An Ultrastructural Study of the Red Pulp of the Spleen in Malaria

By B. Schnitzer, T. M. Sodeman, M. L. Mead, and P. G. Contacos

An ultrastructural study was undertaken of the spleen of a rhesus monkey infected with *Plasmodium knowlesi* to determine whether the spleen is able to pit malaria parasites from red cells. It was found that in the spleen parasitized cells are: (1) phagocytized in toto by cordal macrophages, (2) pitted of parasites, and (3) hemolysed in the splenic microvasculature. Phagocytosis of the entire parasitized red cell appears to occur most frequently of the three mechanisms and probably accounts for most of the anemia. Pitting of parasitized red cells may account in part for the hemolysis in excess of the number of parasitized red cells seen in malaria. The red cells that have had their parasites removed would become spherocytic and hence, would be more susceptible to removal from the circulation during subsequent passages through the spleen.

The spleen, because of the peculiar microcirculation of its red pulp, is capable of recognizing and removing defective or damaged red blood cells from the circulation. The spleen is also able to "pit" inclusions, such as Heinz bodies or iron granules, from red cells without destroying these cells. Conrad and Dennis suggested, on the basis of studies with transfused chromium-labeled red cells from monkeys infected with *Plasmodium knowlesi*, that the spleen of recipient monkeys removed parasites from the transfused cells without destroying them. In this study, we present morphologic evidence that the spleen of rhesus monkeys infected with *Plasmodium knowlesi* handles parasitized red cells in several ways: (1) by phagocytosis in toto by cordal macrophages, (2) by pitting or fragmenting the parasite-containing portion of the red cells, or (3) by hemolysis of red cells in the splenic microvasculature.

MATERIALS AND METHODS

A rhesus monkey (*Macaca mulatta*) was infected with $4.5 \times 10^5$ parasites of *Plasmodium knowlesi*, H strain. Six days after inoculation, the parasitemia reached 360,000 parasites/cu mm, and the monkey was splenectomized. The splenic tissues from the infected monkey and from a noninfected monkey were fixed for $5 \frac{1}{2}$ hr in 3% gluteraldehyde containing 4% sucrose, buffered with phosphate solution at pH 7.3, washed in phosphate buffer, and postfixed for 1 hr with phosphate-buffered 11/2 osmium tetroxide. The tissue was then dehydrated in a series of ethanols and propylene oxide and embedded.

From the Department of Pathology, The University of Michigan Medical Center, Ann Arbor, Mich. and the Laboratory of Parasitic Diseases, Section of Primate Malaria, National Institutes of Health, Chamblee, Ga.

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B. Schnitzer, M.D.: Associate Professor of Pathology, The University of Michigan Medical Center, Ann Arbor, Mich. T. M. Sodeman, M.D.: Assistant Professor of Pathology, The University of Michigan Medical Center, Ann Arbor, Mich. M. L. Mead: Electron Microscopy Technologist, Department of Pathology, The University of Michigan Medical Center, Ann Arbor, Mich. P. G. Contacos, M.D., Ph.D.: Head, Section of Primate Malaria, Laboratory of Parasitic Diseases, National Institutes of Health, Chamblee, Ga.
Fig. 1. (A). A cordal macrophage contains phagocytized, parasitized red cell (arrow) × 1800. (B). Remnants of phagocytized, parasitized red cells (arrow) are present within cordal macrophage. × 1800. (C and D). Junction of sinus (s) and cord (c). Nonparasitized part of two red cells is in sinus, while parasitized part is trapped in cord. Two parts of red cell are connected by a drawn-out stalk of red cell (arrow); (e) sinus endothelial cell projecting into the sinus. × 1800.
Fig. 2. Large parasite (P) in red cell is trapped in splenic cord (C) and remains attached by a tenuous stalk of red cell to parasite-free part of cell (R) that has passed between basement membrane (Bm) and endothelial cells (En) into the sinus. Thin rim of hemoglobin surrounds parasite. Another parasitized part of a red cell is present in the cord. × 17,600.

in Epon 812. One micron-thick sections were viewed with the light microscope for orientation. Thin sections were cut with a diamond knife on a Porter-Blum MT-2B ultramicrotome, stained with uranyl acetate and lead citrate,7 and viewed and photographed with an RCA EMU-3H electron microscope.

RESULTS

Light Microscopy

The cords of Billroth and, to a lesser degree, the sinuses of the red pulp of the spleen were congested with predominantly parasitized red cells. The macrophages lining the narrow passages of the cords appeared enlarged, and many contained phagocytized, parasitized red cells in various stages of digestion (Fig. 1A, B). Nonparasitized red cells were rarely observed. Parasitized red cells in transit between cords and sinuses were also observed, the
Fig. 3. Doubly parasitized red cell is seen partly within sinus (S) and partly in cord (C). Portion of red cell (R) containing smaller parasite has squeezed through fenestration in basement membrane (Bm) and between endothelial cells (En) lining sinus, while part of cell with large parasite (P) is trapped in cord. Small break (arrow) in the stalk that connected the two parts of the red cell is seen. × 18,300.

parasitized part apparently trapped in the cords (Fig. 1C, D). Sections of spleens from a control monkey showed no congestion, no malaria parasites, and only rarely a phagocytized red cell in the cordial macrophages.

