The Role of the Sinus Wall in the Passage of Erythrocytes Through the Spleen

By Li-Tsun Chen and Leon Weiss

The passage of erythrocytes through the walls of splenic sinuses was studied in rats treated with phenylhydrazine. The sinus wall contained no preformed apertures. Instead, erythrocytes and other cells passing through the spleen entered interendothelial slits and squeezed through. The presence of phenylhydrazine-induced Heinz bodies within erythrocytes impeded their transmural passage. Normally, few erythrocytes were found in transit across the walls of sinuses. However, after phenylhydrazine, many of the interendothelial slits in sinuses were occupied by damaged erythrocytes. Virtually all of the interendothelial slits in sinuses appeared capable of passing erythrocytes. The slits seldom exceeded 0.2-0.5 μ in width, even with an erythrocyte in passage. Bands of intraendothelial microfilaments running alongside the interendothelial slits appeared to have a major role in causing the slits to remain narrow. Thus, the sinus wall, reinforced by microfilamentous bands, significantly controlled the circulation of erythrocytes through the spleen. Normal red cells were pliant enough to squeeze through, but cells containing large rigid inclusions were held in the slits or delayed in their passage. When many of the slits were occupied by cells slow in passage, the outflow track became blocked, and circulation through the spleen was impeded. Therefore, cells pooled in the cords, macrophages increased in number, and the sequences initiated that resulted in splenomegaly.

The mammalian spleen selectively removes certain aged, damaged, or abnormal red blood cells from the circulation. The sequestering mechanisms that are distinctive to the spleen, are believed to depend on its distinctive vasculature and reticulum. The electron microscope studies of the spleen in hemoglobin H disease, after partial splenectomy, and in animals treated with phenylhydrazine strongly suggest the wall of the splenic sinus as a critical site in the control of erythrocyte passage through red pulp. There are no preformed apertures in the sinus wall. Instead, slits between the sinus endothelial cells, which are otherwise closed, are widened while cells pass through them. Even when widened, the slits remain narrow passages, so that an erythrocyte must be pliant in order to squeeze through them.

In this paper, we present electron microscopic findings on Heinz body-bearing erythrocytes crossing the sinus wall in rats treated with phenylhydrazine. We demonstrate the extent of the surface of the sinus wall capable of permitting cellular passage, provide additional evidence that the inter-
endothelial slits in the sinus wall constitute a major basis by which the spleen selectively processes red cells, and make references to the causes of splenomegaly.

MATERIALS AND METHODS

Ninety young male, Sprague-Dawley albino rats obtained from Charles River Breeding Laboratories, Wilmington, Mass. and weighing 240–280 g were used. Acute hemolytic anemia was induced by phenylhydrazine hydrochloride (Eastman Organic Chemicals, Rochester, N.Y.), prepared as a 0.1% solution in sterile saline and administered as a single intraperitoneal injection 0.1 mg/g body weight. Rats injected with saline alone served as controls. Hematocrits, reticulocyte counts, and spleen weights were determined 4 hr, 12 hr, and 1, 3, 5, and 7 days after injection. Five experimental and five control animals were used at each interval.

Fig. 1. Spleen weight and hematocrit of rats following a single intraperitoneal injection of phenylhydrazine (0.1 mg/g). Values indicate mean ± SE. Each point represents a group of five rats. Open circles are obtained from rats treated with phenylhydrazine and closed circles from rats treated with saline alone.
PASSAGE OF ERYTHROCYTES THROUGH SPLEEN

For light and electron microscopy, the spleens, after weighing, were cut into pieces 1 mm thick and were fixed in Karnovsky's fixative in cacodylate buffer, pH 7.2, for 6–12 hr at room temperature. The tissue was then placed in three changes of the buffer for 6–12 hr and was postfixed in 1% OsO₄ in cacodylate buffer for 2 hr at room temperature. The tissues were then dehydrated through graded ethanol, embedded in Araldite, sectioned in a Reichert Ultratome, stained with uranyl acetate and lead citrate, and studied in a Siemens Elmiskop I.

