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Oxygen radical release by adherent cell populations during the initial stages of a lethal rodent malarial infection

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Summary. A series of experiments was carried out to assess the levels of reactive oxygen intermediates (ROI) released by macrophages and monocytes during an acute malarial infection, and to consider the importance of oxidant-induced parasite killing in host protection. Adherent cell populations were removed from the peritoneum and spleen of BALB/c and B10/D2/n mice between Days 0–5 of a *Plasmodium yoelii nigeriensis* infection. These cell populations were quantified, characterized and their ROI-releasing capacity was measured by following ferricytochrome *c* reduction upon stimulation with phorbol myristate acetate (PMA). Both strains of mice displayed higher numbers of macrophages and macrophage precursors as the infection progressed; this rise was more marked and accompanied by splenomegaly in BALB/c mice. A concurrent decrease in peritoneal cell numbers was observed. Splenic adherent cell populations released much lower levels of ROI than peritoneal macrophages upon triggering. The levels of ROI released from BALB/c splenic adherent cells rose gradually until Day 3, when the parasitaemia was slightly decreased. In contrast, splenic populations from B10 mice had a decreased capacity to release ROI, particularly after Day 3, when the parasitaemia rose sharply. In further studies, electron microscopy was used to detect H_2O_2 release during the *in vitro* interaction of peritoneal macrophages and parasitized erythrocytes. Cerium chloride staining techniques demonstrated that H_2O production was not dependent on phagocytosis or the presence of immune serum, although levels were increased by the presence of the latter.

Abbreviations: HBSS, Hanks' buffered salt solution; H_2O_2, hydrogen peroxide; NCS, newborn calf serum; O_2^-, superoxide anion; PMA, phorbol myristate acetate; ROI, reactive oxygen intermediates; SOD, superoxide dismutase; SRBC, sheep red blood cells.

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INTRODUCTION

There is a great deal of evidence to suggest that erythrocytes parasitized with malaria are highly susceptible to damage by oxidant stress (Friedman, 1979). Recent studies have demonstrated the *in vitro* killing of rodent Dockrell & Playfair, 1984) and human (Wozencraft *et al.*, 1984) malaria parasites by reactive oxygen intermediates (ROI) released by enzyme-substrate systems. Macrophages, monocytes and neutrophils release ROI under suitable conditions of activation and triggering, and it is therefore important to consider their role as effector cells in parasite killing during an immune response. During rodent malarial infections, increased numbers of these cells can be detected in the peripheral bloodstream, the liver and the spleen, and contact with parasitized erythrocytes may be increased (Allison & Eugui, 1983).

Ockenhouse & Shear (1984) demonstrated that the *in vitro* killing of *Plasmodium yoelii* 17XL by peritoneal macrophages resulted from the release of ROI, and that this killing was not dependent on phagocytosis or cell contact.

In the present study, a rodent malaria model has been used to quantify ROI release *in vitro* from peritoneal macrophages and splenic adherent cells removed during the initial stages of a lethal infection. These cell populations were removed from two strains of mice, which differed in their capacity to control parasite numbers during the initial stages of a *Plasmodium yoelii nigeriensis* infection. The cells were quantified, characterized and their capacity to release ROI was measured by following ferricytochrome *c* reduction. In addition, the release of hydrogen peroxide (H_2O_2) during the *in vitro* interaction of peritoneal macrophages and parasitized erythrocytes was examined by electron microscopy utilizing enzyme staining techniques. The findings have been considered with respect to the relevance of oxidant-induced killing during rodent and human malarial infections, and the influence of other cells involved in the immune response.

MATERIALS AND METHODS

Parasite maintenance

The rodent malaria parasite *P.y.nigeriensis* was derived from LUMP No. 1766 (London School of Hygiene and Tropical Medicine) and maintained by blood passage in 6-week-old outbred mice (A. J. Tuck and Son Ltd, Battlesbridge, Essex). Male BALB/c and B10/D2/n (6-week-old, OLAC 1976 Ltd) were used for experiments concerning ROI release. All mice were infected by the i.p. inoculation of 1×10^6 infected erythrocytes, and the parasitaemia followed daily by the preparation and examination of Giemsa-stained tail blood films (expressed as the percentage infected cells from 2000 examined under × 100 oil immersion).

Preparation of peritoneal macrophage populations

Mice were killed, and peritoneal lavage carried out using 5 ml ice-cold RPMI-1640, containing 1 mU/ml heparin. Following harvesting, the cells were centrifuged at 500 ***g*** for 8 min, washed twice with RPMI-1640 and resuspended in RPMI-1640 supplemented with 10% newborn calf serum (heat-inactivated; NCS), 20 mM L-glutamine, 100 μg/ml steptromycin and 100 U/ml penicillin to a final concentration of 5×10^5/ml (all reagents from Gibco Ltd, Paisley, Renfrewshire). Finally, the cells were plated out in 1-ml quantities onto sterile 13-mm coverslips placed in the wells of 24-well tissue culture plates (Linbro, Flow Labs, Irvine, Ayrshire), and incubated at 37° with 5% CO_2 for 2 h. Non-adherent cells were then removed by thorough washing, and the remaining cells were incubated for a further 24 h.

