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ANTIOXIDANTS IN PLASMA FROM MICE INFECTED WITH PLASMODIUM VINCKEI

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SUMMARY: The late stage of infection of mice with the malarial parasite Plasmodium vinckei was accompanied by significant changes in the content of most antioxidants within plasma. The plasma concentrations of uric acid and vitamin C increased, in contrast to those of vitamin E and total plasma proteins, whilst the activity of superoxide dismutase did not change significantly. In contrast to the situation within erythrocytes, the ratio of partly oxidized forms of vitamin C (dehydroascorbate and diketogulonic acid) to reduced ascorbic acid failed to decrease as a result of malarial infection. These results are consistent with earlier findings and add to the idea that malarial infection may result in oxidative tissue damage.

Antibody-independent immune mechanisms play a significant role in the protection of the host against acute malarial infection (1,2) and the involvement of mononuclear phagocytes in this process seems likely (3). Phagocytic cells are known to release highly reactive oxygen radicals upon stimulation (4) and these molecules, produced chemically or by immune cells, have recently been shown to kill intra-erythrocytic parasites under experimental conditions in vivo or in vitro (5,6). Furthermore, these toxic forms of oxygen are thought to inhibit parasite growth in some host red blood cell variants in which the antioxidant protection is insufficient (7,8), a hypothesis which implies that parasitized red blood cells are exposed to oxidative stress during the natural course of a malarial infection. A number of studies have investigated the changes in the antioxidant capacity of erythrocytes that occur following their invasion by

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different strains of malarial parasites (9-13). In general, the stability of reduced glutathione and ascorbic acid within these cells is enhanced rather than diminished, a finding which is at variance with the expected presence of oxidative damage within parasitized erythrocytes and which is difficult to reconcile with the reported accumulation of oxidation products of hemoglobin within the latter (14). However, little information is available about possible changes to the antioxidant capacity of plasma obtained from malarious subjects, although this may be of importance in limiting the extent of the oxidative damage to endothelial cells and tissues that is often associated with malarial pathology (15). Therefore, we measured the activities of the most significant antioxidants within plasma from control and P. vinckei-infected mice.

MATERIALS AND METHODS: Hydrated 2,4-dinitrophenylhydrazine was obtained from Flucka, tributylcitrate from Merck; ascorbic acid from BDH, xanthine oxidase and cytochrome c from Boehringer, uric acid, xanthine, vitamin E acetate and ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine] from Sigma. Divicine was synthesized by Dr W.B. Cowden (Canberra, Australia). All chemicals used were of analytical grade.

Male CBA/CaH mice, 6-10 weeks old, were infected intraperitoneally with 10^6 red cells containing the malarial parasite Plasmodium vinckei subsp. vinckei. Blood from control or infected mice, the latter showing parasitemias > 80%, was collected into sodium heparin (20 units/ml of blood) and the plasma obtained after centrifugation of the blood (2200 x g, 10 min, 4°C).

Vitamin E was determined fluorimetrically (16). Analysis for vitamin C alone was performed as described (17) while that for ascorbic acid and its oxidation products was carried out differently (18). Prior to both analyses, samples were gassed with carbon monoxide as described elsewhere (13). The activity of superoxide dismutase within plasma was estimated on the basis of its ability to inhibit xanthine/xanthine oxidase-dependent reduction of ferricytochrome C (19). Uric acid was determined by HPLC-analysis (20) using a Waters Model 441 Absorbance Detector connected to a Waters Data Module (Model 730). Uric acid was separated on a 30 cm x 2.9 mm C18 "μ Bondapak" column (Waters, 5 μm particle size) and detected using a UV-filter with maximum transmission at 313 nm. Plasma protein was determined according to the method of Lowry et al. (21).

RESULTS AND DISCUSSION: Infection of mice with P. vinckei affected the plasma content of individual antioxidants within plasma in an apparently idiosyncratic, rather than consistent, manner: the concentrations of uric and ascorbic acid increased significantly, whilst those of total protein

Table 1. Contents of antioxidants in plasma obtained from control and P. vinckei-infected mice

Antioxidant	control	infected
uric acid (μg/ml)	8.7 ± 4.1 (32)	17.5 ± 7.9* (31)
ascorbic acid (μg/ml)	7.6 ± 0.9 (11)	11.7 ± 2.5* (7)
superoxide dismutase (units/ml)	12.7 ± 0.9 (5)	12.3 ± 1.5 (5)
vitamin E (μg/ml)	2.4 ± 0.6 (17)	1.3 ± 0.4* (12)
protein (mg/ml)	63.7 ± 1.1 (19)	61.9 ± 3.1* (32)

Results represent mean values ± S.D. with numbers of determinations in parenthesis. The asterisks indicate values significantly different from those found in the control plasma (for all, $P < 0.005$).

and vitamin E decreased and there was no change in the activity of superoxide dismutase (Table 1).

The most striking change observed was a doubling in the plasma concentration of uric acid (Table 1), a metabolite formed during the degradation of purines. This is in agreement with the observation that malarial infection of rats is accompanied by increases in the activity of nucleic acid-degrading enzymes within liver, spleen and kidney and that the malarial parasite, which is itself incapable of de novo purine biosynthesis, utilizes this host-derived supply (22). Amongst the enzymes which show increased activity during infection with P. berghei is xanthine oxidase (22), which catalyses the formation of uric acid from hypoxanthine and xanthine with concomitant production of $O_2^{\bullet -}$. The increased plasma content of uric acid in P. vinckei-infected mice could also be a response to increased oxidative stress, as suggested by Ames et al. (23).

