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Research Article

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Splenohepatomegaly in Protein-Malourished Mice Infected with Plasmodium Vinckei

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Abstract

Cellular and histopathological observations were made with light microscopy during the Plasmodium vinckeiinfection in protein-malnourished mice (fed on 2% casein diet and para-aminobenzoic acid deficient diet (PABA-DD).In the infected spleen, hyperplasia of the red as well as the white pulp was evident; active erythropoiesis and lymphopoiesis. Hyperemia and deposition of malarial pigments inside the macrophages.

Cloudy swelling was noticed in the swollen hepatic cells of the uninfected liver due to protein deficiency. This lesion had developed to fatty infiltration in the infected protein-malnourished mice. These new and interesting changes represent the combined deleterious effect upon the host of infection and protein-malnutrition. Hypertrophy of the kupffer cells due to progressive phagocytosis of malarial pigments. In addition, focal necrosis surrounded by inflammatory cells was seen.

Keywords: Liver, Mice, Plasmodium Vinckei, Protein-Malnutrition, Rodent Malaria, Spleen

Introduction

Two billion people in the world suffer from different forms of malnutrition. Malnutrition is the cause of death of 2.6 million children every year – a third of child deaths globally. The most common and significant form of malnutrition is protein-malnutrition [1, 2].It is likely that both malarial infection and protein-malnutrition frequently occur together in the populations of tropical countries, and it is important to discover whether relationships occur between these 2 diseases.

Under nutrition predisposes the host to the morbidity of infection due to both impaired immune competence and nutritional deficiency [3, 4], It has been believed that the levels of nutrition are important components of the ecological balance between man, his micro and macrobiological predators and his environment.

Dietary factors may suppress, stimulate or produce no detectable effect on blood parasitaemia. Therefore, the aim of this study is to demonstrate by histopathological observations evidence of relationships between malarial infection and protein-malnutrition in laboratory mice.

Materials and Methods

Fifteen mice infected with *Plasmodium vinckeivinckei* and fed ad *libitum* on low protein diet (2% casein w/w) were sacrificed by exposure to diethyl ether just before death was expected to occur. Their mean parasitaemia and survival time recorded was 69.05±1.91% and 10.88±0.81 days respectively. This study did not include the mice which survived the infection. Ten uninfected mice which had been fed in the same manner on low protein diet were killed at the same time. Kidneys of all mice were removed, fixed in Helly's solution for 24 h, dehydrated, embedded in paraffin wax (56°C), sectioned, stained with H and E stain and mounted with D.P.X for histopathological examination by light microscopy [5].

Results

Spleen: In the uninfected mice, no evidence of pathological lesion was observed (Figure 1). The spleens of the infected mice were enlarged in size and dark in colour. Microscopically, hyperplasis of the white pulp was noticed but there was little sign of myeloid activity (myelopoiesis) (Figure 2). The transitional zone which separates the white pulp from the red pulp seemed to have disappeared in the spleens of the infected mice. Depositions of black or dark-brown granules of malarial pigments were seen in the macrophages as a result of a marked erythrophagocytosis (Figure 2).

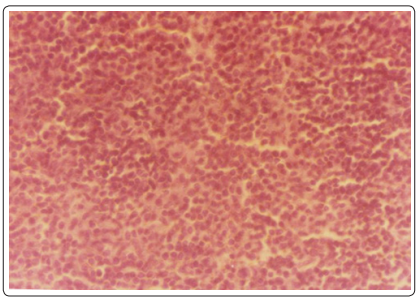

Figure1: Section of spleen from an uninfected protein-malnourished mouse. No evidence of pathological lesions was observed (X550)

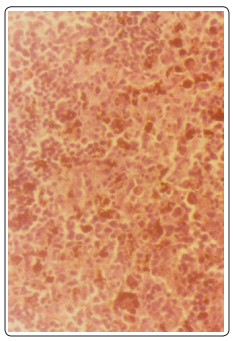

Figure 2: Section of spleen from an infected protein-malnourished mouse. Hyperplasia of the white and red pulp is evident. Note the hyperemia of the spleenic tissue and deposition of black or dark-brown granules of malarial pigments inside the macrophages. Haemosiderosis is prominent within the spleen (X700)

Small amount of malarial pigments appeared yellow-brown but later and after accumulation, become brown-black (Figure 3). The pigment particles varied considerably in size and shape. Due to progressive phagocytosis, these pigments also appeared as clumps in many macrophages. Free pigment could be seen scattered throughout the spleen. As a result of breakdown of haemoglobin, haemosiderosis was prominent within the spleen (Figure 2, 3). Accompanying the phagocytosis, cellular debris was observed within the macrophages. The splenic tissue was hyperaemic and, especially in the transitional zone, was obscured by erythrocytes. There was progressive active erythropoisis everywhere in the spleen except in the white pulp, giving the impression that the organ was mainly engaged in erythropoiesis (Figure 2, 3).

