Study on the Induction of Immunity by Purified Cysteine Protease against *Plasmodium berghei* Infected Mice

ABSTRACT

This study was aimed at inducing immunity against *Plasmodium berghei* infection using cysteine protease in mice. Twenty laboratory mice were used for the study, they were grouped into 4 groups of 5 mice each. The first and second groups were immunized with 100ug/l and 5ug/l of purified cysteine protease respectively, the third group was administered with adjuvant which served as positive control while the fourth group (negative control) was not administered. All the groups were challenged with the parasite *Plasmodium berghei.* Results for percentage parasitaemia, Packed Cell Volume, Total Protein and Enzyme assay was recorded. Findings revealed that the mice immunized with the higher concentration of the cysteine protease had the lowest load of infection. The mice that were not immunized had the highest load of infection which showed that the cysteine protease conferred protection against infection.

Keyword: Cystein, Immunity, Malaria, Plasmodium, Protease

1.0 Introduction

Plasmodium berghei is a unicellular parasite and is one of the species of malaria parasites that infect mammals other than humans. *Plasmodium berghei* is one of the four Plasmodium species that have been described in African murine rodents. The four parasites are; *Plasmodium berghei, Plasmodium vinckei, Plasmodium yoelli* and *Plasmodium chabaudi* [1]. These parasites are not of direct practical concern to man or his domestic animals. The interest of these parasites is that they are practical model organisms in the laboratory for the experimental study of human malaria. *Plasmodium berghei* is found in the forest of central Africa where its natural

cyclic hosts are the thicket rat (*Grammomys surdaster*) and the mosquito (*Anopheles dureni)* [2].

Rodent Malaria parasites are used in many research institutes for studies aiming at the development of new drugs or a vaccine against malaria. In the laboratory, the natural host has been replaced by a number of commercially available laboratory mouse strains. Rodent parasites are recognized as valuable model organisms for the investigation of human malaria in most essential aspects of morphology, physiology and life cycle and the manipulation of the complete life cycle of these parasites is simple and safe [3].

Like all malaria parasites of mammals, including the four malaria parasites (*Plasmodium* ovale, *Plasmodium falciparum, plasmodium vivax,* and *Plasmodium malariae*), *Plasmodium berghei* is transmitted by *Anopeheles* mosquitoes and it infects the liver after being injected into the blood stream by a bite of an infected female mosquito [4,5]. After a short period of development and multiplication, these parasites leave the liver and invade erythrocytes. The multiplication of the parasite in the blood causes the pathology such as anemia and damage of essential organs of the host such as lungs, liver, and spleen [6]. *Pasmodium Berghei* infections may also affect the brain and can be the cause of cerebral complication in laboratory mice. These symptoms are to a certain degree comparable to symptoms of cerebral malaria on patients infected with the human malaria parasite, *Plasmodium falciparium* [7].

The complete genome of *P. berghei* has been sequenced and it shows a high similarity, both in structure and gene content with the genome of the human malaria parasites *Plasmodium falciparium*. *Plasmodium berghei* can be genetically manipulated in the laboratory using Standard genetic engineering technologies. Consequently this parasite is often used for the analysis of the function of malaria genes using the technology of gene modification [8]. Malaria is a disease which is claiming the lives of 300 million people in Africa of which women and children are the highest victims [7]. Because of the resistance to treatment, vaccine is the best alternative. Therefore, this study is aimed at inducing immunity against *Plasmodium berghei* infection using cysteine protease in mice.

2.0 Materials and Methods

2.1 Laboratory Animals and Grouping

Twenty laboratory mice were purchased from Department of Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The mice were acclimatized for two weeks after which they were divided into 4 groups of 5 mice each.

Group 1: Consists of mice that were immunized with the higher concentration of the cysteine protease (100ug/l)

Group 2: Consists of mice that were immunized with the lower concentration of the cysteine protease (50ug/l)

Group 3: Consists of mice that were administered with adjuvant only (complete freund adjuvant) and also served as the positive control.