Preparation of splenic macrophage populations

Spleens were removed, placed in sterile petri-dishes with 5 ml cold RPMI and gently teased to release a single cell suspension. This suspension was centrifuged at 4°, 500 ***g*** for 5 min, resuspended in 3 ml erythrocyte lysis solution (0·15 M NH_4Cl, 9 mM $KHCO_3$, 1mM EDTA) and incubated on ice for 3 min. After this, 7 ml ice-cold PBS (pH 7·4) were added dropwise, the cells were recentrifuged and the pellet resuspended in RPMI-1640 supplemented as above, but with an increased (20%) NCS concentration to give a final concentration of 5×10^7/ml. The cells were plated onto coverslips in 24-well tissue culture plates. Following a 2-h incubation period, the non-adherent cells were removed from each well by pipetting the contents several times and replacing the supernatant with fresh medium. For quantification, the adherent cell population was harvested by the addition of 0·5 ml Versene (Flow Labs) to each well. Following 15 min incubation at 37°, the supernatant was harvested by pipetting, and more Versene was repeatedly added over the plating surfaces. Finally, the supernatants were pooled, spun at 500 ***g*** for 5 min, and the cell yield was quantified by haemocytometer counts.

Characterization of macrophages within adherent cell populations

Fc receptor characterization. This was carried out by the detection of SRBC adherence and uptake in the presence of specific rabbit antisera, using the method of Crofton, Diesselhoff-Den Dulk & van Furth (1978). SRBCs were opsonized with specific antibodies at a subagglutinating titre, washed three times with PBS, and subsequently 5×10^6 were added to each test well. Following 30 min incubation at 37°, 5% CO_2, the adherent cells were washed, methanol-fixed and Giemsa-stained. The percentage of cells with attached or internalized SRBC was evaluated.

Phagocytosis. Phagocytic functions of the cells were assessed by the non-specific uptake of heat-killed *Candida albicans*. The organisms were washed three

times with PBS, resuspended in PBS to a concentration of 5×10^6/ml and added in 1-ml quantities to the test wells. Following 1 hr incubation at 37°, the cells were washed, fixed and stained as above. The percentage of cells which had phagocytosed one or more organisms was recorded.

Peroxidase staining. The technique described by Page & Garvey (1979) was used to detect neutrophil and monocyte peroxidase.

Esterase staining. Qualitative assessment of non-specific esterase staining was carried out by the addition of 5μg/ml fluorescein diactetate to each well. Following 5 min incubation, the extent of green fluorescence within the treated cell populations was assessed by examination under a Vickers UV microscope.

Qualitative assessment of ROI release. The ability of the adherent cells to undergo an oxidative burst was measured by the superoxide anion (O_2^-)- dependent reduction of NBT to insoluble formazan (Wilson, Tsai & Remington, 1980). One-hundred μl of 1 mg/ml NBT solution was added to each well, and the respiratory burst was stimulated by the addition of 100 μl of a 2·5 μg/ml PMA solution (both reagents from Sigma, Poole, Dorset). Following 1 hr incubation (as outlined above), the cells were washed, methanol-fixed and counter-stained in 0·2% safranin for 3 min before examination.

The measurement of ROI release by adherent and peritoneal macrophage populations

The ROI-releasing capacity of B10 and BALB/c mice was assessed between Days 0 and 5 of a *P.y.nigeriensis* infection. This technique was based on that of Johnston, Godsik & Cohn (1978). Cells were plated 24 h previously, as outlined above. The cells were then washed thoroughly with PBS, and 0·45 ml 80 μM ferricytochrome *c* (Sigma) prepared in 1 × HBSS (phenol-red free; Gibco Ltd) with 0·01 M phosphate buffer at pH 7·4 was added to each well. Duplicate test wells were additionally given: (i) 25 μl of 10 μg/ml PMA solution (Sigma) and 25 μl HBSS (phenol-red free); and (ii) 25 μl of 10 μg/ml PMA and 25 μl of 200 U/ml superoxide dismutase (SOD; provided by Sigma). Control wells received (i) 50 μl HBSS only; (ii) 50 μl HBSS, in the absence of adherent cells. The plates were then incubated at 37°, 5% CO_2 for 1 hr. The supernatants were then removed from each well, added to 1·5 ml ice-cold HBSS and the absorbance of each sample was read between 540 nm and 560 nm using a Pye-Unicam 1200 spectrophotometer with a chart recorder attachment. Finally, the contents of each well were washed three times with PBS, 0·1 ml 0·1 M NaOH was added and a Lowry protein estimation was carried out. The ferricytochrome *c*-containing supernatants were read against the ferricytochrome *c* control incubated in the absence of macrophages, using a light path of 1 cm and a slit width of 3 mm. The peak height at 550 nm was calculated for each sample, and ferricytochrome *c* reduced calculated using the extinction coefficient $2{\cdot}1 \times 10^{-4}$/M/cm.

