Impaired Endotoxin Detoxification as a Factor in Enhanced Endotoxin Sensitivity of Malaria Infected Mice[1] (35669)

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The fixed macrophages of the reticuloendothelial system (RES) have been reported to be of major importance in natural immunity to malaria (1–5). The liver and spleen, which contain the largest populations of fixed macrophages (6), are organs most intimately involved in a malarial infection. Liver and spleen are also major organs responsible for the removal of endotoxin from the blood (7) and its subsequent inactivation (8). Alteration of RES activity either by glucan, an RES stimulant, or methyl palmitate, an RES depressant, has been shown to increase or decrease, respectively, host sensitivity to endotoxin (9). Similarly, other RES stimulating agents such as *Mycobacterium bovis* BCG cells (10) or zymosan (11) have been demonstrated to induce endotoxin hypersensitivity.

Since RES activity has also been shown to be increased in a malaria infection as evaluated by colloidal carbon (3, 12, 13) or ^{125}I microaggregated human serum albumin (5) and since stimulation of the RES is an event usually associated with endotoxin hypersensitivity (9–11), the susceptibility of malaria-infected mice to endotoxin was initially evaluated. The observed enhancement in endotoxin susceptibility, coupled with the development of procedures to measure endotoxin detoxification (8), prompted a study on the influence of malaria infection on endotoxin detoxification ability of liver and spleen.

Materials and Methods. Animals. White male mice (Carworth Farms), weighing 20–25 g, were used in all experiments.

Endotoxin. The endotoxic polysaccharide of the Boivin type prepared from *Salmonella enteritidis* was obtained from Difco Laboratories, Detroit, Michigan (LPS B, 599008).

Actinomycin D. Actinomycin D (Meractinomycin) was prepared in sterile 0.9% sodium chloride solution at a concentration of 500 μg/ml.

Malaria. Mice were injected intraperitoneally (ip) with 1×10^7 *Plasmodium berghei*-infected syngeneic erythrocytes. Endotoxin susceptibility was tested 5 days after the inoculation of the parasite and was determined as the proportion of animals dead 24 hr after ip challenge with various doses of endotoxin. Experimental animals had a mean percentage parasitemia of 25.6% on day 5.

Endotoxin inactivation by liver and spleen. As previously described (8, 14), the bioassay of endotoxin was based on the 100,000-fold sensitization to lethal endotoxic shock induced in mice by actinomycin D administration. Homogenates of mouse liver and spleen were prepared by previously described procedures (8). In essence, 1% (w/v) homogenates of liver and spleen in 0.01 *M* sodium phosphate buffer were prepared from normal and malaria-infected mice. The homogenates (4.0 ml) were incubated with 1.0 μg (0.5 ml) of LPS at 37° for 3 hr. Following incubation, 0.5 ml of actinomycin D (250 μg) was added to each flask. Each normal mouse was then injected ip with 0.5 ml of this preparation.

Control groups consisted of those animals which received either homogenate preparations incubated with isotonic saline rather

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than endotoxin or those which received a preparation of LPS incubated with phosphate buffered saline.

The chi-square test was employed to determine significance which was set at the 95% confidence level.

Results and Discussion. The presence of *P. berghei* infection profoundly enhanced the lethality of *S. enteritidis* endotoxin. The administration of 100 μg of LPS, which produced no mortality in normal mice, was associated with an 82% mortality in malaria-infected mice (Table I). Normal mice receiving 500 μg of LPS had a 27% mortality at 24 hr. This is in marked contrast to the 100% mortality observed in malaria-infected mice. Sensitivity to endotoxin is also indicated by the finding that a dose of 25 μg of LPS induced, in the malaria-infected mice, a 50% mortality, while a 4-fold increase of this dose administered to normal mice did not result in any mortality. The LD_{50} dose in the malaria-infected mice was 25 μg (1 mg/kg) of endotoxin in contrast to an LD_{50} of 1020 μg (40.80 mg/kg) in the normal noninfected mice, indicating an approximate 41-fold enhancement in endotoxin susceptibility in malaria-infected mice.

To determine if alterations in hepatic or splenic endotoxin detoxification ability were responsible for the increased sensitivity to endotoxin, liver and splenic homogenates from malaria-infected and normal animals were assayed for endotoxin detoxification ability (8). In essential agreement with the observation of Trejo and Di Luzio (8), who used rat liver and spleen homogenates to inactivate endotoxin, a significant ($p<.001$) endotoxin detoxification ability was present in mouse liver and spleen homogenates. The incubation of endotoxin with liver and spleen homogenates prepared from normal mice reduced endotoxin induced mortality approximately 50% compared to the mortality observed in the control group which received endotoxin preparation incubated in buffer (Table II).

TABLE I. Effect of *P. berghei* Infection[a] on the Lethality[b] of *S. enteritidis* Endotoxin.

Group	Endotoxin (μg)	Deaths/ total	Mortality (%)
Normal	100	0/11	0
	200	1/10	10.0
	500	3/11	27.3
	1000	5/12	41.7
	1200	10/10	100
Malaria	—	2/20	10.0
	25	5/10	50.0
	50	8/10	80.0
	100	9/11	81.8
	200	9/10	90.0
	500	10/10	100.0

[a] Normal mice received 1×10^7 *P. berghei*-infected erythrocytes intraperitoneally, 5 days prior to endotoxin challenge.

