected. The animals died because of high parasitaemia between day 13 to day 17 .

Group C :

This group contained 6 mice (C_1 to C_6) . In this group the parasite was observed on 14^{th} day of post inoculation in 4 mice . Whereas , two mice became infected on 5^{th} day . There was Zig – Zag course of parasitaemia . In all the mice initially most of the erythrocytes were infected along with a few reticulocytes (Fig . 3) with the gradual increase in parasitaemia , reticulocytosis occurred and the post migrated from red cells to the reticulocytes and finally the parasite was exclusively confined to the reticulocytes . An increase in number of WBC_S were observed during later days . Futher smears showed decrease in parasitaemia between day 15 and day 20 and finally no parasite was observed in

the smear of this group after 25th day . Indirect Fluorescent Antibody (IFA) test: IFA test was employed to detect the presence of antibodies in parasitized mice . The IFA slides were counter stained with Evan's blue to avoid any non-specific reaction . The appearance of antimalarial antibodies in mice of different groups and the titre of antibodies are depicted in Table 1 .

Group A :

Antibodies appeared in one of the mice on day 9 whereas , on 10th day all the mice showed positive titre of 1 : 32 (Fig. 4) . Sera collected on day prior to the death of infected animals showed varying IFA titre . Four mice (A_1 , A_2 , A_6 and A_7) showed varying IFA titre between 1 : 256 and 1 : 1024 while others showed 1 : 2048 (Fig . 5) .

IFA that was significant ($P < 0.05$) with the infection percentage .

Group B :

In this group antimalarial antibodies were observed on day 9th in 2 mice (B_1 to B_4) whereas , by next day appeared in other 5 mice (Fig. 6) . The IFA titre ranged between 1 : 64 which died on day 13th and 1 : 512 which died on day 15th an 1 : 1024 which died on 16th and 17th day (Fig . 7) .

NO or almost negligible fluorescence was observed with normal red cell antigen . no significant ($P < 0.05$) between IFA and infection percentage .

Group C :

The antimalarial antibody was observed on day 10th post inoculation in all the 6 mice (Fig . 8) . IFA titre of 1 : 4096 was observed in the sera of these mice when sacrificed on 36th day . The IFA titre of this group showed in (Fig . 9) . A very strong IFA reaction was observed against all the stages of the parasite in case of parasitized erythrocytes or reticulocytes . The reaction with the host cell antigen revealed that more fluorescence was observed with erythrocytes as compared with the reticulocytes .

IFA that was significant ($P < 0.05$) with the infection percent .

DISCUSSION

Susceptibility of Balb / C strain of white swiss mice to NK – 65 strain of *P.berghei* as an asynchronous parasite it was well established the suscep [14] .

Preferential invasion of reticulocytes by merozoites of *P . berghei* was also observed by others [15]; [16] ;[17] . *P. vivax* invades reticulocytes exclusively and reticulocyte binding gene expressed PvRBP-1 and PvRBP-2 , two proteins which are shown to be responsible for preferential invasion of *P . vivax* in reticulocytes . *P . berghei* has shown preference for reticulocytes , however , PvRBP gene probes did not cross hybridise to DNA of *P . falciparum* , *P . knowlesi and P . berghei* [18] .

***In Vivo* reticulocytes mature to red cells within 24-36 hours after release from bone marrow into circulation [19] . During the maturation process of erythrocytes a number of constituent proteins from the membranes of the reticulocytes are lost [20] ; [21] ; [22] . The phenomenon of invasion of red blood cells by the parasite is a multistep process involving attachment of the host cell and internalization of the parasite [23] ; [24] . The relationship between age of the host cell and invasion by *P.falciparum* indicated that young erythrocytes in particular have an increased susceptibility to the parasite [25] . In *P . berghei* the parasite parasitizes reticulocytes preferably initially and exclusively later , if it remains in the host cell for 2-3 weeks or more .**

Although the IFA is useful in estimating exposure to malaria , previous work has indicated that many antibodies directed against some parasite antigens detected by IFA , depicted appearance of antibodies between 7th to 10th day of post inoculation . There was a gradual increase in antibody level in different mice . Some mice died due to low parasitaemia with IFA titre of 1 : 2048 after 24 days whereas IFA titre of 1 : 1024 was observed in some mice which died due to high parasitaemia between

16th and 17th day , or some mice recovered from infection and the parasite was not observed in the smear with IFA titre of 1 : 4096 . An increase in WBC'S was observed during later days depicting some role of cell mediated immunity along with humoral immunity , both playing a major role in protection .

