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SPLEEN WEIGHT, LIVER WEIGHT AND LEVELS OF CIRCULATING IMMUNE COMPLEXES IN VITAMIN DEFICIENT MICE INFECTED WITH *PLASMODIUM BERGHEI*

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Three groups of mice viz: well fed mice, vitamin deficient mice and vitamin deficient *Plasmodium berghei* infected mice were studied. In these groups of mice, the weights of the liver and spleen were determined using a weighing balance and the levels of circulating immune complexes measured spectrophotometrically using polyethylene glycol precipitation method. The mean spleen weight, liver weight and CICs of vitamin deficient mice or vitamin deficient *P. berghei* infected mice were reduced compared with those of well-fed mice. However, the reduction in spleen weight was significant in vitamin deficient mice from day 15-post vitamin deficiency compared with well-fed mice. Also, the reduction in liver weight was significant in vitamin deficient mice at day 5- and day 10-post vitamin deficiency compared with well-fed mice while the reduction in liver weight was significant in vitamin deficient *P. berghei* infected mice at day 5-, day 10-, day 15- and day 20- post *P. berghei* infection compared with well-fed mice. The reductions in the levels of CICs were significant in both vitamin deficient mice and vitamin deficient *P. berghei* infected mice compared with well-fed mice from day 5-post *P. berghei* infection or day 5-post vitamin deficiency. The observed decreased CICs in vitamin deficient mice accompanied by reduction in liver and spleen weights showed that vitamin is essential in mounting effective immune response against malaria.

INTRODUCTION

Malaria infection has been associated with great morbidity and mortality with severe consequential effect on children under five year of age and pregnant women (1) living in malaria endemic regions of the world. In 1999, it was estimated that there were some 261 million cases of malaria and 870,000 deaths in malaria endemic areas (2).

Several investigators have provided evidence that cell-mediated immunity and humoral immunity act in concert or sequentially to control and clear a blood-stage malaria infection (3, 4). The prolonged release of different types of antigens in large quantity into the circulation induces a massive immunological response. The endo-erythrocytic stage of malaria parasite when present in numbers induces a lot of immunological responses. This commences with proliferation of phagocytes of the reticuloendothelial system particularly in the spleen, liver and bone marrow. These cells phagocytose parasitized and unparasitized red blood cells, free malaria parasites and malaria pigments. The action is controlled by thymus-derived lymphocytes (4, 5).

During the early phase of infection, both reactive oxygen and reactive nitrogen metabolites produced by non-specific immune cells participate in controlling the primary parasitaemia (6). This initial CD4+ Th1 cell response is followed by a switch to Th2 cytokine production, stimulating antibody-dependent mechanisms involved in the final control and clearance of the parasite (3, 4, 5). Malaria antigen causes proliferation of splenic lymphocytes that leads to macrophage proliferation and active immunoglobulin synthesis (3, 4).

Transient splenomegaly occurs with acute malaria in children and non-immune adults. In malaria endemic areas, a chronic

form of splenomegaly can be found in children (7). Persons with hyper-reactive malaria splenomegaly are immune adults in malarious areas who have gross and chronic splenomegaly, with an overproduction of IgM, high levels of malaria antibody and circulating immune complexes, and a moderately enlarged liver with hepatic sinusoidal lymphocytosis (8).

Resistance to malaria depends on nutritional status of the host among others factors. Most studies on nutritional effects on malarial severity were concentrated on protein energy malnutrition (9). There are scarce literature on vitamins and malaria severity. Deficiency of vitamin A results in reduced weight of thymus and decreased lymphocyte proliferation in response to mitogens (20-22). Feeding diets deficient in vitamin B1, or B2 decreases antibody responses in rodents. Vitamin B6 or vitamin C deficiency results in marked reduction in cell-mediated immune responses and decrease in production of thymic inductive factors. Vitamin E is associated with reduction in T cell, NK cell and phagocyte responses (11, 12).

