ELECTRON MICROSCOPIC STUDY OF PHAGOCYTOSIS IN HUMAN SPLEEN IN FALCIPARUM MALARIA

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INTRODUCTION

The ability of a host to overcome malaria infection by the phagocytic system is an important mechanism of defence (Taliaferro and Mulligan, 1937) particularly by the spleen (Maegraith *et al.*, 1980; Wyler, 1983). The mechanisms of splenic host defence include both immunological and nonimmunological interaction with parasitized erythrocytes. Morphological evidences of phagocytosis and destruction of malaria parasites by the spleen have been well documented (Taliaferro and Mulligan, 1937; Schnitzer *et al.*, 1973; Pongponratn *et al.*, 1987). The killing processes may be assisted by the altered rheological factors of the infected erythrocytes, which are non-immunological splenic factor (Wyler, 1983). It has been also postulated that parasites avoid circulation through the spleen and thereby avoiding exposure to the antibody produced by spleen cells and destruction by specific and nonspecific clearance mechanisms localized in this organ (Kreier and Green, 1980).

Since the spleen is a complex organ endowed with multiplicity of functions, study of phagocytosis in spleen by electron microscopy will clarify some mechanisms involved in the interaction between the phagocytic cells and the parasitized erythrocytes. We therefore, study the red pulp, which is a reticular mesh through which blood passes to enter the sinusoids and where the removal of damaged and infected cells takes place.

MATERIALS AND METHODS

Case history: The 13-year-old boy from malaria endemic area was admitted to the Bangkok Hospital for Tropical Diseases with a chief complaint of fever for 6 days. Liver and spleen were not palpable. Blood examination revealed 7.3% of ring forms of *Plasmodium falciparum*. Quinine HCl was given intravenously. On the second day the parasitaemia was slightly increased, then gradually decreased to 0.9% on the fourth day with 0.22 haematocrit and 7.69% haemoglobin. The patient developed renal failure and pneumonia and died on the fifth day of hospitalization.

Autopsy was carried out 3 hours after death. The spleen weighed 140g, was mahogany brown, congested and firm.

Small pieces of the spleen were fixed in modified Millonig's phosphate buffered formalin (Carson *et al.*, 1973) at room temperature for two hours followed by the usual

procedures for electron microscopy. The sections were cut with glass knives and stained with uranyl acetate and lead citrate before examination by a Hitachi HU-12A electron microscope.

RESULTS

The spleen cells which showed phagocytic activity were polymorphonuclear leukocytes (Fig. 1a), littoral or endothelial linnig cells (Fig. 1b), reticular cells (Fig. 1c) and mononuclear cells (Fig. 1d). The phagocytic cells had shown high phagocytic activity including the presence of surface processes or pseudopods appearing as flaps and ruffles, fingerlike projections or long delicate irregular extensions.

Phagocytosis was more predominantly associated with cordal macrophages than endothelial cells.

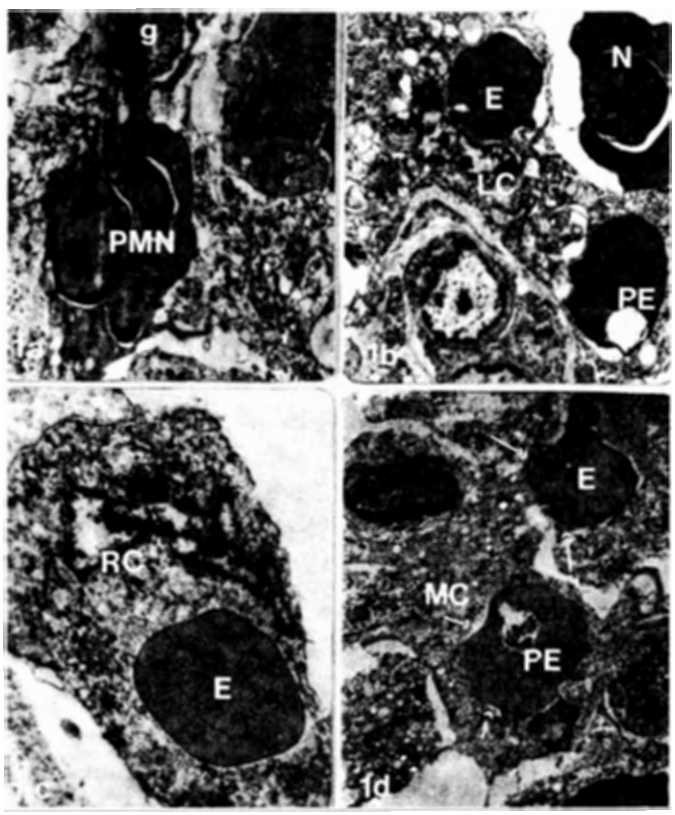

Fig. 1–Phagocytic cells in the spleen

1a). Polymorphonuclear leukocyte (PMN). The cytoplasmic process of PMN was enveloping some granular material (g). × 7,000