Finally humoral and cell mediated immunity along with some genetic factors may be responsible for protective immunity in these mice.

Table1 : Appearance of antimalarial antibodies in mice of different groups and the tutre of antibody .

Group	Antibody appearance		Maximum Antibody Titre in sera collected *
	Day	No . of mice	
A	9 10	1 6	1 : 2048
B	7 8	2 5	1 : 1024
C	10	6	1 : 4096

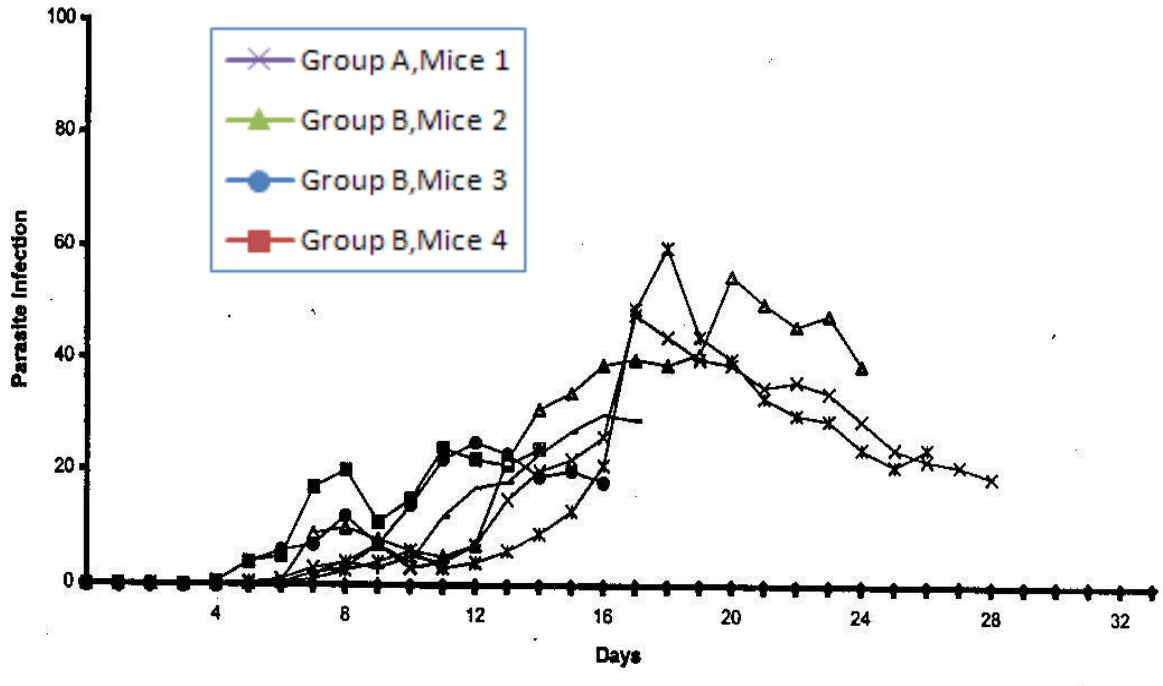

Fig. 1 : Course of P. berghei in mice resulting in death of animals due to low parasitaemia.