The importance of liver and spleen in controlling *Plasmodium* infection and the involvement of vitamins in disease control cannot be underrated. This study is designed to find out the effects of vitamin deficiency on the immune system of experimental animal (mice) infected with *P. berghei*. This was carried out by determining the levels of soluble circulating immune complexes and weights of spleen and liver of normal mice, vitamin deficient and vitamin deficient *P. berghei* infected mice.

MATERIALS AND METHOD

Two hundred and twenty-five albino mice (6-10 weeks of age and weighing 9-14kg) were distributed into well-fed mice (Group 1), vitamin-deficient mice (Group 2) and vitamin-deficient *P. berghei* infected mice (Group 3). The animals in Group 1 were fed with complete normal diet while animals in Groups 2 and 3 were fed with vitamin deficient diet. The diet composition is as shown below.

Ingredients	Normal complete diet	Vitamin deficient diet
Maize starch	71.8g	71.8g
Casein	18g	18g
Palm oil	5g	5g
Mineral mixtures	5g	5g
Vitamin mixtures	0.2g	nil

Vitamin deficiency in the mice of Groups 2 and 3 commences on weaning (4 weeks post birth). 3.0 x 10^8 *P. berghei* parasitized red blood cells were inoculated intra-peritoneally at the volume of 0.2ml into experimental mice. Organs (liver and spleen) and blood samples were obtained from etherized dissected mice on days 1, 5, 10, 15, 20 post *P. berghei* infection and/or vitamin deficiency. The weights of the spleen and liver were taken using a sensitive weighing balance while the level of circulating immune complexes were determined as previously described using polyethylene glycol precipitation method (13).

RESULTS

Table 1 compared the spleen weights in the 3 groups of mice. There was no significant reduction in the spleen weights of vitamin deficient *P. berghei* infected mice compared with well-fed mice. Significant reduction in the weight was noted in vitamin deficient mice compared

with well-fed mice from day 15-post vitamin deficiency. The weights of the liver of vitamin deficient mice showed significant reduction only at days 5 and 10 post vitamin withdrawal while the liver of vitamin deficient *P. berghei* infected mice showed significant reduction from day 5 to the end of the end of the study when compared with well-fed mice (Table 2). In Table 3, the levels of CICs were reduced in both of vitamin deficient mice and vitamin deficient *P. berghei* infected mice compared with well-fed mice.

Table 1: Comparison of spleen weights in well-fed mice, vitamin deficient mice and vitamin deficient-*P. berghei* infected mice.

Groups	n	Day 1	Day 5	Day 10	Day 15	Day 20
1	5	0.06±0.23	0.06±0,32	0.07±0.03	0.07±0.52	0.07±0.35
2	5	0.06±0.001	0.05±0.002	0.05±0.002	0.05±0.30	0.05±0.30
3	5	0.06±0.20	0.05±0.33	0.05±0.35	0.06±0.50	0.05±0.22
t-, p-values[a]		0.19, >0.20	0.49,>0.02	0.30,>0.20	2.30,<0.05	2.2,<0.05
t-, p-values[b]		0.56, >0.20	1.06,>0.02	0.93,>0.20	0.80,>0.02	1.2,>0.10

a = Well-fed mice compared with vitamin deficient mice.
b = Vitamin deficient-*P. berghei* infected mice compared with normal mice.
Group 1 = Well-fed mice.
Group 2 = Vitamin deficient mice.
Group 3 = Vitamin deficient-*P. berghei* infected mice.

Table 2: Comparison of liver weights in well-fed mice, vitamin deficient mice and vitamin deficient *P. berghei* - infected mice

Groups	n	Day 1	Day 5	Day 10	Day 15	Day 20
1	5	0.76±0.26	0.70±0.61	0.72±0.11	0.75±0.11	0.79±0.11
2	5	0.66±0.03	0.53±0.51	0.51±0.10	0.67±0.05	0.59±0.24
3	5	0.66±0.02	0.50±0.53	0.45±0.15	0.56±0.10	0.55±0.12
t-, p-values[a]		0.00,>0.20	2.89,<0.02	3.30,<0.01	1.30,>0.2	0.87,>0.2
t-, p-values[b]		0.56, >0.20	4.06,<0.01	4.93,<0.01	3.80,<0.01	3.42,<0.01

a = Well-fed mice compared with vitamin deficient mice.
b = Vitamin deficient-*P. berghei* infected mice compared with normal mice.
Group 1 = Well-fed mice.
Group 2 = Vitamin deficient mice.
Group 3 = Vitamin deficient-*P. berghei* infected mice.

