differences. Staphylococcal penicillinase activity was inhibited by both homologous and heterologous antiserum. *B. cereus* penicillinase was neutralized to lesser degree by the heterologous antiserum.

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Malaria Induced Endotoxin Tolerance. (29820)

MELVIN RUBENSTEIN, JOHN H. MULHOLLAND, GEOFFREY M. JEFFERY AND SHELDON M. WOLFF (Introduced by Vernon Knight)
Laboratory of Clinical Investigations and Laboratory of Parasitic Chemotherapy, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

Hyperreactivity of the reticuloendothelial system (RES) has been considered to be one of the major mechanisms of endotoxin tolerance, the state of resistance to the pyrogenic properties of endotoxin induced by a series of injections(1). Diminished febrile reactivity to endotoxin, noted in patients recovering from malaria, who are known to develop hepatosplenomegaly, the major organs of the RES, has been cited as supporting evidence for this theory(2). This evidence was based primarily on a report that the febrile response to injection of heat killed typhoid vaccine was less marked in neurosyphilis patients recently treated with malaria than in untreated controls(3). In addition, experimental malaria in mice has been reported to induce increased RES activity as measured by the rate of clearance of colloidal carbon particles(4).

We have recently had the opportunity to study febrile reactivity to a well characterized endotoxin in the same subjects before and after induction of experimental malaria. Volunteers, inoculated with malaria parasites, who developed hepatosplenomegaly but no parasitemia, were studied in addition to those who developed clinical malaria. Endotoxin tolerance was observed in those subjects who did develop malaria. In addition, a preliminary report on the functional capacity of the RES is presented.

Materials and methods. Eight normal white male federal prisoner volunteers, age 21 to 37, comprised the study group. Four of the subjects were inoculated with *Plasmodium cynomolgi* (M. strain) by mosquito bites; the remaining 4 were inoculated with *Plasmodium vivax* (Chesson strain), 2 by mosquito bites and 2 by intravenous administration of blood from infected patients. After inoculation Giemsa stained thick and thin blood films were examined daily for malaria parasites. A minimum of 750 oil immersion fields was inspected before concluding that a preparation was negative.

All patients were examined daily by the same physician and particular attention was paid to the possibility of hepatosplenomegaly. Rectal temperatures were taken at least 4 times daily.

Subjects with *P. vivax* malaria were treated with chloroquine and primaquine after 2-3 weeks of illness while treatment was not instituted in the patients inoculated with *P. cynomolgi* until all endotoxin studies were completed. Endotoxin was administered to the patients with *P. vivax* malaria before

TABLE I. Febrile Response to Endotoxin Before and After Malaria.

Patient	Inoculation	Clinical illness	Parasitemia	FI before malaria	FI after malaria
WR	*P. vivax*	Typical	Yes	63*	21
RA	"	"	"	54*	10
ED	"	"	"	39	16
RL	"	"	"	25	25
LR	*P. cynomolgi*	"	" (rare)	56*	14
DM	"	No fever; transient hepatosplenomegaly; thrombocytopenia	No	21*	35*
JG	"	No fever; hepatosplenomegaly	"	40*	34
JC	"	None	"	75*	55*

* Chills and/or nausea.

inoculation and within one week after institution of therapy. In patients WR and RA (Table I) 21 days elapsed between the premalaria and postmalaria endotoxin studies while in RL and ED the interval was 35 days. Patients WR and RA were given endotoxin a third time 21 days after their postmalaria endotoxin study. Prior to the second challenge with endotoxin all 4 of these patients had been treated with chloroquine and primaquine.

In a similar experiment endotoxin was given to 4 men prior to and 36 days after inoculation with *P. cynomolgi,* but before antimalarial therapy had been instituted.

Lipexal®, an endotoxin preparation derived from *Salmonella abortus equi,* has been used extensively in human studies and offers the advantage of eliciting highly reproducible febrile and subjective responses in the same subjects tested at adequate intervals (*ca.* 21 days)(5). This endotoxin was diluted in pyrogen-free physiological saline immediately prior to use; each volunteer received 2.5 mμg per kilogram of body weight. All subjects remained in bed and were allowed food and water as desired; rectal temperatures were taken half-hourly for one hour prior to and 7 hours following endotoxin injection; blood pressure and pulse were measured hourly. Endotoxin was not given if the baseline temperature was greater than 37.5°C. The area in cm^2 under a 7-hour fever curve was measured with a compensating polar planimeter and recorded as the fever index (FI).

Using I^{131} labeled aggregated human serum albumin Iio and Wagner were able to measure the functional capacity of the RES in man (6). These same techniques were employed to measure the activity of the RES before and after *P. cynomolgi* inoculation. RES function was expressed as the time required for the patient to clear 50% of the injected material circulating at 5 minutes (t ½). From the 2 dosages of protein injected, 0.025 and 5 mg/kg, the maximum rate of clearance (*V. max*), *i.e.,* the phagocytic capacity of the RES, was determined.