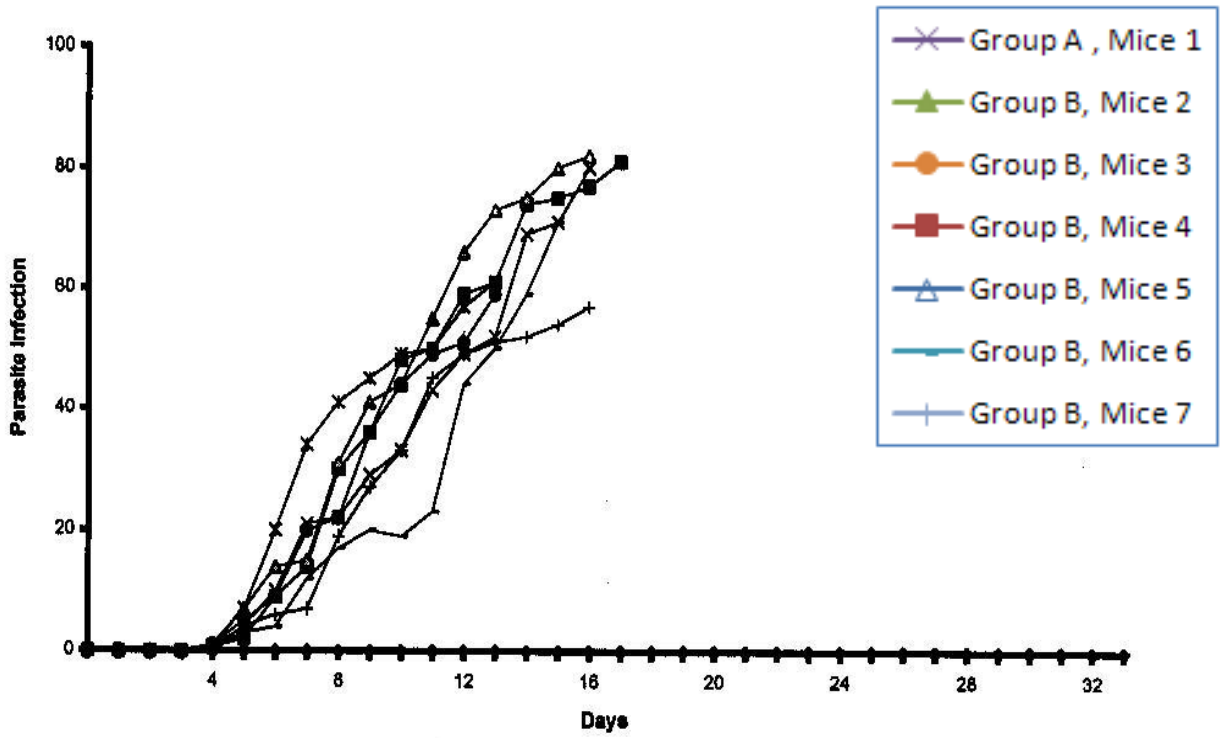

Fig. 2 : Course of P. berghei in mice resulting in death animals due to high parasitaemia

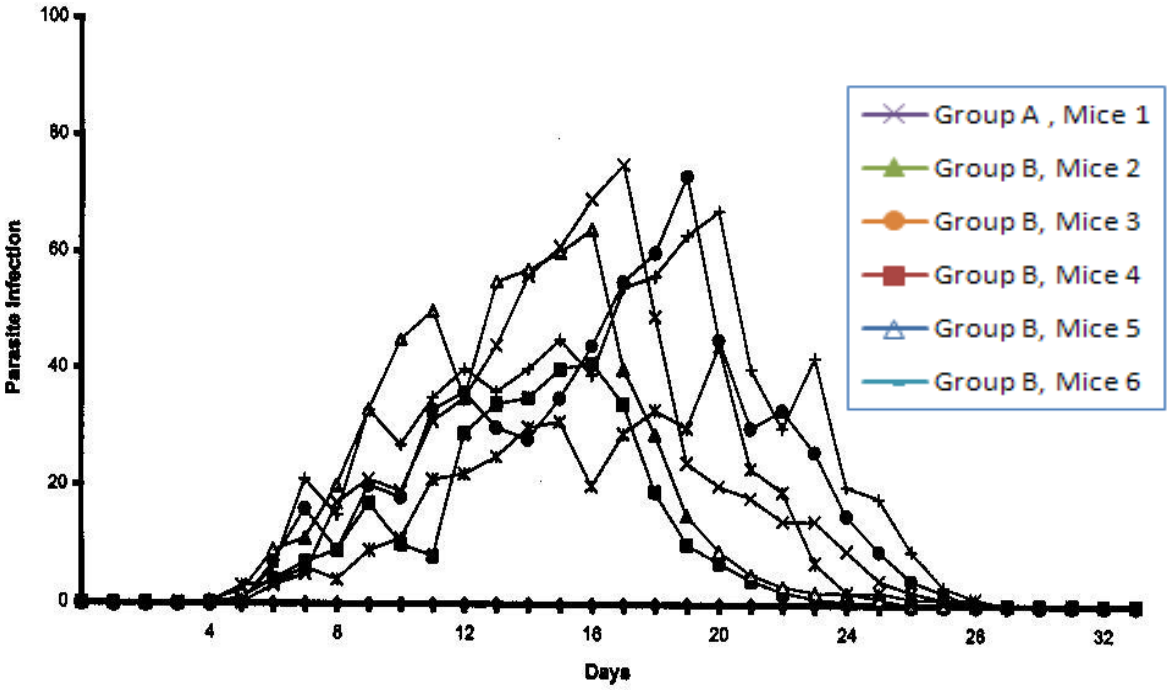

Fig. 3 : Course of P. berghei infection in mice and subsequent protection of the animals