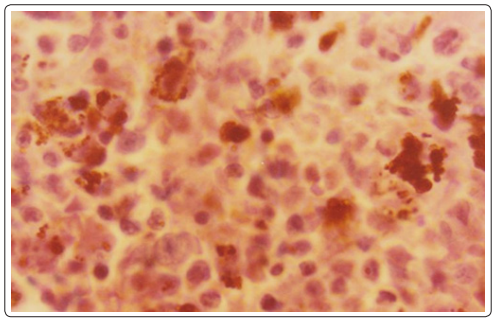

Figure 3: Section of spleen from an infected protein-malnourished mouse. The yellow-brown colour is caused by small amounts of malarial pigments which after accumulation, become brown-black. Haemosiderosis is seen as a result of haemoglobin breaking down. There is progressively active erythropoiesis (X1375)

Liver: In the uninfected protein-malnourished mice, the liver was pale in colour. Microscopically, the hepatic cells were swollen in size and contained vacuoles (Figure 4). The occurrence of pinkish filamentous materials in some the parenchymal cells makes it difficult to decide about the nature of the material inside the vacuoles (Figure 4). The highest concentration of affected cells was around the portal vein; away from this region the effects became less severe and the cells appeared normal. These morphological changes are known as cloudy swelling. Nevertheless, transparent fatty droplets, which are typical of protein deficiency when energy in excess could be seen in some of the hepatic cells. More intense accumulation led to coalescence of the small droplets into large vacuoles. These vacuoles often appeared to cause compression of the nucleus against the cell wall. The nuclei were normally stained blue but some shown up only very faintly which may have been due to some degree of the degeneration (Figure 4). Similarly, a few nuclei which had possessed small vacuoles were seen. The hepatic sinusoids were reduced in size, presumably because of the swelling of the hepatic cells (Figure 4). Local hyperaemia was observed. Cellular infiltration, mainly of mononuclear cells in the central and portal vein and to a lesser extent in the sinusoids was evident. Nevertheless, regenerative activity and mitotic divisions were observed in the parenchymal cells (Figure 4).

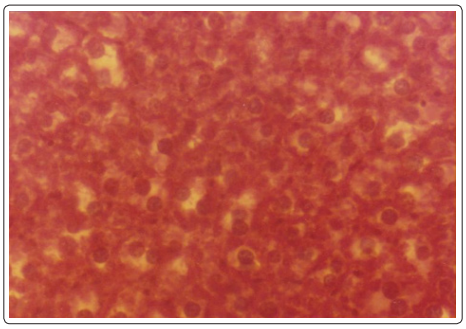

Figure 4: Section of liver from an uninfected protein-malnourished mouse showing a cloudy swelling of the hepatic parenchymal cells. Not some degree of nuclear degeneration and size reduction of the hepatic sinusoids (X700)

The liver of the infected protein-malnourished mice were large in size and dark-brown in colour. Microscopically, the normal architecture of the tissue seemed to have been disrupted and the portal and hepatic vessels appeared to be congested as well as there being few sinusoids. Transparent fatty droplets were dominant in the cytoplasm of the paranchymal cells while the pinkish materials which were seen in the liver section prepared from uninfected mice (Figure 4) seemed to have disappeared resulting in a lesion of fatty infiltration (Figure 5). One or 2 hepatic cells had degenerated and there was evidence of inflammatory cell infiltration suggesting that focal necrosis had occurred (Figure 5). Numerous phagocytic cells were observed inside the sinusoids. The kupffer cells had hypertrophied and appeared to have been fixed in a highly active state; they contained ingested pigments (Figure 5). As a result of progressive phagocytosis, some of these pigments appeared as clumps and were much darker in colour (Figure 6). Phagocytic cells, contained malarial pigments, were evident in the sinusoids and the blood vessels. Mononuclear cells were also observed in the interlobular areas and around the bile ducts suggesting infiltration associated with infection (Figure 7).

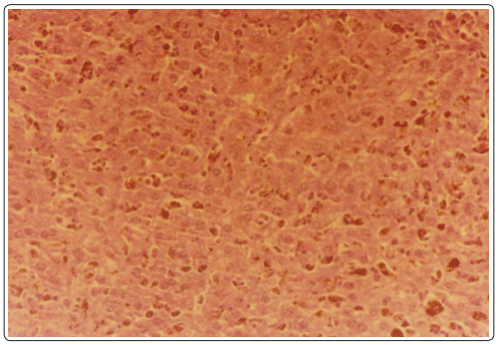

Figure 5: Section of liver from an infected protein-malnourished mouse showing a lesion of fatty infiltration. Focal necrosis

surrounded by inflammatory cells is seen. The hypertrophied kupffer cells contain ingested malarial pigments (X550)