Results. All 4 subjects inoculated with *P. vivax* developed a syndrome of acute malaria with parasitemia, thrombocytopenia(7), rigors, fever and hepatosplenomegaly. In 3 of these 4 patients, the febrile response to endotoxin after malaria was unequivocally diminished when compared to the premalaria response (Table I). When endotoxin was given to WR and RA on a third occasion, 21 days after the diminished febrile response had been observed, the FI had risen to 40 cm^2 in WR and in RA it had returned to its baseline value of 53 cm^2.

Results were not as consistent in the patients who were inoculated with *P. cynomolgi,* however, and as can be seen in Table I only one patient (LR) developed malaria. Two patients (DM and JG) developed hepatosplenomegaly but no parasitemia and no fever. Transient thrombocytopenia was observed in both LR and DM. No clinical or laboratory changes compatible with malaria were noted in subject JC.

In LR, the only patient who developed unequivocal *P. cynomolgi* malaria, the fever

TABLE II. Clearance of I^{131} Aggregated Albumin Before and After Inoculation with *P. cynomolgi* Malaria.

Patient	Days after inoculation					
	−1	0	10	22	25	36
LR	3.0 *	3.0		3.0		2.9
	6.6 †	6.0	6.2	6.3	6.1	6.7
	.89‡	1.06		.97		.84
DM	3.0	2.7				3.4
	6.4	7.0	6.0	6.1		8.2
	.94	.75				.67
JG	2.8	3.0				3.3
	8.4	6.9	7.8	6.7		8.1
	.58	.82				.67
JC	2.8	3.1				3.2
	7.7	6.6	7.2	6.2		7.2
	.66	.91				.82
Mean ± S.E.	2.9 ± .08	2.95 ± .12				3.2 ± .15
	7.2 ± .67	6.6 ± .32	6.8 ± .42	6.3 ± .19		7.5 ± .51
	.77 ± .12	.88 ± .09				.75 ± .07

* t ½ of .025 mg/kg dose. † t ½ of 5 mg/kg dose. ‡ V max.

index following endotoxin administration decreased from 56 cm^2 (premalaria) to 14 cm^2 (postmalaria). DM and JG who developed hepatosplenomegaly, but no fever and no parasitemia, and JC who experienced no clinical change after inoculation, did not exhibit tolerance, and, in fact, DM developed an increased febrile response (Table I). Although patient JC did exhibit a fall of FI from 75 cm^2 to 55 cm^2, it is to be emphasized that both of these values represent significant febrile responses (peak temperature rises of 2.7°C and 2.3°C respectively), and that an FI of 55 is a considerably greater response than that seen in tolerant individuals.

During the study there was no change in functional capacity of the RES in the 4 individuals inoculated with *P. cynomolgi* (Table II). This finding is especially noteworthy in that 3 of the patients (LR, DM and JG) developed hepatosplenomegaly and one of these (LR) exhibited tolerance to the postmalarial injection of endotoxin.

Discussion. The only study of which we are aware describing diminished febrile reactivity to endotoxin in subjects with malaria is that of Heyman and Beeson, who studied patients given malaria for therapy of asymptomatic neurosyphilis(3). In their experiments, typhoid vaccine was administered to postmalaria patients and the febrile response during the ensuing 8 hours was compared to that of control neurosyphilis patients who had not received malaria. Mean FI in the 16 control subjects was 148 vernier units as compared to 63 units in 20 postmalaria patients. This decreased reactivity noted in the postmalaria group was interpreted as having a similar mechanism to that of tolerance induced by a series of endotoxin injections, *i.e.*, hyperfunction of the RES.

We have confirmed the above observations using a different experimental model. Our subjects were normal adult volunteers without evidence or history of significant illness. The experiment was designed so that pre- and postmalaria endotoxin studies were performed on the same individual. In addition, a purified endotoxin with an established reproducible dose-fever response relationship was used. The time lapse between the endotoxin studies, a minimum of 21 days, precluded the possibility that the diminished reactivity following malaria was induced by the first endotoxin injection. In addition, in 2 patients (RA and WR) endotoxin was administered a third time, 21 days later, and both subjects had marked increases in FI with one returning to his baseline value.

Since hepatosplenomegaly is a common manifestation in malaria, a suggested mechanism for diminution of febrile reactivity to endotoxin following malaria has been an increase in the rate of endotoxin clearance due to hyperreactivity of the RES(3). This hy-

pothesis is supported by the observations of increased rates of clearance of colloidal carbon particles in mice infected with murine malaria(4). However, we are unaware of any previous studies of the functional capacity of the RES in humans with malaria; and, although preliminary because of the small number of subjects, the data reported here raise questions which merit further investigation. Despite the presence of hepatosplenomegaly in 3 of 4 subjects in whom the functional capacity of the RES was measured, no increase was noted in rate of clearance of aggregated albumin. These findings indicate that an increase in size of these major organs of the RES is not necessarily accompanied by enhancement of phagocytic capacity as measured by clearance of aggregated albumin. Furthermore, the fact that LR had clinical malaria as well as endotoxin tolerance, but no change in clearance of aggregated albumin, would suggest that endotoxin tolerance is not necessarily accompanied by increased RES activity in man. This supports the observations of Greisman *et al* who demonstrated that changes in the functional capacity of the RES did not accompany the development of classical endotoxin tolerance in man(8).