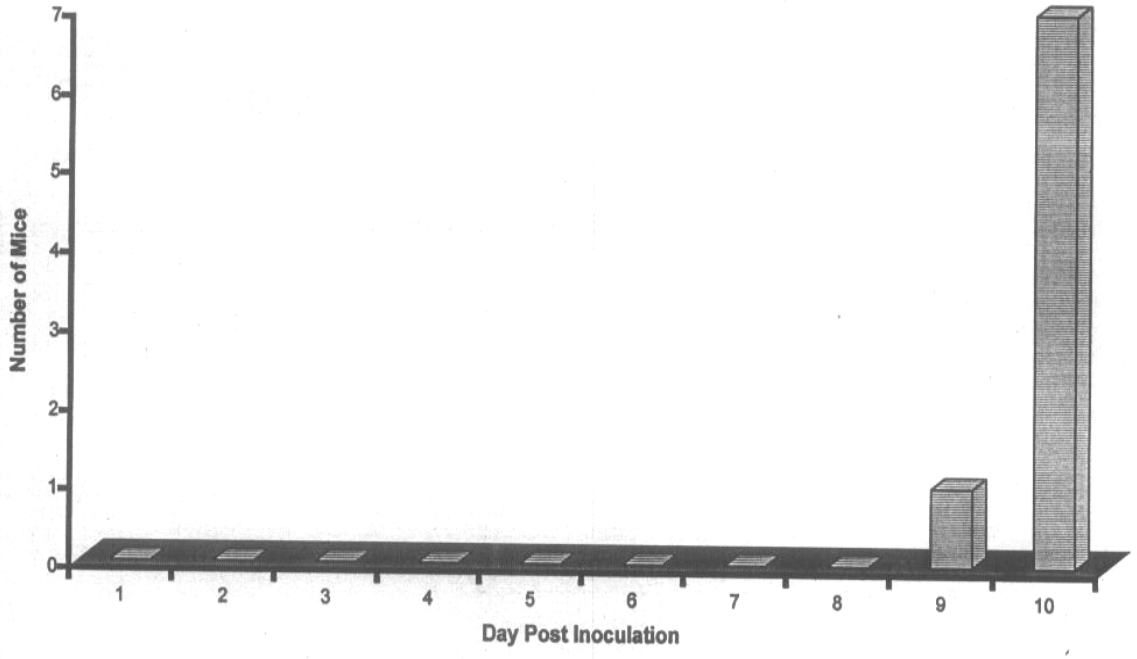

Fig. 4 : Histogram showing antimalarial antibodies in the sera of mice of group A. Animals showing 1 : 32 or more titre were positive.