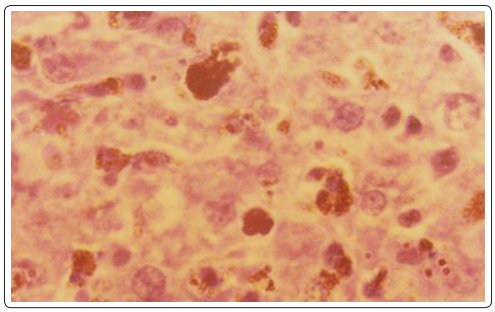

Figure 6: Section of liver from an infected protein-malnourished mouse showing the large, darkly coloured malarial pigments accumulated by progressive phagocytosis (X1375)

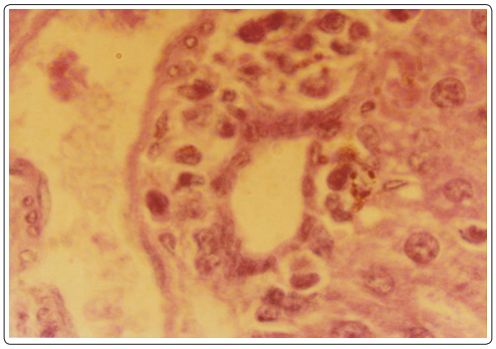

Figure 7: Section of liver from an infected protein-malnourished mouse showing the infiltration of mononuclear cells around the bile duct (X 1375)

Discussion

Spleen enlargement is associated with malarial infection. Lymphopoiesis and erythropoiesis were known to occur in the splenic tissues as a result of the presence of an infectious agent. Due to a massive destruction of the erythrocytes, the spleen was very much involved in the erythopoietic activity, trying to compensate for the numbers of lost erythrocytes (Figure 1). The cellular proliferations as well as the accumulation of the malarial pigments inside the macrophages are signs of the mouse's defense reactions against *P. vinckei*. The severity of the lesions was found to be related to the ability of the host firstly to provide a substrate for the parasite, secondly to damage the parasite in the erythrocytes, and thirdly to substitute the destroyed cells [6].

Splenic cords lie between the sinuses, separating them from one another. The reticular cells are present in the splenic sinuses of red pulp. The blood cells like macrophages are found in sinuses, as are plasma cells. The splenic cords contained lymphocytes and monocytes (T-cells) in high level [7].

Malarial parasites cause the rupture of erythrocytes liberating haematin which is the insoluble breakdown product of haemoglobin. If the infection is cured, the pigment will be processed by the host and will disappear ultimately but in fact can be seen for at least one year [8]. The degree of accumulation of malarial pigments in the spleen's phagocytic cells was found to be directly related to both the level and the duration of parasitaemia [8]. It would seem that the splenic reaction against the infection is marked and thus the parasitaemia was increased in splenectomizedmice. In human malaria, rupture of the spleen is a not uncommon complication and antimalarial therapy should be considered after splenectomy to prevent the development of a severe infection [9]. The usual consequence of malarial infection is tropical splenomegaly syndrome [10].

Hepatic pathological lesions seem to reflect concurrent changes in the spleen. The new and interesting point to note is that the cloudy swelling which occurred in the hepatic cells of the uninfected protein-malnourished mice, developed to a characteristic fatty infiltration in the infected protein-malnourished mice. This was characterized by the accumulation of fat droplets within the hepatic cells. Although this change is reversible, it often implies severe injury and may predict cell death [11]. It would seem that, the injurious influence caused by a lack of protein first induced a primary cellular injury in the term of cloudy swelling followed by a severe cellular injury in the term of fatty infiltration resulted from the *P.vinckeiinfection*. Mean while, focal necrosis was observed. Similarly, focal necrosis has been noticed in the liver of mice fed on standarad protein diet infected with *Plasmodium yoelii* [12].

The response of the liver to *P.vinckei* infection was evidenced by a deposition of the pigments inside the very active hypertrophied kupffer cells. It has been suggested that the endothelial cells lining the liver sinusoids are phagocytic in nature and that they can transform into kupffer cells [13]. In fact, the highest amount of macrophage activity has occurred in the spleen and liver. Hyperplastic Kupffer cells and portal tract inflammation are two main features found in the liver tissues of severe *P. falciparum* malaria cases. In addition, NF-κB is associated with Kupffer cells and lymphocyte apoptosis in severe *P. falciparum* malaria [14].

Mononuclear cells were seen to have infiltrated into the liver. The infiltration of the mononuclear cells around the bile ducts is difficult to explain and has not been described by other workers. Therefore, a further investigation is needed to decide about the nature of this interaction. As a result of progressive phagocytosis the clumps of the pigment within the kupffer cells increased into even larger masses.

As would be expected the most marked changes in the uninfected protein-malnourished mice, were seen in the liver. Fatty infiltration of the liver is a common feature of kwashiorkor in children [15, 16]. In conclusion, mice are suitable model for histopathological studies. The deleterious effects of malaria upon the nutritional status of the host have been confirmed by histopathological investigation. These results would provide a better understanding for malarial disease in protein-malnourished populations.

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