RESULTS

The Pattern of Anemia and Splenomegaly

Following administration of phenylhydrazine, the hematocrit and spleen weight were followed for a period of 7 days (Fig. 1). The hematocrit fell from 45% to 35% as early as 2 hr following phenylhydrazine injection. On day 3, the hematocrit dropped to 25%. The spleen weight increased slightly 4 hr after phenylhydrazine injection, doubled on day 3, reached its highest measurement on day 5, and declined slightly after day 7. Splenomegaly appeared proportional to the number of erythrocytes in both cords and sinuses of the red pulp.

The structure of the red pulp has been described. Briefly, it is composed of cords and sinuses. Arterial capillaries travel and terminate in cords. Sinuses receive blood from cords through the sinus wall. The sinuses are the first segment of the venous outflow track of the spleen through which blood must pass to leave the spleen. From the sinuses blood flows into pulp veins, trabecular veins, and the splenic veins. The sinus wall consists of endothelial cells, basement membrane, and adventitial cells. The endothelial cells are rod shaped, run parallel to the longitudinal axis of the sinus, and appear bound to the ringlike component of basement membrane that runs transverse to the endothelial cells. In the basal part of the endothelial cells, bundles of filamentous bands in sinus endothelial cells of splenic red pulp and the role of these bands in the passage of blood cells through sinus wall. Filamentous bands, composed of microfilaments of 30–50 Å in diameter, lie in the sinus along interendothelial slit in the basal part of the endothelial cell and arch between ring components of basement membrane. Each end of the band appears to insert on plasmalemma overlying basement membrane and is very close to slits. Middle portion of band is a little away from slit while slit is closed. It appears subjacent to slit, separated only by lateral plasmalemma, while slit is widened due to cellular passage. Filamentous bands on either side of sinus slit restrict width of slit during passage of cells from cords into sinuses. Narrow slits play important role in filtering out aged or damaged blood cells from circulation. Drawing was made by Mrs. Ranice W. Crosby.
microfilaments arch between ring components of the basement membrane and run parallel to and along the interendothelial slits (Fig. 2). The slits are covered, as a rule, by either adventitial cells or blood cells. The slits are closed except when penetrated by blood cells, platelets, or macrophages.

**Availability of Interendothelial Slits for Cellular Passage**

In sections of control spleens, few blood cells appeared in the interendothelial slits of the sinus wall. In the experimental rats, many red cells occupied the slits as early as 2 hr after phenylhydrazine injection (Fig. 3). Every interendothelial slit not covered by basement membrane could contain red cells with Heinz bodies. Not uncommonly, red cells appeared squeezed between adventitial cells and the basal (cordal) surface of sinus endothelial cells, presumably en route to the sinus. With increasing time, up to 5 days after injection of phenylhydrazine, more and more red cells were in the cords as well as in the sinuses. The great majority of the sinuses was well packed with red cells. Increasing numbers of macrophages and platelets were also found in the cords.

Fig. 3. Tracing for electron micrograph that appears on the next page.
In phenylhydrazine-treated spleens, red cells in interendothelial slits of

Control of Cellular Passages Through the Interendothelial Slits

In phenylhydrazine-treated spleens, red cells in interendothelial slits of
Fig. 4. Spleen, phenylhydrazine-treated rat, 2 hr after injection. An interendothelial slit occupied by a red cell is shown transversely. Basement membrane (BM) is present on either side of the slit. The microfilamentous bands appear in cross section and are indicated by arrows. Two different cytoplasmic densities of the red cell are noted; the dark portion containing most of the Heinz bodies remains in the cord, while the light portion including few Heinz bodies (HB) appears in the sinus. Hemolyzed red cells (LE) containing Heinz bodies lie in the lumen of the sinus as well as in the cord. E: sinus endothelial cell.
sinuses were numerous and thereby facilitated assessment of the role of the microfilamentous bands in control of cellular passage through the slits. Whenever a red cell squeezed through a slit, the width of the slit (0.2–0.5 μ) appeared limited by the two microfilamentous bands that lay alongside that slit. The length of the slits, 2–3 μ, was the interval between the ring