Group 4: Consists of mice that were not administered with anything at all. This served as the negative control group.

2.2 First Immunization

The first immunization was given to the mice following the method carried by Goh and Goh [9]. An emulsion of the cysteine protease of about 100ug/l with complete adjuvant was given to the first group of mice. The second group of mice were immunized with 50ug/l of the cysteine protease with complete freund adjuvant, the third group of mice were not immunized but was administered with complete freund adjuvant only. The fourth group of mice were not given cysteine protease or adjuvant at all.

2.3 Booster Immunization

After 14 days of first immunization, a booster immunization was administered (to further raise antibodies against the parasite) in the order of first immunization with the addition of

Incomplete Freund adjuvant given to groups 1, 2 and 3. However, group 4 was not administered again.

2.4 Challenge of Mice with *Plasmodium berghei* parasite

Seven days after the booster immunization, all the 4 groups of mice were challenged with *Plasmodium berghei* parasite intraperitoneally using 1ml syringe.

2.5 Determination of parasitaemia

Blood was harvested from each mice and placed on a microscopic glass slide to prepare a thin film. The film was fixed with absolute ethanol and letter stained with Giemsa stain as described by Cheeshbrough [10]. It was viewed under the microscope at x100 objective. The infected cells were expressed in percentage as percentage parasitaemia by counting the infected red blood cells over the total red blood cells multiplied by 100%.

2.6 Determination of Packed Cell Volume

Microhaematocrite method was used to determine the PCV as described by Cheeshbrogh [10].

Two meals (2ml) of blood from EDTA bottle was allowed by capillary action to flow through the capillary tube and one end of the tube was sealed using plaster seal. It was then centrifuged at a speed of 3000rpm for 10 minutes. The PCV was estimated using a microheamatocrit reader and expressed as percentage erythrocytes in the blood.

2.7 Total Protein

The capillary tube was crushed and the plasma content was poured on a refractometer and the values were viewed and recorded [11].

2.8 Assay for protease activity

Protease activity with gelatin as substrate was determined according to Dominguez [12]. About 50ul of serum with and without inhibitor (antibody) in five tubes each were incubated with 100ul of the protease and substrate of varying concentrations (1, 0.5, 0.33, 0.13, and 0.63) mg/ul at 37^0C for 30 minutes. Sodium acetate buffer was added and incubated for 1 hour, after which the reaction was stopped by adding 200ul of 20% v/v trichloroacetic acid. The tubes

were centrifuged and the supernatant was decanted into a cuvette and read with a spectrophotometer at 360nm. The corresponding absorbance value was read as enzyme activity in minutes per microgram and recorded. The principle behind the assay was to check for the presence of antibody, by the reaction of enzyme with the antibody in the presence of a substrate. Antibody present, inhibited the enzyme (protease) to bind with the substrate.

2.9 Ethical Approval

The ethical approval for the use of laboratory animals was obtained from the Ethical committee, Ministry of Agriculture and Forestry, Kaduna State, Nigeria.

3.0 Results

3.1 Percentage Parasitaemia

Results for percentage parasitaemia is shown in Table 1. Group of mice that were immunized with 100ug/l of cysteine protease recorded the lowest average parasitaemia of 36% after 15 days of post infection. The highest load of average parasite was recorded in the negative control group 90.5% after 15 days of post infection.

3.2 Packed Cell Volume

Results for Packed Cell Volume is shown in Table 2. The average packed cell volume is highest in the group of mice immunized with 100ug/l (44%) after 15 days of post infection. The least average packed cell volume was recorded in group immunized with 50ug/l (40.3%) after 15 days of post infection.

3.3 Total protein

Results for total protein is shown in Table 3. It revealed that the positive and negative control groups had the least average total protein of 4.5 and 4.7 respectively after post infection of day 15. The groups immunized with 100ug/l and 50ug/l had the average higher values of 5.0 and 4.9 respectively after post infection of day 15.