Table 3: comparison of circulating immune complexes in well fed mice, vitamin deficient mice and vitamin deficient *P. berghei* infected mice

Groups	n	Day 1	Day 5	Day 10	Day 15	Day 20
1	5	5.70 ± 0.57	6.00 ± 2.3	6.0 1± 3.2	6.50 ± 3.1	6.60 ± 4.1
2	5	5.21 ± 1.93	2.70 ± 3.2	0.5 ± 0.5	UD	UD
3	5	5.11 ± 1.54	2.80 ± 3.5	1.5 ± 0.5	UD	UD
t-, p-values[a]		0.59,>0.20	3.20,<0.01	4.20,<0.01	UD	UD
t-, p-values[b]		0.66, >0.20	3.60,<0.01	3.93,<0.01	UD	UD

a = Well-fed mice compared with vitamin deficient mice.
b = Vitamin deficient *P. berghei* infected mice compared with normal mice.
Group 1 = Well-fed mice.
Group 2 = Vitamin deficient mice.
Group 3 = Vitamin deficient *P. berghei* infected mice

DISCUSSION

Malnutrition and malaria affects both cellular and humoral immune systems (5, 11, 12). Malnutrition has been known to occur in many children especially in areas endemic for malaria (9). Inhibition of protein synthesis, disturbance of normal function of T lymphocytes and macrophages are possible methods by which *P. berghei* suppresses the immune system (3, 4). The present study gives an insight to the effects of vitamin deficiency and malaria infection on liver weight, spleen weight and levels of circulating immune complexes in mice. Sowunmi (7) reported increase spleen weight during malaria.

The result of the present study showed decrease in spleen weight in vitamin deficient mice or vitamin deficient *P. berghei* infected mice compared with well-fed mice. Therefore in vitamin deficiency vital organs like spleen are readily affected. Suskind (14) previously observed this. This observation may be accounted for by the fact that vitamins are essential for the synthesis of proteins and are needed for maintainance and growth of body tissues. The clearance of antigens from the blood has been considered indicative of non-specific activity of spleen. Also, spleen is the site of contact between antigens and immunocompetent cells (15).

Malaria parasite destroys red blood cells that are usually removed from circulation by spleen; therefore the weight of the spleen is expected to be increased in *P. berghei* infected mice. This is corroborated by WHO (16) report that treatment of malaria infection results in return of the spleen size to normal.

The study also showed that in vitamin deficient *P. berghei* infected mice, the reduction in the weight of the liver was significant earlier (day 5 and day 10) than of the spleen (day 15). Withdrawals of dietary vitamins cause reduction in the synthesis of proteins by the liver because some vitamins are co-factors for the synthesis of proteins that are use for the repair of body tissues.

Reduced circulating immune complexes (CICs) observed in this study could be due to reduced formation but not rapid clearance. CICs are macromolecules consisting of immunoglobulins bound to different antigens. Malaria infection results in reduced bone marrow function (17), and vitamin deficiency cause reduced DNA synthesis (10, 12), lymphocyte proliferation and B lymphocyte function (10, 11). Since bone marrow produces B lymphocytes plasma cells that synthesize immunoglobulins, the effects of both vitamins and malaria could lead to reduced levels of immunoglobulins. Therefore low levels of antibodies will lead to reduced CICs formation.

This study revealed that vitamin deficiency causes reduction in CICs formation, spleen weight and liver weight in experimental animals and that these are aggravated by *P. berghei* malaria. It is therefore recommended that vitamin supplement be given during treatment of malaria.

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