1b). Littoral cell (LC). The littoral or endothelial cell lining sinusoid appears to phagocytose a parasitized erythrocyte (PE) and erythrocyte (E). A normoblast (N) is seen nearby. × 6,000

1c). Reticular cell (RC). Phagocytosed erythrocyte (E) in cytoplasm of the reticular cell. × 12,000

1d). A macrophage (MC). A phagocytosed parasitized erythrocyte (PE) lying intact in a single membrane bound vacuole (⟶), long projections from the macrophage also appear to grasp an erythrocyte (E) (⟶⟩). × 6,000

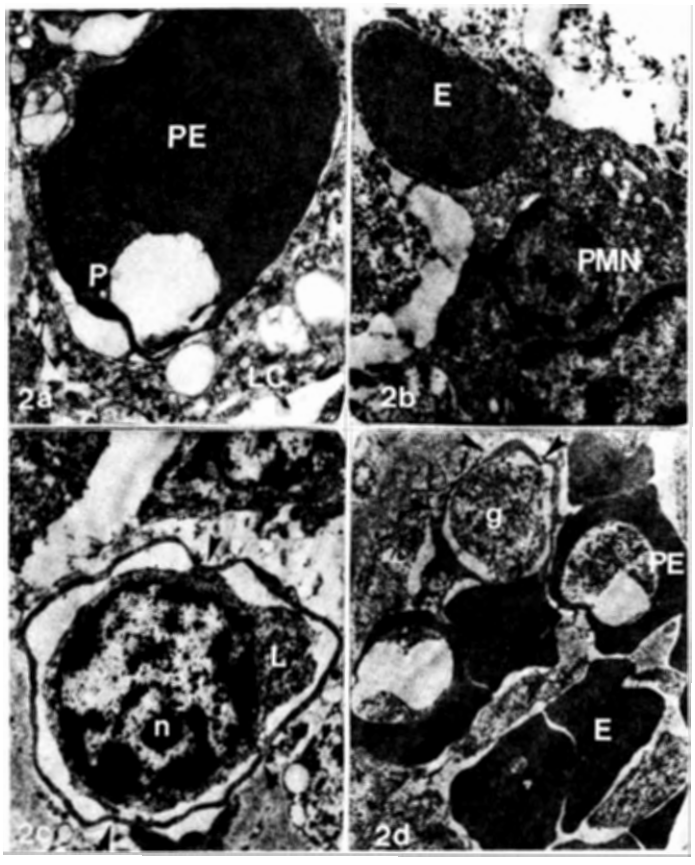

Fig. 2–Phagocytosed materials

2a). Parasitized erythrocyte (PE) is seen phagocytosed by a littoral cell (LC), the intra-erythrocytic parasite (P) posses large food vacuole and the erythrocyte that it infected is devoid of knob. × 15,000

2b). An erythrocyte (E) is phagocytosed by a polymorphonuclear leukocyte (PMN). × 10,000

2c). Long process of a macrophage encircled a lymphocyte (L), Note the points of attachment (arrow). n = nucleus of the lymphocyte. × 10,000

2d). Long process of a macrophage encircled granular material (g) (arrow) among the congested parasitized (PE) and non-parasitized (E) erythrocytes. × 7,000

Parasitized erythrocytes were always seen phagocytosed by phagocytes (Fig. 2a). Ultrastructurally the intra-erythrocytic parasites were mostly yong and growing trophozoites and rarely schizont. The growing trophozoites possessed large food vacuoles with haemozoin pigments inside, the knobs on the surface of the infected erythrocytes were rarely present.

Non-parasitized erythrocytes were also seen phagocytosed in large numbers (Fig. 2b). Lymphocyte (Fig. 2c) and granular materials (Fig. 2d) were occasionally encircled by long processes of phagocytes.

The sequential steps of phagocytosis are depicted in Fig. 3a-3d. Initially the materials were touched (Fig. 3a) then enclosed by their pseudopods of phagocytes followed by engulfment. The completely enveloping processes of the phagocytes could be seen in three forms: the loose enveloping or only encircle (Fig. 3b), the tight enveloping (Fig.