3.4 Enzyme Assay

Results for enzyme assay is shown in Table 4. From the values, the enzyme activity in serum with inhibitor is high with varying substrate concentration with highest activity of 9.5min/µg recorded after post infection of 16days. The enzyme activity without inhibitor is low compared to the ones with inhibitor, with the highest activity of 5.4min/µg recorded after infection of day 16.

Table 1: Average Percentage Parasitaemia

Days (Post infection)	Groups			
	100ug/l	50ug/l	Positive contr	Negative contr
8	**26.4**	**30.3**	**60.6**	**52.0**
13	**13.3**	**42.2**	**52.0**	**37.5**
15	**36.0**	**50.1**	**72.0**	**90.5**

Table 2: Average Packed Cell Volume

Days (Post infection)	Groups			
	100ug/l	**50ug/l**	**Positive contr**	**Negative contr**
8	48.4	53.0	39.5	48.4
13	46.0	49.8	46.0	46.4
15	44.0	40.3	41.0	42.0

Table 3: Average Total Protein

Days (Post infection)	Groups			
	100ug/l	50ug/l	Positive contr	Negative contr
8	5.0	5.0	4.6	5.0
13	5.2	5.8	4.9	4.5
15	5.0	4.9	4.5	4.7

Table 4: Results showing enzyme activity

Substrate Concentration	Enzyme activity min/ug (No inhibitor)	Enzyme activity min/ug (with inhibitor)
1	2.3	2.6
2	2.7	3.0
3	3.1	4.2
8	3.3	5.4
16	5.4	9.5

4.0 Discussion

4.1: Implication of cystein protease on malaria in mice

There was an increase in percentage parasitaemia for all groups that were challenged with *Plasmodium berghei*. The cysteine protease antigen did not raise enough antibody to clear off the parasite but was able to reduce the parasite load as compared with the control. In addition, the 100ug concentration conferred better protection than the 50ug and the 50ug conferred better protection than the positive and negative control groups. This result is similar to the works of Rosenthal [13] which reported a delay in the onset of infection and a significant reduction in the total parasite load from 55% to 30% when mice, immunized with cysteine protease were challenged with *Plasmodium falciparum.*

The normal total protein in mice is between 5.0 – 5.5 [4]. The total protein comprises of globulin and albumin. High total protein tells us the tendency of contracting a disease. The total protein in general tells us how well the liver is functioning. From the results, the positive and negative controls had the least total protein which could be as a result of infection of the liver by the parasite while the high value recorded initially in the negative control could be as a result of onset of infection. This agrees very well with the results from Schlagenhaul [4] where the immunized mice had total protein within 5.0-5.4.

The PCV values for all the groups reduced. Low PCV could be as a result of malfunction of the kidney which is unable to produce erythropoietin a hormone that induces the production of red blood cell. The unexpected low value in the group immunized with 50ug/l could be attributed to physiological differences. Our results is in agreement with the works carried out by Oladede [14], the PCV for the challenged mice that were immunized was between 43-46% while the unimmunized mice had low PCV ranging between 38-40%.

The high enzyme activity recorded in the serum with the antibody is due to the binding of the enzyme to the antibody. The values of the Michaelis menton constant from the lineweaver burk's plot were calculated to be 0.8 and 1.25 for the serum without inhibitor and with inhibitor respectively. The implication for these values is that low value means there is great affinity for substrate meaning inhibitor is not present while high value means low affinity for substrate because of the presence of an inhibitor.

In other words, antibodies were produced in the groups immunized and no antibodies in un immunized groups. According to Dominguez [12], protease antigen raise antibodies in mice that act as inhibitors, preventing the enzyme from binding to the substrate. This agrees very well with the result obtained as the serum from the mice that were immunized was able to raise antibodies that acted as inhibitors of the substrate.

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