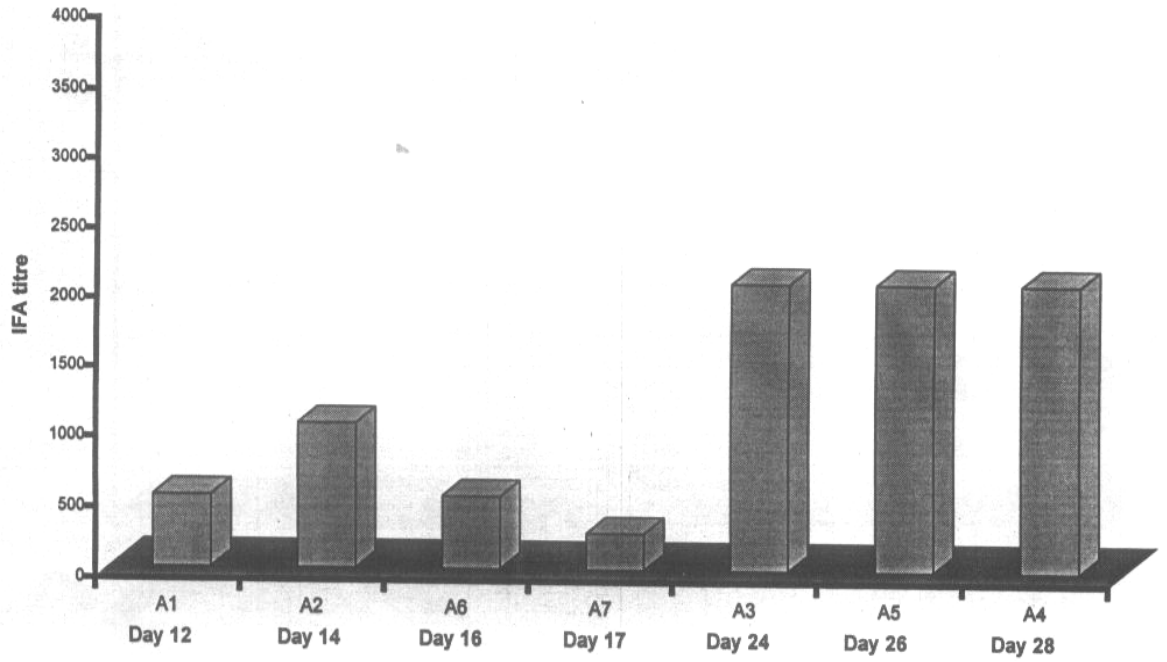

Fig. 5 : Histogram showing IFA titre in the sera samples collected in group A mice.

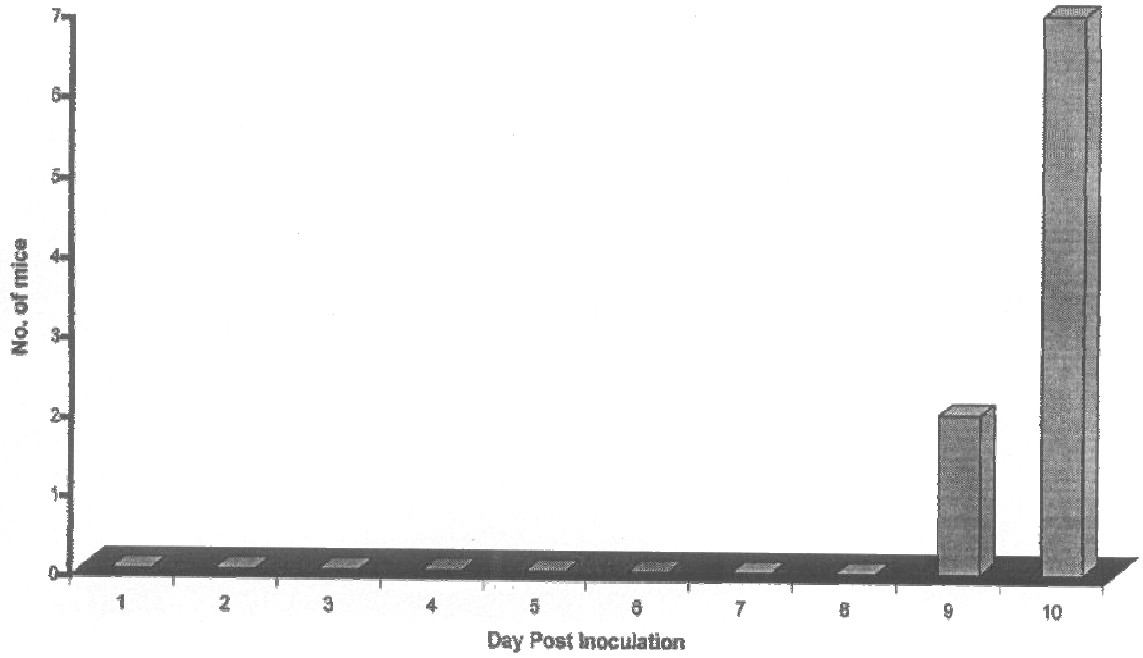

Fig. 6 : Histogram showing antimalarial antibodies in the sera of mice of group B. Animals showing 1 : 32 or more titre were positive.