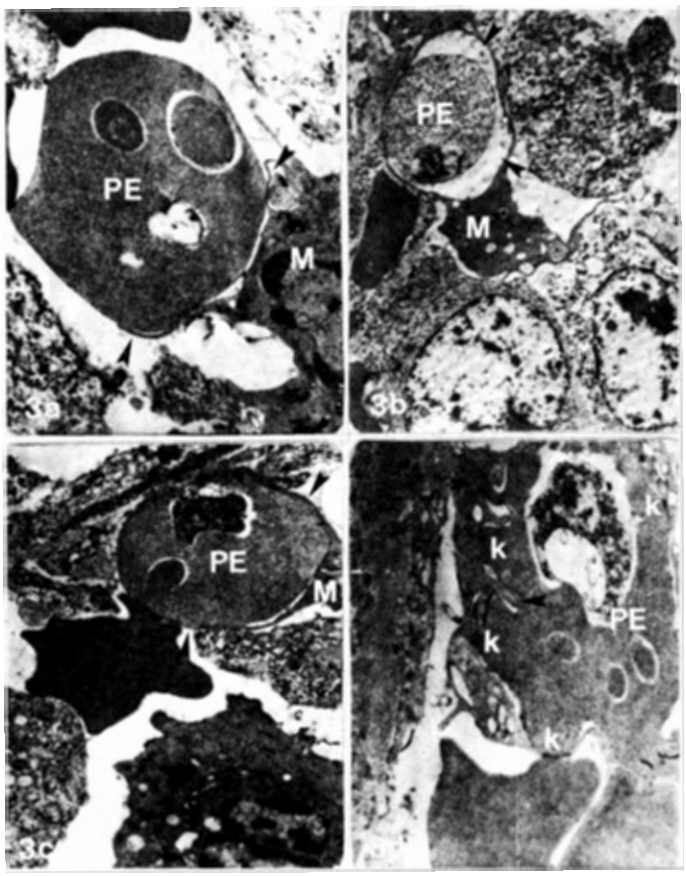

Fig. 3–Phagocytosis

3a). Thin pseudopods of a macrophage (M) appear to touch and grasp (arrow) parasitized erythrocyte (PE). × 10,000

3b). The cytoplasmic process of a macrophage (M) entirely enveloped a parasitized erythrocyte (PE), a wide space exists between the membrane of the macrophage and the parasitized erythrocyte (arrow). × 4,800

3c). The cytoplasmic process of a macrophage (M) entirely enveloped a parasitized erythrocyte (PE), the macrophage cytoplasmic membrane is directly apposed to the phagocytosed membrane of the PE (arrow). × 7,000

3d). Complex interdigitation between short villous processes from the macrophage and the parasitized erythrocyte (PE) (arrow). k = knob. × 12,000

3c) and the complex interdigitation between short villous processes from the phagocyte with indentation in the infected erythrocyte membrane (Fig. 3d).

The most common finding was the complete enclosement of phagocytosed materials by extension of phagocyte cytoplasm.

In many instances, intact parasitized erythrocytes infected with young forms of parasites were enclosed in a single membrane bound vacuole (Fig. 4). These parasitized erythrocytes were knobless.

Pitting of parasitized erythrocyte by two enclosing ends of phagocyte pseudopods was occasionally seen (Fig. 5).

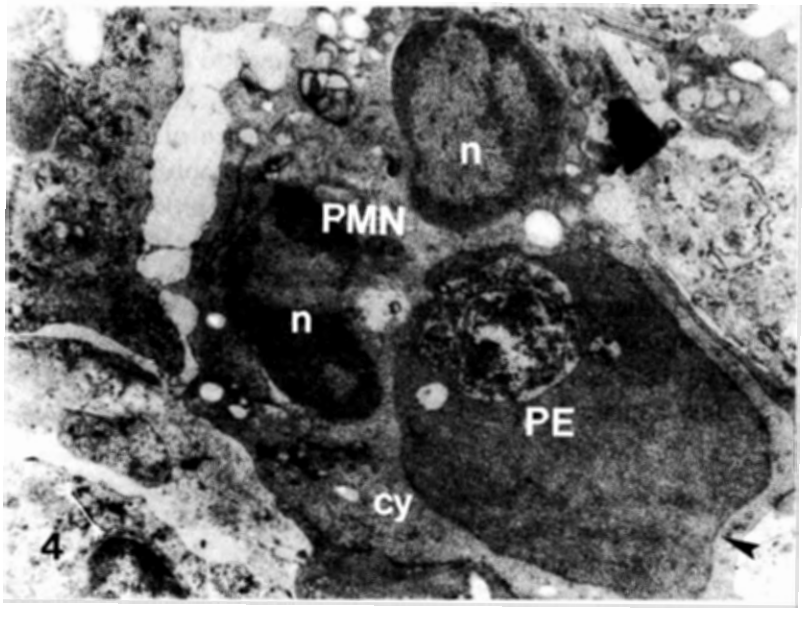

Fig. 4—A parasitized erythrocyte (PE) is phagocytosed intact in the cytoplasm (cy) of a polymorphonuclear leukocyte (PMN), n = nucleus, enclosed in a single membrane bound vacuole which probably lysosome (arrow). × 9,000