Electron Microscopic Observations

We detected three main ways in which the parasite-containing red cells or the parasites were destroyed in the spleen. Most frequently, the entire parasitized part apparently trapped in the cords (Fig. 1C, D). Sections of enlarged cordal macrophages. Various stages of breakdown of these phagocytized, parasitized red cells could be noted. Some of the parasitized red cells were in the process of being engulfed by cordal macrophages; other red cells with parasites were largely intact within the cytoplasm of macrophages, while still other red cells had undergone digestion and only fragments of
recognizable red cell and/or parasites remained. In some macrophages, malaria pigment and membranes (presumably red cell plasma membranes and parasite membranes) remained as evidence of previous red cell and parasite digestion. Other macrophages contained only malaria pigment that appeared to be relatively resistant to digestion. Phagocytized, nonparasitized red cells were only rarely seen in cordal macrophages in the spleens of both infected and noninfected monkeys.

Parasitized red cells, or parts of red cells containing parasites, appeared to be detained in their passage from cord to sinus via the basement membrane fenestrations and between endothelial or littoral cells lining the sinuses. The nonparasitized part of some red cells could be observed within the sinus, while the parasitized portion remained in the cord apparently unable to pass into the sinus, the two parts of the red cell remaining connected by a long tenuous stalk of hemoglobin (Fig. 2). Cells containing two parasites were also observed in transit from cord to sinus (Figs. 3 and 4). The part of the red cell containing the smaller parasite was usually seen in the sinus; the part of the red cell with the larger parasite appeared trapped in the cord, apparently unable to squeeze through the opening in the basement membrane to the sinus. The narrow stalk of red cell membrane and hemoglobin between the two parts of the cell was often extremely thin and sometimes revealed small fissures and breaks. The stalks of other red cells were severed, and the nonparasitized part of the cell was seen in the sinus, while the part of the erythrocyte containing the parasite had been left behind in the cord (Fig. 5). The parasitized fragments of red cells were sometimes seen in the process of being phagocytized by cordal macrophages (Fig. 4). Some parasitized cells with one or two drawn-out and rounded processes of cytoplasm pointing in the direction of the sinus were seen immediately on the cordal side of the basement membrane (Fig. 4). These rounded parts of red cells appeared to have been severed from another part of the erythrocyte that had passed through into the sinus. Trapped or pitted parts of parasitized red cells were seen at least once at the junction of almost every cord and sinus.

Within the cords and sinuses, ghosts of hemolyzed red cells or red cells in a prelytic state were seen (Fig. 6). Some of these cells still contained a parasite (Fig. 7), while other ghosts of red cells, having lost all their hemoglobin, consisted merely of an empty membrane-bound sac. Free parasites were also occasionally seen in the sinuses, some of these showing evidence of degeneration. Also observed were deformed parasitized red cells in the cords, or red cells that had successfully passed from cord to sinus but had not yet returned to their original shape and still trailed a portion of red cell containing a parasite (Fig. 7). Pitting of parasite-containing parts of red cells by macrophages was occasionally observed in the cord.

**DISCUSSION**

It is well known that following splenectomy, parasitemia in experimental malaria is frequently increased. It is also established that hemolysis in malaria occurs in excess of that expected from the number of parasitized red
Fig. 4. Three parasitized red cells at junction of cord (C) and sinus (S). Parasitized (P) part of red cell remains in cord, while two rounded stalks of red cell reach into sinus between two littoral cells (En), apparently having been severed from another part of red cell. A doubly parasitized (P1) red cell is in transit from cord to sinus, and the larger part is trapped in cord. A third parasitized and pitted part of a red cell (P2) is being engulfed (arrowheads) by cordal macrophage (M). Thin rim of hemoglobin surrounds parasite and forms a projection (arrow) that probably represent region of severance from another part of red cell that passed into sinus. N, nucleus of sinus endothelial cell; arrowheads, processes of macrophage enclosing parasitized and pitted red cell. × 16,000.
Fig. 5. Severed (long arrow) red cell (R), one part having passed into sinus (S), with the parasitized (P) part remaining trapped in cord (C). Break has occurred in thin stalk that lies in the fenestration of basement membrane (Bm) and between two endothelial cells (En). Within the cord are two other parasite-containing pitted red cells, one showing a broken red cell stalk (short arrow). × 11,700.
Fig. 6. Perisinusoidal part of cord containing a parasitized osmotic previllic state. To the right are basement membrane (Bm) and endothelial cells (En) lining sinus (S), × 12,200.
cells in the peripheral blood.\textsuperscript{10} This increased parasitemia after splenectomy can be accounted for by the absence of the phagocytic function of the spleen, a function that contributes significantly to the anemia in malaria. There has been considerable controversy, however, about the possible mechanisms of the development of the excess of anticipated hemolysis. Some of the factors that are known to cause or are suspected of contributing to the excessive hemolysis in malaria are: (1) autoimmune mechanisms directed at either the malaria parasite and/or the red cell,\textsuperscript{11,12} (2) a direct toxic effect of circulating antigen on the red cell,\textsuperscript{13} and (3) the action of the spleen on parasitized and nonparasitized red cells.\textsuperscript{14,15}