Detection of H_2O_2 release by electron microscopy

Hydrogen peroxide release by peritoneal macrophages was detected by following the formation of cerium perhydroxide, using the electron microscopy method outlined by Briggs, Karnovsky & Karnovsky (1975). Peritoneal macrophages were harvested from normal and infected BALB/c mice on Day 3 of a *P.y.nigeriensis* infection. The cells were plated in 0·5 ml volumes at a concentration of 5×10^5/ml into the wells of Lab-Tek slides (Miles Labs Ltd, Slough, Berks). Following 24 hours incubation at 37°, 5% CO_2, the cells were washed once with RPMI-1640 and overlaid with 0·5 ml RPMI-1640 and supplements containing (i) 5×10^5 normal mouse erythrocytes; (ii) 5×10^5 mouse erythrocytes parasitized with *P.y.nigeriensis* at various stages of development (separated by method outlined by Knight & Sinden, 1982); (iii) 5×10^5 parasitized erythrocytes and 1:1000 titre of hyperimmune serum (see below); and (iv) medium only. Additional wells were pretreated for 30 min with 0·5 ml RPMI containing 5 μg/ml cytochalasin B (Aldrich Chem. Co. Ltd, Gillingham, Dorset). After 1 hr incubation, the macrophages were washed briefly in 0·1 M tris-maleate buffer and 7% sucrose at pH 7·5, 4°. They were then preincubated for 10 min in buffer containing 1mM 3-amino 1, 2, 4-triazole. Finally, they were incubated for 10 min at 37° in buffer containing 1 mM cerium chloride, 10 mM 3-amino 1, 2, 4 triazole and 0·71 mM NADH at pH 7·5 (all reagents from Sigma). Duplicate control wells without $CeCl_3$ and NADH were included in each test. After 10 min, the cells were again washed in tris-maleate buffer and fixed in 3% (v/v) glutaraldehyde in 0·066 M cacodylate buffer at pH 7·4 for 1 hr, followed by post-fixation for 1 hr at 1% (w/v) OsO_4 in 0·066 M cacodylate buffer. The cells were dehydrated through a graded ethanol series, and the wells filled with Epon 812 (Araldite, EMscope Ltd, Ashford,

Kent). After allowing the samples to polymerize overnight, the glass slide at the base was removed and the remaining blocks from each well were sawn into 1-mm squares. Finally, monolayers were cut and the sections viewed using a Joel 100cx electron microscope at 80 kV without further staining.

Preparation of hyperimmune serum

BALB/c mice infected with *P.y.nigeriensis* were treated with 15 mg chloroquine/kg body weight/day for 14 days. Additional uninfected mice were treated the same way. After 14 days, both groups of mice were rechallenged with 1×10^6 parasitized cells. Only the previously infected mice were partially protected, indicating that residual levels of chloroquine were not present. IFAT tests were carried out using the serum to indicate the presence of specific antibodies.

RESULTS

Macrophage migration

The mean percentage parasitaemia during the initial stages of a *P.y.nigeriensis* infection in (i) BALB/c, (ii) B10/D2/n mice is summarized in Fig. 1. From Day 4 onwards, the parasitaemias of the latter strain were found to be significantly higher, culminating in death from Day 7 onwards. The accompanying changes in total spleen cell numbers (as assessed by the spleen teasing technique), and in adherent cell numbers in the peritoneum and spleen during the first 5 days of infection are given in Fig. 2. The levels of peritoneal macrophages had similar profiles for both species: on Day 3, the levels dropped, indicating possible migration of the cells to other areas. The lower parasitaemias found in BALB/c mice correlated to a signifi-

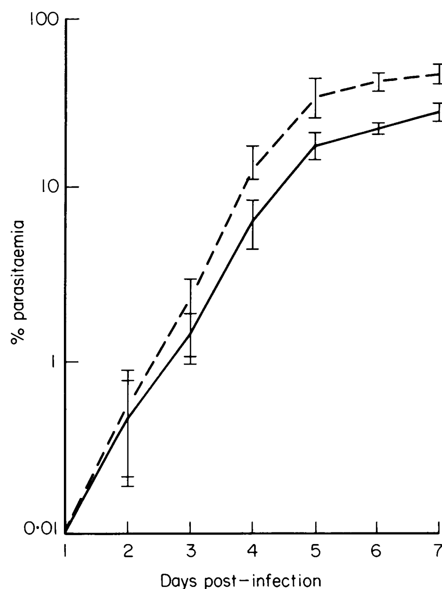

Figure 1. The initial course of a *Plasmodium yoelii nigeriensis* infection in BALB/c (————) and B10/D2/n (— — — —) mice (percentage parasitaemia represented in log cycle). Values given are the means of six test groups containing three mice. Parasitaemias were significantly higher from Day 4 onwards for B10/D2/n mice, as determined by Student's *t*-test (95% confidence limits).