The significant increase in the plasma concentration of ascorbic acid, an efficient scavenger of $O_2^{\bullet -}$ (24), parallels its higher level in P. vinckei-infected red blood cells (13). However, in contrast to the situation within parasitized erythrocytes, where the ratio of oxidized to reduced forms of vitamin C is decreased (13), the ratio within the plasma, which may also correlate with the degree of oxidative stress (25), increased slightly (Table 2). Within erythrocytes, the concentration of

Table 2. Total and relative levels of vitamin C in its reduced and oxidized forms, dehydroascorbic acid and diketogulonic acid, in plasma obtained from control and heavily-parasitized mice

SAMPLE	AA µg/ml (%)	DHA µg/ml (%)	DKA µg/ml (%)	TOTAL µg/ml (%)	DHA + DKA / AA
control plasma	4.24 ± 0.82 (58.4)	2.96 ± 0.41 (40.8)	0.06 ± 0.12 (0.8)	7.26 ± 0.69 (100)	0.71
infected plasma	5.43 ± 0.59* (56.8)	3.99 ± 0.61* (41.8)	0.13 ± 0.29 (1.4)	9.55 ± 0.57* (100)	0.76

Results represent mean values ± S.D. from five independent experiments, of which each represents pooled plasma from 6 (control) and 5 (infected) mice, respectively. The asterisks indicate values significantly higher than those found in control plasma. ($P < 0.05$ for AA; $P = 0.01$ for DHA; $P < 0.005$ for total ascorbic acid).

AA: reduced ascorbic acid; DHA: dehydroascorbic acid; DKA: diketogulonic acid.

reduced glutathione approximately doubles from 1 to 2 mM as a result of malarial infection (9,11,12) and this may be associated with increased redox cycling of ascorbic acid and maintenance of the more reduced steady-state (13). Glutathione, at a concentration about 100-times smaller in mouse plasma than that within red cells (26), is not an important antioxidant in plasma and may not be readily available for redox cycling of ascorbic acid. Also, it is unlikely that the increase in plasma ascorbic acid is due to its enhanced hepatic biosynthesis by the murine host, since this activity is actually decreased during malarial infection (Stocker, et al., submitted for publication). Other factors, such as increased uptake of the partly oxidized form of vitamin C, dehydroascorbate, by parasitized erythrocytes, followed by its intracellular reduction (13) and subsequent release into the circulation, may be responsible for the increased amount of plasma ascorbic acid (27) and its protection against oxidation.

Vitamin E is an important inhibitor of oxidative peroxidation through its own radical-chain breaking activity as well as through its interaction with other antioxidants like ascorbic acid (28). At late stages of malarial infection the plasma concentration of vitamin E decreased significantly (Table 1). This is unlikely to be a direct

Table 3. Changes in vitamin E content in plasma obtained from mice subjected to the indicated treatment

	treatment			
	none	saline inject.	divicine inj.	bleeding
Vit. E [µg/ml]	2.41 ± 0.60 (17)	3.93 ± 0.37* (10)	5.44 ± 1.29* (14)	4.61 ± 0.58* (8)

Control mice were treated daily for seven days by intraveneous injection of either 150 µl of divicine (25 µg/kg) or 0.9% (w/v) saline or by bleeding (150 µl) from the tail vein. Results represent mean values ± S.D. with numbers of determinations in parentheses. The asterisks indicate values significantly different than those observed in untreated controls (for all, $P < 0.005$).

consequence of infection-induced oxidative stress, since injection of divicine, an agent which generates hydrogen peroxide in the presence of oxygen (29), caused a significant rise in plasma vitamin E (Table 3). A similar effect, although less pronounced, was achieved by repetitive bleeding of the mice from the tail vein or by intravenous injection of saline (Table 3), suggesting that part of these changes may be a response to stress. Vitamin E exchanges rapidly between plasma and red cells and it has been suggested that conditions of lowered hematocrit and plasma lipid concentration, which occur in P. vinckei infection, favour erythrocytic localisation of vitamin E, thus lowering its concentration in the plasma (13, 30).

In a recent report, Wayner et al. (31) showed that uric acid, vitamin C and vitamin E together contribute only 27-47% of the total peroxyl radical-trapping capacity of human plasma and that the remaining, previously unrecognized, portion of the antioxidant activity can be attributed largely to plasma proteins, particularly albumin. The late stage of malarial infection of mice with P. vinckei resulted in a significant decrease in total plasma protein (Table 1). Although small, this decrease may be of quantitative importance in reducing the total antioxidant capacity of mouse plasma, an argument strengthened by the finding that, during the active stages of the disease, the relative

concentration of albumin tends to fall (15) while that of hemoglobin increases significantly as a result of extensive hemolysis (32). Hemoglobin can act as a "Fenton" reagent, having the potential to catalyze hydroxyl-radical formation in the presence of superoxide anion or hydrogen peroxide (33). Furthermore, the increase in stimulus-induced chemiluminescence by peripheral blood leukocytes observed at high parasitemia (32) may indicate that the activated oxygen species necessary to drive the Fenton-reaction are indeed present at this stage of malarial infection.

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