These observations raise some questions about the mechanism of tolerance to endotoxin induced by malaria. Since the antigens of *Plasmodia* are not identified, one might speculate that these organisms possess polysaccharide structures similar to those known to be characteristic of the gram-negative bacteria. It is well known that febrile tolerance developed with one endotoxin is reflected in tolerance to others as well(1). The possibility follows therefore that the tolerance noted after malaria is a result of exposure to endotoxin-like substances derived from the parasites. It has recently been suggested that a macroglobulin antibody plays a major role in the development of tolerance to the febrile effect of endotoxin(9). Should this prove to be the case, then the presence of common antigens in *Plasmodia* and gram-negative bacteria might provide yet another explanation for endotoxin tolerance following malaria. Furthermore, the recent demonstration that macroglobulin levels are significantly elevated during the course of malaria in man(10), suggests the possibility that malaria is associated with either specific or nonspecific elevations in "natural antibodies" to gram-negative organisms. These antibodies are produced in increased quantity after endotoxin injection and are known to be of the macroglobulin type(11). However, it is emphasized that the role of the RES and of antibody in the development of endotoxin tolerance is still unresolved and the subject of intensive study.

Summary. The febrile reactivity to endotoxin before and after malaria was studied in normal human volunteers. Four of the 5 subjects who developed malaria showed unequivocal diminution of febrile response. Two of 3 other inoculated subjects developed hepatosplenomegaly, but none of these 3 had parasitemia, nor did they exhibit endotoxin tolerance. Preliminary studies in the latter 3 men and one other who had malaria and developed endotoxin tolerance revealed no changes in the phagocytic capacity of the reticuloendothelial system. Some of the possible mechanisms of malaria induced tolerance are discussed.

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Differentiation of Pulmonary Congestion and Edema in the Rat, Induced by Epinephrine, and Their Modification by Various Procedures.* (29821)

KERMIT A. GAAR, JR. AND LLOYD D. SEAGER
Department of Pharmacology, University of Arkansas Medical Center, Little Rock

Various techniques have previously been designed to measure shifts of blood in the pulmonary circuit and to attempt to determine the relative degrees of pulmonary edema and congestion when present. The methods used in this study were designed to determine how the changes in wet lung weight were related to the directional shifts of blood within the pulmonary circuit and the relative extent of pulmonary edema formation. The modification of these parameters by several agents was studied.

Methods. Sprague-Dawley albino rats usually weighing over 400 g were divided into 9 experimental groups of 8 animals per group. A tracheotomy was performed on each animal after anesthesia with ether or pentobarbital (40-50 mg/kg). Pressure was monitored from the femoral artery using a polyethylene cannula connected to a Statham pressure transducer and a Sanborn recorder. Epinephrine, 0.01%, was infused through a femoral vein at a rate adjusted to give maximum mean systemic arterial pressures for each rat, free of irregularities. The length of infusion varied from 25 to 35 minutes for each group of animals during which time approximately one milliliter of solution was infused. At the end of each experiment, the animals were sacrificed by removing the arterial cannula to allow hemorrhage and opening the chest to prevent intrathoracic pressure. The lungs were immediately removed and their wet weight recorded. Drying of the lungs was accomplished by placing them under a lamp at a temperature of 50°C until a constant weight was attained.

Some of the experiments were modified by giving each animal a single intraperitoneal dose of atropine sulfate (5 mg/kg) 10 to 20 minutes prior to beginning the epinephrine infusion. In other experiments a Physiograph small animal respirator was used to supply an inspiratory pressure of 15 cm of water at a rate of 70 inspirations per minute and an inspiratory-expiratory ratio of 1:2, respectively; expiration was passive.

Wet lung weight to animal body weight ratios and wet lung weight to dry lung weight ratios were calculated for each experimental animal. These ratios were then totaled for each experimental group and the arithmetic mean and standard deviation (SD) were calculated for each group.

Computations. An accepted method for determining the extent of pulmonary edema and congestion is the index of the wet lung weight to body weight ratio(1). Various increments above control level in this ratio indicate a progressive increase in degree of pulmonary edema and/or congestion. It is often difficult to separate the relative extent of the two conditions. Guyton and Lindsey(6) used a method for determining relative increases in the amount of edema fluid formation in the lungs. Using wet lung weight to dry lung weight ratios they were able to establish an index of edema fluid formation. They based

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