[b] Mortality recorded at 24 hr following endotoxin administration.

The splenic hyperplastic reaction in malaria infection, which results in considerable loss of lymphoid tissue and degeneration of cord cells (15), did not modify the endotoxin detoxification ability of the spleen. The induced mortality of mice which received endotoxin, which was incubated in splenic homogenates of malaria-infected mice, was comparable to that observed with splenic preparations of normal mice. However, when endotoxin was incubated with liver homogenate prepared from malaria-infected donors, the protective effect of liver homogenates was abolished. Indeed, livers from malaria-infected mice showed a complete absence of endotoxin detoxification ability as mortality patterns were comparable to that observed when endotoxin was incubated with buffer.

Malaria infection has been demonstrated to be associated with hepatic structural and functional alterations. Hepatic circulatory disturbances result in congestion and centrolobular cellular changes (15). The congestion may result from parasitic-induced changes in erythrocytes and from enlarged, parasite-laden, swollen macrophages, either free or lying in hepatic sinusoids (15).

Focal necrosis of the liver and distortion of normal hepatic architecture has been shown to occur during a malaria infection with a concomitant depletion of fat, glycogen, and other materials (16). In addition to the aforementioned disturbances, increases in serum glutamic pyruvic transaminase and

TABLE II. Effect of *P. berghei*[a] Infection on Detoxification of *S. enteritidis* Endotoxin by Liver and Spleen Homogenates.[b]

Treatment	Tissue homogenate	Endotoxin (0.1 μg)	Actinomycin D (25 μg)	Deaths/ total	Mortality (%)
None	Buffer	+	+	26/29	89.7
	Liver	+	+	12/29	41.4
	Liver	—	+	1/27	3.7
	Spleen	+	+	13/29	44.8
	Spleen	—	+	1/27	3.7
Malaria	Liver	+	+	11/14	78.6
	Liver	—	+	1/12	8.3
	Spleen	+	+	5/14	35.7
	Spleen	—	+	0/12	0

[a] Normal mice received 1×10^7 *P. berghei*-infected erythrocytes intraperitoneally, 5 days prior to the preparation of liver and spleen homogenates.

[b] 4.0 ml of 1% liver and spleen homogenates incubated with 1.0 μg (0.5 ml) of endotoxin in saline at 37° for 3 hr. After incubation, 0.5 ml (250 μg) of actinomycin D was added, and 0.5 ml of the mixture was administered ip to normal mice. Mortality was recorded 48 hr following the administration of the preparation.

serum glutamic oxalacetic transaminase, hypoglycemia, reduced alkaline phosphatase and albumin levels, increased bromsulfalein retention and positive cephalin fluocculation reactions have also been observed (17). Ultrastructural alteration, consisting of decreases in the amount of smooth endoplasmic reticulum (SER) of liver cells in malaria-infected mice, has been associated with an impairment in drug metabolism (18).

The contribution of ultrastructural alterations (18) or functional alterations of hepatic cells (17) to the observed impairment in endotoxin detoxification in malaria-infected mice cannot presently be ascertained.

Since endotoxin detoxification has been reported to be a function of Kupffer cell lysosomes (19), the inability of liver of malaria-infected mice to detoxify endotoxin would suggest a lysosomal defect. The impairment in endotoxin detoxification could be the result of lysosomal enzyme inhibition or depletion due to the previously ingested materials, either cell or parasite in nature. The accumulation of hemozoin in the phagolysosomes (20) could be a contributing factor.

The present findings indicate that malaria infection is associated with a state of endotoxin hypersensitivity due to an impairment of endotoxin detoxification by liver. The enhanced phagocytosis and reduced endotoxin inactivation observed in malaria-infected mice appears to parallel those results observed in lead acetate- (8) and glucan- (21) treated animals in which a hyperphagocytic state was associated with an endotoxin hypersensitivity, and loss of detoxification ability. A pattern, therefore, appears to be emerging where enhanced phagocytosis and increased sensitivity to endotxin is associated with an impaired ability of liver to detoxify endotoxin. The enhancement in endotoxin susceptibility in malaria infection may under certain conditions be a factor in malaria-induced mortality.

Hepatic dysfunctions associated with a malaria infection are related mainly to hepatic parenchymal cell activity (16). Since endotoxin detoxification has been shown to be a property of hepatic Kupffer cells and not of parenchymal cells (19), this study adds a new dimension to disturbances in liver function associated with a malaria infection, *i.e.*, an inability to detoxify endotoxin.

Summary. The influence of *P. berghei* infection on endotoxin susceptibility and detoxification in white mice was studied. Malaria-infected mice showed a 41-fold increase in endotoxin susceptibility. Liver and spleen homogenates from normal control animals possessed a significant endotoxin detoxification ability. In contrast, liver homogenates

prepared from malaria-infected mice possessed no significant endotoxin detoxifying ability. The endotoxin detoxifying ability of splenic homogenates prepared from malaria-infected donors was, however, normal.

These findings indicate that endotoxin hypersensitivity in malaria-infected mice may be due to an impairment in hepatic endotoxin detoxification. The loss of endotoxin detoxification ability by liver adds another dimension to hepatic functional alterations in malaria.

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