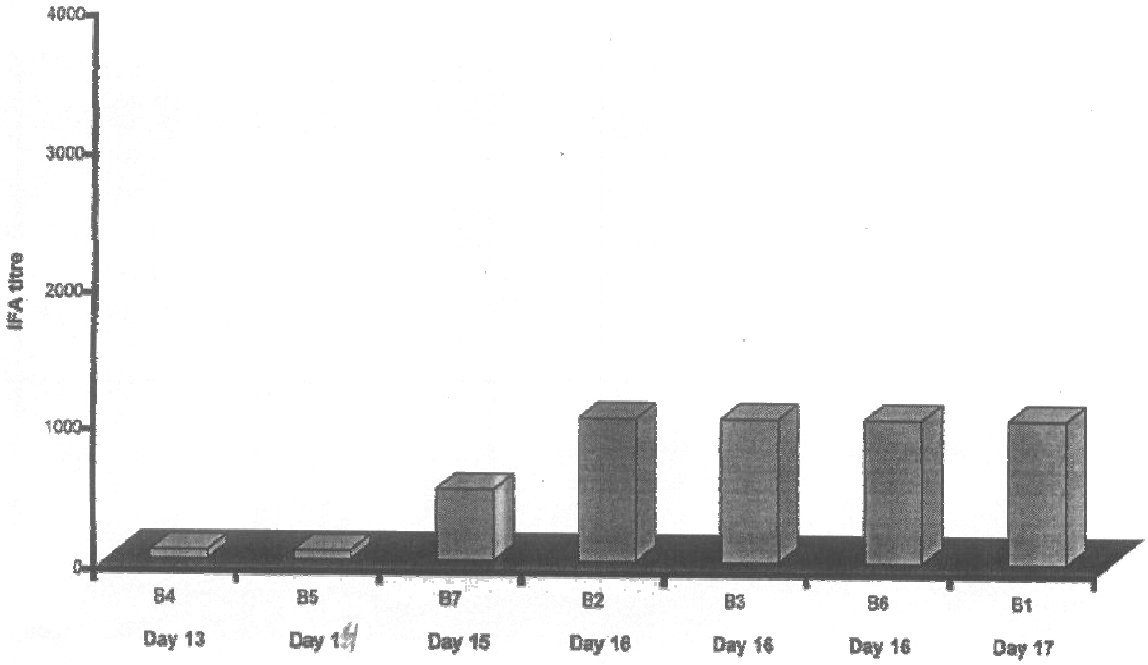

Fig. 7 : Histogram showing IFA titre in the last sera samples collected in group B mice.

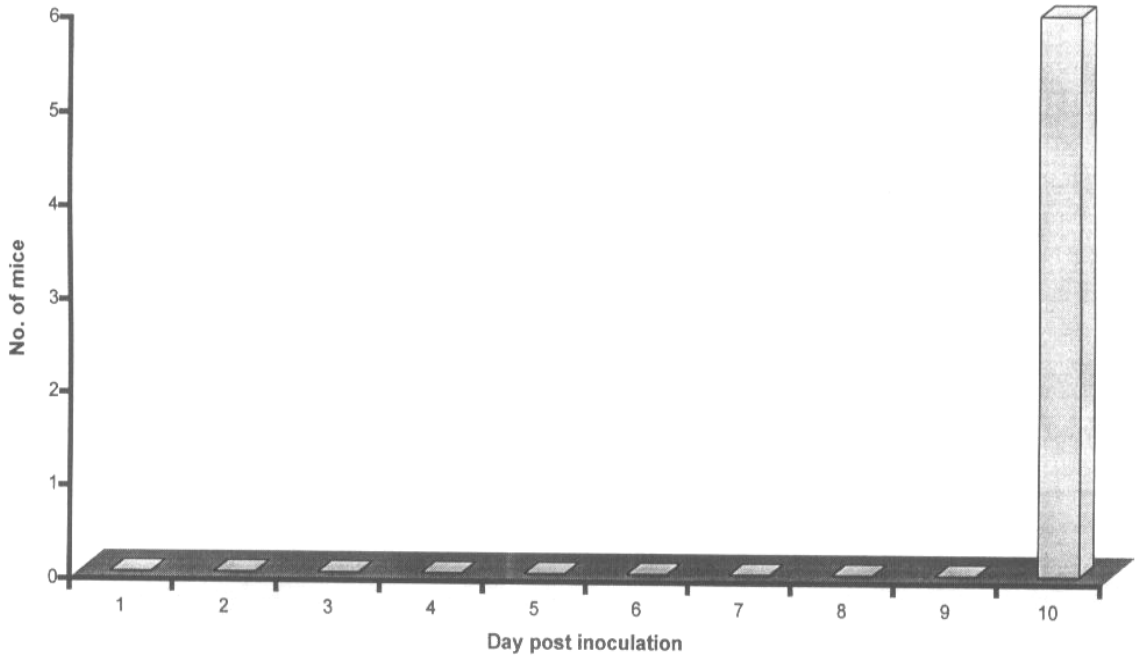

Fig. 8 : Histogram showing antimalarial antibodies in the sera of mice of group C. Animals showing 1 : 32 or more titre were positive.