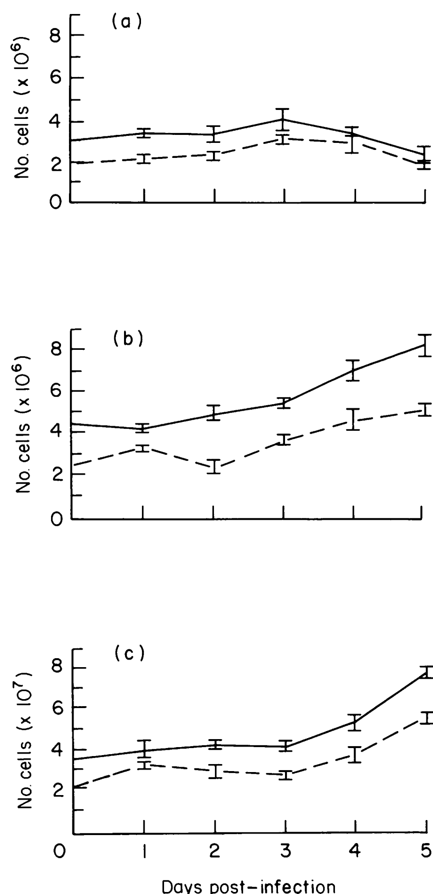

Figure 2. Adherent cell numbers in (a) the peritoneum, (b) spleen and (c) total spleen cell numbers in a BALB/c (————) and B10/D2/n (— — — —) mouse during a *P.y.nigeriensis* infection. Values given are the means of six experimental groups, each consisting of three mice. Total spleen cell numbers and splenic adherent cell numbers were significantly higher from Day 3 onwards for BALB/c, as determined by Student's *t*-test (95% confidence limits).

cantly higher degree of total cellular influx and adherent cell numbers in the spleen from Day 3 onwards. In addition, a much larger increase in spleen size was observed in BALB/c mice.

Macrophage characterization

The average percentage of cells expressing Fc receptors, and phagocytic functions, was high for most adherent cell populations investigated. Between 88% and 98% of the peritoneal cell populations studied were positive in these tests on all days of infection. Splenic adherent cell populations initially contained only 70–78% cells positive in both characterization tests, but this level increased to 86–96% by Days 3–5 of infection for both strains of mice, reflecting increased maturation and activation. Monocytes and neutrophils, characterized morphologically and by peroxidase staining, were only detected in spleen cell populations. Their numbers rose in both strains of mice as the infection proceeded from 5–7% to 15–18%. The levels of both cell types were seen to rise, although this increase was predominantly in monocyte numbers. Strong esterase staining was observed for all populations studied, confirming the presence of large numbers of monocytes and macrophages.

Peritoneal cell populations maintained a 90% capacity to undergo a respiratory burst, as measured by NBT reduction. In contrast, 70–80% of splenic adherent cells from BALB/c mice displayed NBT reduction between Day 0 and Day 5. The reducing capacity of splenic populations from B10/D2/n mice dropped from 68–81% at the start of infection to 40% on Days 4 and 5. NBT staining also gave an indication of the capacity of individual cells to release ROI. A gradual increase in formazan deposit was observed in both peritoneal and BALB/c splenic adherent cell populations as the infection progressed, although levels were consistently greater in peritoneal cells from both strains of mouse. During the latter stages of the characterization studies, parasite pigment was observed in splenic adherent cells removed from BALB/c and B10 mice; these cells displayed low levels of NBT reduction. No fibroblast contamination was evident in the splenic populations examined in these studies.

Ferricytochrome *c* reduction

The release of O_2^- from peritoneal macrophages removed from the peritoneum and spleen of the two strains of mouse during the initial stages of a *P.y.nigeriensis* infection is represented by Figs 3 and 4. O_2^- release was expressed in terms of ng ferricytochrome *c* reduced per mg protein, as cell numbers could vary between different test wells, and this provided a suitable method of standardization. Test wells contained 40–199 μg protein, suggested by Johnston *et al.* (1978) to be the optimal levels for ROI release. Peritoneal macrophages from both strains (including those removed from uninfected mice) demonstrated relatively high levels of O_2^- release, rising to a peak on Day 4 for BALB/c and Day 3 for B10 mice. Splenic adherent cells removed uninfected mice released only 10–20% of O_2^- released by peritoneal cells removed from the same animals: however, these levels rose sharply by Day 3 for BALB/c mice, whereas B10 mice appeared to display only a small increase by Day 2. On Days 3 and 4, the splenic adherent cells from the former strain released significantly higher levels of O_2^- than B10 populations. These findings reflected those of the characterization experiments in which B10 splenic cells had a decreased capacity to reduce NBT,