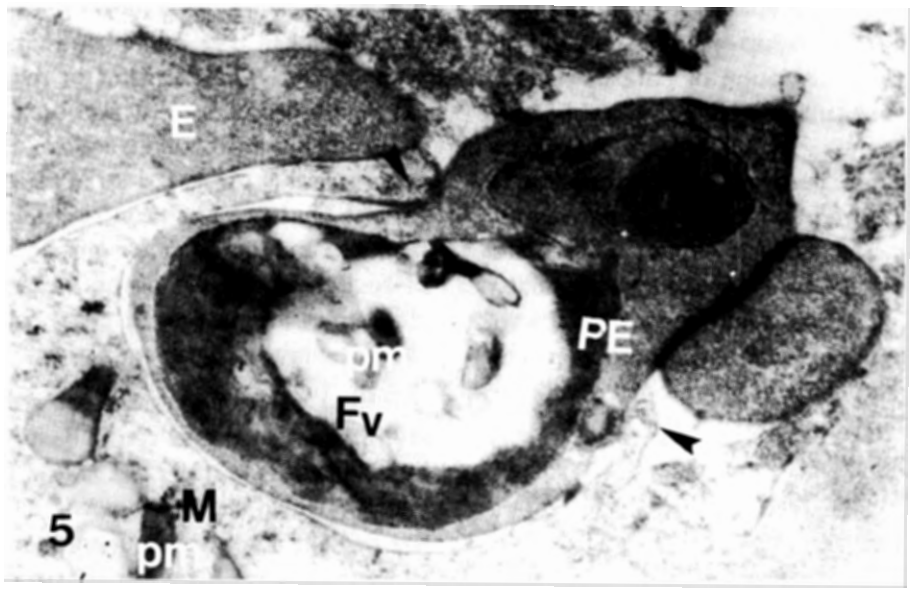

Fig. 5—A parasitized erythrocyte (PE) is being pitted off its parasitized part by two pseudopods of a macrophage (M) (arrow). Pigments (pm) exist in the large food vacuole (Fv) of the parasite and also in the cytoplasm of the macrophage. E = erythrocyte. × 18,000

DISCUSSION

The spleen selectively removes abnormal erythrocytes from circulation (Rifkind, 1965; Simon and Burke, 1970; Schnitzer *et al.*, 1972; 1973). In human malaria, the cells exhibiting phagocytic activity include neutrophils, lymphocytes, endothelial or littoral cells and mononuclear cells (Taliaferro and Mulligan, 1937). In this study, phagocytes in the splenic cords comprising macrophage and polymorphonuclear cells were shown to exert more phagocytic activity than endothelial cells.

Many investigators suggested that altered rheological properties of the parasitized cells (Wyler, 1983) especially their decreased deformability (Miller *et al.*, 1972) may result in their trapping and subsequent phagocytosis by the cordal macrophages in the spleen. Our previous paper (1987) provided additional ultrastructural supportive evidences. We showed in this study that factors other than simple clogging-up or simple recognition of the surface alteration of parasitized erythrocytes may play a role in natural phagocytosis. The time taken for the patient to develop natural immunity to malaria is that it is spleen centred and the killing mechanism has immunological basis (Wyler, 1983). The role of the spleen in protective immunity mediated by splenic macrophages is often an early feature of immune response. The mechanism of immunity depends on the activity of both humoral and cellular factors, although the physiological condition of the host also plays some part (Bruce-Chwatt, 1985).

Cellular factors are related to the activity of two populations of lymphocyte :- T-lymphocyte and B-lymphocyte. T-lymphocytes play an important role in splenomegaly enhanced phagocytosis. Splenomegaly and enhanced phagocytosis have been shown to be T-cell dependent (Roberts and Weidanz, 1978). In Fig. 2c, ultrastructural evidence of a T-lymphocyte was encircled by cytoplasmic process of some phagocytes. This process might be mediated by either reticular cells or cordal macrophages since the irregular network of the red pulp consists of the reticulum and cytoplasmic processes of cordal macrophages which have phagocytic activity. The T-cell-phagocyte interaction needs further study in relation to activation of phagocytic cells during malaria. Humoral and cellular immunity are in many ways interrelated. The T-cells that act on macrophage to induce cell mediated immunity may also help B-cells produce antibody (WHO, 1984).