Fig. 5. Spleen, phenylhydrazine-treated rat, 2 hrs after injection. A red cell R1 containing Heinz bodies (HB) lies in the interendothelial slit. The light portion of the red cell is in the sinus while the dark portion containing Heinz bodies trails. The width of the slit is limited by the microfilamentous bands which are cut transversely and surrounded by the broken lines. Another red cell (R2) is in the cord. E: sinus endothelial cell; BM: basement membrane. × 50,000
components of the basement membrane. The depth of the slits (3–5 μ) was the height of the endothelial cells. The first two parameters were rather constant, and the last one varied with the content of blood cells in the sinus. The height of the endothelial cells was lower in the sinuses well packed with the blood cells than in either constricted sinuses or in sinuses containing few luminal cells. The width of a slit varied somewhat, moreover, being greater on the luminal and abluminal surfaces than at lower third, the level at which the microfilamentous bands were located. (Figs. 2-5). Where slits were closed, the microfilamentous bands were not straight but were rather wavy. They ran a short distance from the lateral plasmalemma forming the boundary of the slit. At their ends, they turned down to the basal plasmalemma. When the slits were widened due to cellular passage (Fig. 2), the whole band along the slit was stretched out without any waviness and appeared very close to the lateral plasmalemma. The slit was narrower and more nearly constant in width in erythrocytic passage than in leukocytic passage.

DISCUSSION

The large number of phenylhydrazine-damaged red blood cells appearing in the interendothelial slits of splenic sinuses strongly suggests that virtually all the slits are potential passageways for cells moving from the cords into the sinuses. Moreover, in the spleen remaining after partial splenectomy in the dog, a large number of interendothelial slits in the sinus wall contain erythrocytes. This further supports the conclusion that virtually all the slits in a sinus wall are available for cellular passage from cords to sinuses. Our results also indicate that injured red cells containing bulky, rigid Heinz bodies have great difficulty in passing through a slit. With a slit blocked or occupied for a long time by a slowly passaging cell, other red cells in the cords must enter other slits in passage into the sinuses. As more and more slits in a sinus wall become blocked by damaged erythrocytes, blood flow out of the spleen would be impeded. Blood cells would gradually accumulate in the cords and contribute to splenomegaly.

Splenomegaly may well result from the combined actions of various factors, in addition to the accumulation of damaged red cells in cords. These additional factors may include the following: (1) an increase in the number of macrophages in the cords consequent to the accumulation of damaged cells. By their bulk, the macrophages may fill the interstices of the cordal reticulum, cover the cordal surfaces of sinuses, enter the interendothelial slits of sinuses and thereby obstruct cellular passage; (2) interference with movement of fluids across the spleen into lymphatic vessels, and thereby interference with lymphatic drainage; (3) splenic erythropoiesis, as occurs with continued phenylhydrazine treatment; (4) a possible direct effect of phenylhydrazine on endothelial cells, interfering with the contractile capacities of the cells and thereby causing red cells to clog the sinuses. This possibility is suggested by Björkman’s observation that epinephrine causes contraction of splenic sinuses in normal spleens but not in phenylhydrazine-treated spleens.

The microfilamentous bands along either side of the sinus slits undoubtedly restrict the width of the slit during the cellular passages. Because of the narrow
slit, cells that are relatively rigid or contain rigid masses will have great difficulty in passing through the slits. Thus, these cells are either removed from general circulation or the rigid body is broken away, with some surrounding cytoplasm and cell membrane to remain in the cords while the remainder of the cell passes through and circulates. Only relatively large, rigid intra-erythrocytic structures are handled in this way. Smaller or more deformable inclusions, as the ferritin particles described by Tanaka, are removed by extrusion or other means. It is at the sinus wall that the removal of the hemosiderin clumps of siderocytes likely occurs. This is the site, moreover, where malarial plasmodia are separated from parasitized erythrocytes along with some host cytoplasm and the parasitized fragments phagocytized. We believe the unique arrangements of the microfilamentous bands and the basement membrane underlie, to a major degree, the unique role of the splenic red pulp in filtering out aged or damaged cells from circulation.

REFERENCES

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