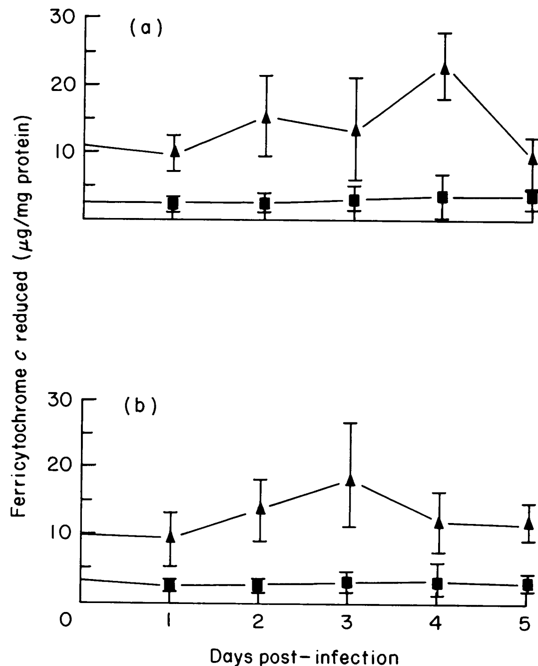

Figure 3. The release of O_2^- from peritoneal macrophages removed from (a) BALB/c mice and (b) B10 mice following (HBSS (■————■) and PMA (▲————▲) treatment. Values given are the means of six experiments, each including duplicate coverslips for test and control groups.

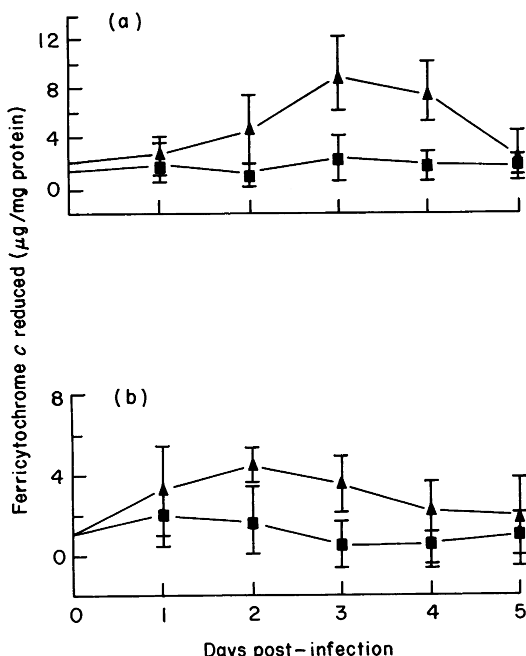

Figure 4. O_2^- release from splenic adherent cells removed from [4·1] (a) BALB/c mice and (b) B10 mice following HBSS (■— — — —■) and PMA (▲— — — —▲) treatment. As in Fig. 3, values given are the means of six experiments.

particularly after Day 3 of infection. Throughout these experiments, PMA was used as a soluble trigger of the immune response. Initial attempts were made to use purified parasite antigen preparations for triggering, but contaminating pigments gave absorption peaks corresponding to those of reduced ferricytochrome *c* and they were therefore unsuitable. In all cases, the addition of SOD to test wells eliminated any ferricytochrome *c* reduction observed, indicating that the reaction was solely a consequence of O_2^- production.

Cerium chloride staining

The results of this series of experiments are illustrated in Fig. 5. During the phagocytosis of erythrocytes by peritoneal macrophages removed from infected mice, cerium perhydroxide deposit could be detected along the plasmalemma at the points of interaction. This clearly demonstrated the localization and extent of activation of the NAD(P)H oxidase responsible for H_2O_2 generation (Briggs *et al.*, 1975). In the absence of $CeCl_3$ or NADH, no deposit was visible (Fig. 5a). It was observed that parasitized cells were preferentially phagocytosed (Fig. 5b), but even when phagocytosis was inhibited by cytochalasin B treatment, phagocyte-erythrocyte interaction led to the release of H_2O_2 (Fig. 5c). The addition of immune serum to test wells resulted in an increase in the close attachment and uptake of parasitized erythrocytes, and therefore an increase in the number of macrophages seen to have undergone a respiratory burst. The resulting layer of cerium perhydroxide deposit between the erythrocyte and the phagosome membrane is shown in Fig. 5d. However, the levels of H_2O_2 release by individual cells did not appear to be greater in the presence of immune serum. In addition, PMA-stimulated macrophages were included as positive controls. PMA treatment resulted in very high levels of H_2O_2 release.