It is shown in the present study that most of the parasitized cells phagocytosed were young and devoid or scarce of knobs. Some of them appeared in tight (Fig. 3a, 3c) and some in loose (Fig. 3b) phagosomes. Morphologically intact parasites in the cytoplasm of erythrocytes were also seen within a single membrane-bound vacuole of phagocytic cells (Fig. 1d, 4). This is probably the earliest phase of phagocytosis. The role of the loose and tight phagosomes and the sites of sequestration may be an important mechanism in the process of phagocytosis and clearance in the spleen.

Pitting or fragmentation of parasitized erythrocytes in the spleen initially reported by Schnitzer *et al.*, (1972, 1973) in monkeys infected with *P. knowlesi* was confirmed in the present study to occur in man (Fig. 5). We agreed with Schnitzer that such pitted red cells would become spherocytic and were therefor more vulnerable to haemolysis leading subsequently to anaemia.

The finding of more young parasite mostly devoid of knob as well as the scarcity of schizonts and merozoites in the splenic red pulp could be attributable to sequestration of mature trophozoite-infected erythrocytes with knobs in other organs (Desowitz *et al.*, 1969; Miller, 1969; Luse and Miller, 1971; Miller *et al.*, 1971; Gutierrez *et al.*, 1976; Walter *et al.*, 1982; Macpherson *et al.*, 1985; Pongponratn *et al.*, 1985; 1987). Those mature parasite-infected cells which escape sequestration in other organs and find their ways to the spleen will be trapped in the splenic sinusoids and thus could not enter the red pulp proper. We showed that they formed complex interdigitation with the phagocytes (Fig. 3d). Such interaction has been described in the bone marrow of patient with cerebral malaria (Wickramasinghe *et al.*, 1987), the significance of which is still unknown. Lodgement of parasitized erythrocytes in the sinusoids will subsequently lead to blood congestion in the spleen. The rareness of mature parasite-infected erythrocytes in the splenic red pulp can be interpreted to favor the immune evasion of the parasites to their survival advantage. In the case when the spleen is removed for whatever reason, mature parasites that fail to adhere to other organs could then bypass the filtering action of the spleen and thus will appear in the peripheral blood. Thus all stages of parasitized erythrocytes will appear in the peripheral blood (Garnham, 1970).

Anti-*P. falciparum* antibodies have been shown to effect the parasites in several ways including reversal of binding of infected erythrocytes to endothelial cells (David *et al.*, 1983), opsonization and phagocytosis of parasitized erythrocytes by PMN (Celada *et al.*, 1983), and arming of macrophages in the uptake of free merozoites (Khusmith and Druilhe, 1983). Opsonization and phagocytosis may serve not only in the protective immune mechanisms for clearance of infected erythrocytes in individuals living in malaria-endemic areas but may also contribute to immunopathological process such as anemia (Ho *et al.*, 1987). In the present study, the precise role of the anti-*P. falciparum* antibody is not known. It is shown that not only parasitized but also non-parasitized erythrocytes were seen in large numbers in the spleen (Fig. 1b-1d and 2b). Since this patient lived in a malaria-endemic area without the history of taking immunosuppressive drug, it is likely that the amount of antibody produced is insufficient qualitatively or quantitatively to control the infection. Phagocytosis of non-parasitized erythrocytes suggests in addition that these erythrocytes might have been altered morphologically and functionally could be regarded as non-self.

SUMMARY

The ultrastructure of the spleen of a patient who died of natural infection of *Plasmodium falciparum* was studied with emphasis on phagocytosis. Parasitized erythrocytes were shown to interact with the heterogenous populations of phagocytic cells. Phagocytosis occurred predominantly in macrophages than endothelial cells and immature forms of parasites were preferentially phagocytosed. Splenic trapping, pitting and destruction of both infected and non-infected erythrocytes were demonstrated. Other forms of interaction between phagocytic cells and parasitized erythrocytes observed include complex interdigitation, association of loose and tight phagosomes, and preferential sites of adherence, the significance of which need further investigation.

ACKNOWLEDGEMENTS

We are grateful to the staff of the Hospital for Tropical Diseases for their help and the Faculty of Tropical Medicine for financial support. Most indebted to Prof. Savanat Tharavanij for his valuable revising this manuscript.

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