Although autoimmunization of the host against its own red cells is a popular and plausible explanation for the excessive anemia in malaria, there is little and largely controversial evidence that malarial antibodies become fixed to red cells to cause their premature destruction.\textsuperscript{14,10} Likewise, evidence for the toxic effects of antigens acting on red cells remains unconvincing.

The ability of the spleen to remove injured or abnormal red cells from
the circulation is well known. The normal biconcave red cell with its excess of surface area to cell volume is very plastic and extremely deformable.\textsuperscript{17} It can, therefore, squeeze through the narrow, circuitous and macrophage-lined cords of Billroth of the spleen and through the small fenestrations\textsuperscript{18} (which may be as small as 0.5 \( \mu \) in diameter) of the basement membrane separating cords from sinuses and then between the endothelial cells lining the sinuses. Abnormal red cells, such as red cells with rigid inclusions (Heinz bodies)\textsuperscript{2,3} or red cells with rigid membranes or contents (sickled red cells, spherocytes),\textsuperscript{19} encounter great difficulty traversing the narrow cords and especially the small basement membrane fenestrations between cord and sinus. Crosby\textsuperscript{4} first presented evidence that iron particles are pitted from siderocytes by the spleen and suggested that other inclusions including malaria parasites\textsuperscript{20} might be removed in the same way. Our ultrastructural observations support Crosby’s suggestion and confirm the functional studies of Conrad and Dennis,\textsuperscript{5} namely that pitting of parasites does occur in the spleen. Pitting or fragmentation of parasitized portions of red cells was seen both within the cords\textsuperscript{8} and, more often, on the cordal side of the basement membrane between cords and sinuses (Figs. 3–5).

The fragmentation of the part of the red cell containing the malaria parasite from its nonparasitized portion may, in part, explain the discrepancy between the degree of hemolysis and the number of parasitized red cells. Pitted or fragmented red cells lose more surface area than volume and, therefore, become spherocytic. Fogel et al.\textsuperscript{21} demonstrated increased osmotic fragility of both parasitized and nonparasitized red cells. George et al.\textsuperscript{22} have shown, by means of osmotic fragility studies, two populations of red cells in malarious animals: one population containing parasitized red cells that had a greater rate of swelling in hypotonic saline than red cells from animals not infected with malaria, and a second population composed of nonparasitized spherocytic cells that were easily lysed in hypotonic saline solution. Such pitted spherocytic cells would be counted on a peripheral blood film as nonparasitized red cells. As spherocytes these cells become rigid\textsuperscript{17} and, therefore, less deformable and more susceptible to hemolysis, especially during subsequent sojourns through the spleen. Thus, ghosts of apparently lysed red cells, which may well have been spherocytes, were at times observed within the splenic vasculature. Additional evidence of lysis of red cells was the observation of free parasites, presumably from hemolyzed, parasitized red cells or from lysed, pitted red cell fragments in the splenic microvasculature.

In addition to its pitting function, the spleen is also active in the phagocytosis of parasitized red cells. Its macrophages possibly recognize parasitized red cells by their injured cell membranes, injuries that might have occurred during parasitization. However, in contrast to the frequent phagocytosis of parasitized red cells, engulfment by cordal macrophages of nonparasitized red cells was rarely observed. It is possible that we did not see more phagocytized, nonparasitized red cells in the spleen because of the relatively high parasitemia in our monkey. The spleen does, nevertheless, play a major role in removing nonparasitized red cells from the circulation. Nonparasitized red cells par-
particularly susceptible to removal in enlarged spleens include cells whose plasma membranes have been injured during pitting, pitted spherocytic cells, senescent cells, and red cells coated with antibody.

A number of factors may be responsible for the anemia in malaria that is in excess of that anticipated from the number of parasitized red cells present. These factors include the pitting function of the spleen, the unfavorable environment, and the increased phagocytic activity seen in enlarged and congested spleens, together with a number of physical, metabolic, and biochemical abnormalities of the parasitized, deparasitized, and non-parasitized erythrocytes.

REFERENCES


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