Macrophages removed from uninfected mice were also included in these experiments. These cells were also found to phagocytose parasitized and normal erythrocytes at low levels, although uptake (which was increased upon immune serum addition) was not always associated with H_2O_2 release. Even after PMA stimulation, cerium perhydroxide deposits were found to be at comparatively reduced levels, indicating that these cell populations were less activated.

DISCUSSION

The characterization and quantification of splenic adherent cells from BALB/c and B10 mice indicated that there was a substantial increase in the number of monocytes and macrophages into this organ during the early stages of a *P.y.nigeriensis* infection. BALB/c mice displayed a higher level of cellular influx than did B10 mice, marked splenomegaly and a greater ability to control parasite numbers during the initial peak of the parasitaemia (although a second subsequent rise led to host death). Several studies have compared the cellular influx into the spleen of mice infected with lethal and non-lethal malarial parasites, and have also concluded that higher phagocyte numbers and greater splenomegaly seem to correspond to increased host recovery (Freeman & Parrish, 1978; Lelchuk *et al.*, 1979). However, an accompanying rise in the general lymphoid population, particularly T cells, is also apparent during non-lethal infections and their contribution to the control of parasite killing processes must also be considered.

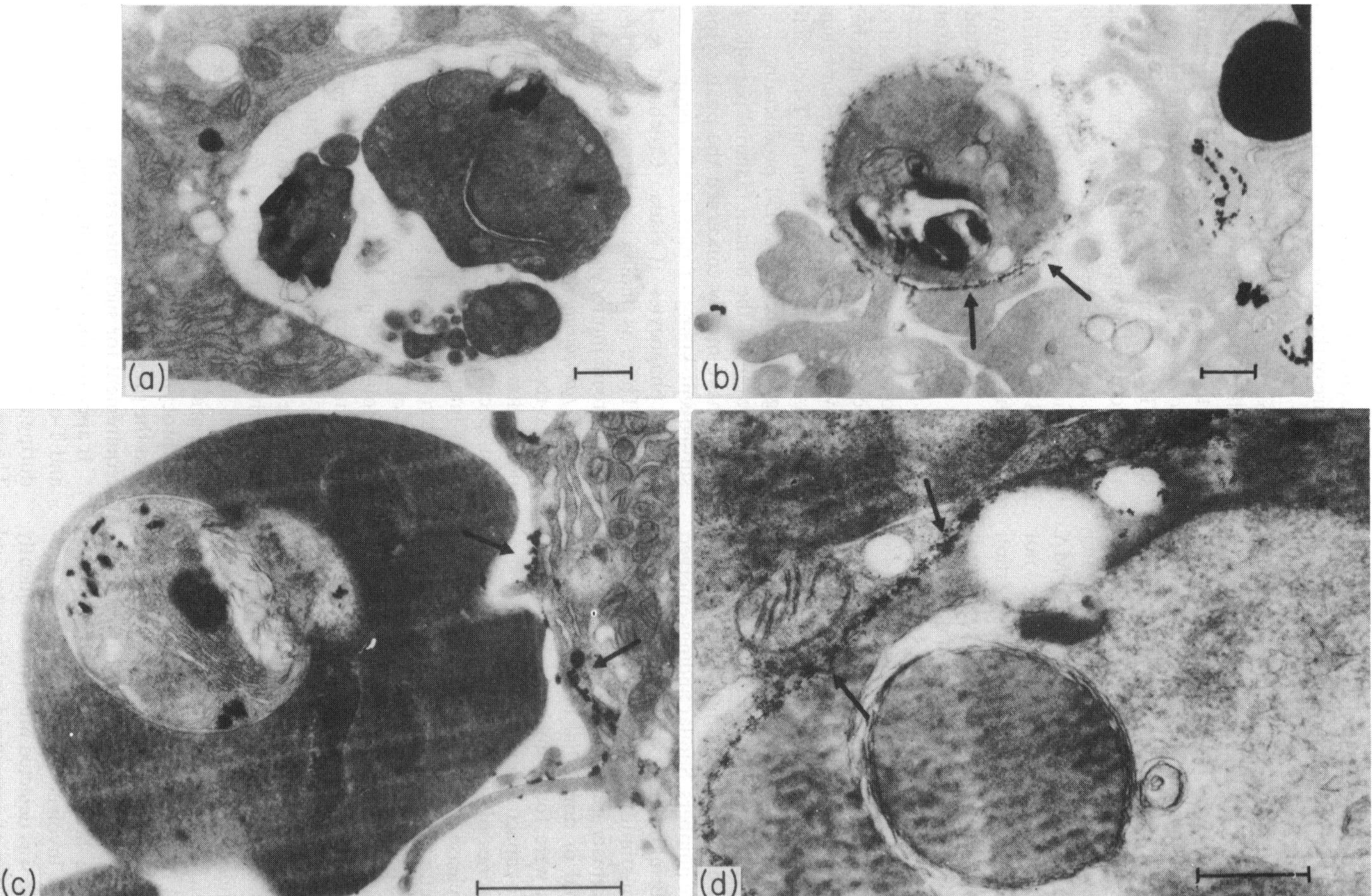

Figure 5. Detection of H_2O_2 release during the *in vitro* interaction of peritoneal macrophages removed from *P.y.nigeriensis*-infected mice and infected erythrocytes, by the formation of cerium perhydroxide deposits. (a) A control preparation showing parasite uptake in the absence of NADH. No cerium perhydroxide deposits are visible. (b) Phagocytosis of a *P.y.nigeriensis*-infected erythrocyte in the presence of $CeCl_3$. Cerium perhydroxide deposits indicate areas of H_2O_2 production. (c) Production of H_2O_2 by cytochalasin B-treated macrophage upon contact with a parasitized erythocyte. (d) H_2O_2 production along the areas of parasite-macrophage contact in the presence of immune serum.

The present study also determined the ROI-releasing capacity of splenic and peritoneal adherent cell populations, using PMA as a non-specific trigger. Peritoneal macrophages from BALB/c and B10 mice released high levels of ROI, peaking on Day 4 and 3, respectively. Splenic populations from both strains of mice released comparatively lower levels throughout infection. However, ROI release from BALB/c cells rose gradually until Days 3–4 of infection, when ferricytochrome *c* reduction was significantly higher than that by equivalent B10 cell populations. After this time, the BALB/c cells were refractory to PMA stimulation, although other stimulants were not tested, and it is possible that selective desensitization had occurred (Berton & Gordon, 1983a). However, it is more probable that they had already undergone a respiratory burst whilst still within the host. Contact with parasitized cells and subsequent phagocytosis could have triggered this response, and many of the cells were observed to contain parasite pigment upon plating. Splenic adherent cells from B10 mice released far lower levels of O_2^-, particularly after Day 3 when their capacity to release ROI appeared to be suppressed. This suppression was accompanied by a sharp increase in parasite numbers. Similar findings emerged from studies by Brinkmann *et al.* (1984), who measured the zymosan-stimulated chemiluminescence responses of splenic macrophages removed from mice infected with lethal or non-lethal malaria parasites. Parasite numbers were found to be higher in mice which had a lower capacity to release ROI.