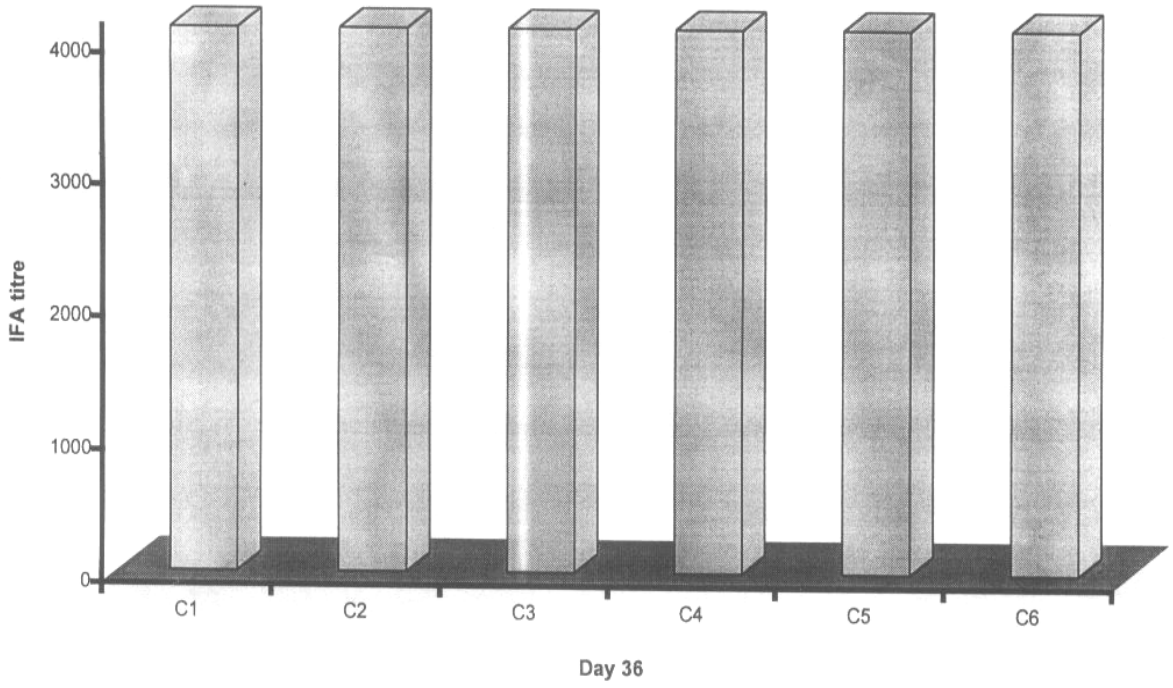

Fig. 9 : Histogram showing IFA titre in sera samples of group C mice.

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تأثير الاجسام المضادة على كريات الدم الحمراء المهاجمة بواسطة الملاريا *P . berghei*

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المستخلص

حقن ٢٠ حيواناً مختبرياً بواسطة 10x1 كريات دم حمراء مصابة بـ P . berghei و اخذت مسحات دم لهذه الحيوانات يومياً ولحين هلاك هذه الحيوانات وقسمت هذه الحيوانات الى ثلاثة مجاميع اعتماداً على الاستجابة للإصابة بعد الحقن وهي مجموعة A ($A_7 – A_1$) اعطت نفس السلوك وقد تم هلاك هذه الحيوانات نتيجة للإصابة بالطفيليات القليلة . المجموعة B ($B_7 – B_1$) اعطت زيادة تدريجية في نسبة الاصابة بالطفيليات ولحين هلاك الحيوانات . مجموعة C ($C_6 – C_1$) اعطت زيادة كبيرة وإصابة كاملة وكانت جميع الحيوانات خالية من الطفيليات .

نسبة الاجسام المضادة حددت بواسطة اختبار فحص الاجسام المناعية (IFA) وقد لوحظت النتائج التالية : 1 : 2048 , 1 : 1024 , 1 : 4096 للمجاميع الثلاثة A , B , C وعلى التوالي . وقد اعطت المجموعة C مناعة كبيرة جداً للإصابة بالطفيليات وقد كانت A ضعيفة جداً .

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