An additional series of studies was carried out to demonstrate the release of H_2O_2 by peritoneal macrophages during interaction *in vitro* with *P.y.nigeriensis*-infected erythrocytes. There is strong evidence to suggest that this is the effector molecule in oxidant-induced malarial parasite killing by macrophages (Ockenhouse & Shear, 1984; Wozencraft *et al.*, 1984). Electron microscopy indicated that cerium perhydroxide deposits, showing the areas of H_2O_2 production, were found at sites of macrophage-parasitized cell contact. Phagocytosis and immune serum were not required for H_2O_2 release, although the frequency of macrophage-erythrocyte contact, and therefore the overall levels of oxidant release, were both increased in the presence of the latter, indicating that specific antibodies may be involved in oxidant killing processes. ROI release was detected much less frequently during the interaction of parasitized erythrocytes and macrophages from uninfected mice, reflecting the lower activation state of the latter population.

A number of factors appear to influence ROI release from macrophages and monocytes *in vitro*, including the nature of the substratum, the duration of culturing and the triggering agent used (Berton & Gordon, 1983a, b). The levels of ROI release *in vivo* are affected by T-cell control, with respect to both macrophage/monocyte migration and their state of activation. Očkenhouse & Shear (1984) found antigen-pulsed splenic T cells removed from mice infected *P. yoelii* 17X produced lymphokines which could activate murine macrophages *in vitro*. These macrophage populations were subsequently shown to kill rodent malarial parasites *in vitro* by H_2O_2-dependent processes that did not require cell contact between the effector and target cell. In the present study, the reduced capacity of splenic macrophages from B10 mice to release ROIs could have been related to low activation levels, resulting from the absence of suitable T-cell derived lymphokines. This may have been due to the overriding effect of a T suppressor cell population, or to a direct reduction in T-cell activation resulting from decreased IL-1 production.

Studies using the human malarial parasite *P.falciparum* have mostly concerned the phagocytosis and subsequent killing of parasites by peripheral bloodstream phagocytes (Celada, Cruchaud & Perrin, 1983). There is indirect evidence to suggest that bloodstream monocytes are more activated during an acute *P.falciparum* infection. Ward *et al.* (1984) found that Fc-receptor expression was increased in this cell population during the course of infection, although non-specific phagocytosis remained at similar levels. The γ-interferon-induced activation of human monocyte-derived macrophages and the subsequent *in vitro* killing of *P.falciparum* has been demonstrated by Ockenhouse, Schulman & Shear (1984). This study again emphasized the role of H_2O_2 in parasite killing, and the involvement of T-cell control mechanisms. The role played by antibodies in facilitating parasite/macrophage contact during a *P.falciparum* infection remains unclear. Mendis *et al.* (1981) have suggested that parasite-induced knobs on the surface of infected erythrocytes may themselves increase this contact, and that the presence of specific Ig is therefore not a strict requirement for such interaction.

It appears, therefore, that both phagocyte numbers and T-cell dependent macrophage activation increase during the initial stages of an acute malarial infection. The macrophage or monocyte may undergo a respiratory burst upon suitable triggering, resulting in oxidant-induced killing of intra-erythrocytic malaria

parasites. This process does not always involve phagocytosis, but it is amplified by the presence of immune serum and depends on close apposition of the parasite and the effector cell. Such contact may occur in the spleen and liver (Allison & Eugui, 1983; Wyler, 1983), where there is also a largely T-cell dependent influx of phagocytes during an acute rodent malarial infection (Lelchuk *et al.*, 1983). The suppression of ROI release by T-cell or macrophage-related mechanisms may result in decreased parasite killing, and it is therefore important to consider how ROI levels are affected by modulating factors, such as vaccination and concurrent infections.

REFERENCES

ALLISON A.C. & EUGUI E.M. (1983) The role of cell-mediated immune responses in resistance to malaria, with special reference to oxidant stress. *Ann. Rev. Immunol.* **1**, 361.

BERTON G. & GORDON S. (1983a) Regulation of superoxide anion release by mouse macrophages in culture. *Trans. R. Soc. trop. Med. Hyg.* **77**, 610.

BERTON G. & GORDON S. (1983b) Superoxide release by peritoneal and bone marrow-derived mouse macrophages. *Immunology,* **49**, 693.

BRIGGS R.L., KARNOVSKY M.L. & KARNOVSKY M.J. (1975) Localization of NADH oxidase on the surface of human polymorphonuclear leukocytes by a new cytochemical method. *J. Cell Biol.* **67**, 566.

BRINKMANN V., KAUFMANN S.H.E., SIMON M.M. & FISCHER H. (1984) Role of macrophages in malaria: O_2 metabolite production and phagocytosis in splenic macrophages during lethal *P. berghei* and self-limiting *P.yoelii* infection in mice. *Infect. Immun.* **44**, 743.

CELADA A., CRUCHAUD A. & PERRIN L.H. (1983) Phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes by human PMN leukocytes. *J.Parasitol.* **69**, 49.

CROFTON R.W., DIESSELHOFF-DEN DULK M.M.C. & VAN FURTH R. (1978) The origin, kinetics and characterisation of Kupffer cells in the normal steady state. *J. exp. Med.* **148**, 1.

DOCKRELL H.M. & PLAYFAIR J.H.L. (1984) Killing of *Plasmodium yoelii* by enzyme-induced products of the oxidative burst. *Infect. Immun.* **43**, 451.

FREEMAN R.R. & PARRISH C.R. (1978) Spleen cell changes during fatal and self-limiting malarial infections of mice. *Immunology*, **35**, 479.

FRIEDMAN M.J. (1979) Oxidant damage mediates variant red cell resistance to malaria. *Nature (Lond.)*, **280**, 245.

JOHNSTON R.B., GODZIK C.A. & COHN Z.A. (1978) Increased superoxide anion production by immunologically activated and chemically elicited macrophages. *J. exp. Med.* **148**, 115.

KNIGHT A. & SINDEN R.E. (1982) The purification of gametocytes of *Plasmodium falciparum* and *Plasmodium yoelii nigeriensis* by colloidal silica gradient centrifugation. *Trans. R. Soc. trop. Med. Hyg.* **76**, 503.

LELCHUK R., TAVERNE J., AGOMO P.U. & PLAYFAIR J.H.L. (1979) Development and suppression of a population of late-adhering macrophages in mouse malaria. *Parasite Immunol.* **1**, 61.

LELCHUK R., DOCKRELL H.M. & PLAYFAIR J.H.L. (1983) T-independent macrophage changes in murine malaria. *Clin. exp. Immunol.* **51**, 487.

MENDIS K., OCKENHOUSE C., SO M., WAHLGREN M., WINCHELL E., ALLEN R., CARTER R., HOMMEL M., MILLER L., PLESSANS W., SCHMIDT J., UDERNYA I. & DAVID P. (1981) Comparative studies on knob positive and knob negative clones of *Plasmodium falciparum* malaria. *Biol. Bull.* **161**, 353.

OCKENHOUSE C.F., SCHULMAN S. & SHEAR H.L. (1984) Induction of crisis forms in the human malaria parasite *Plasmodium falciparum* by γ-interferon activated, monocyte-derived macrophages. *J. Immunol.* **133**, 1601.

OCKENHOUSE C.F. & SHEAR H.L. (1984) Oxidative killing of the intra-erythrocytic malaria parasite *Plasmodium yoelii* by activated macrophages. *J. Immunol.* **132**, 424.

PAGE D.T. & GARVEY J.S. (1979) Isolation and characterization of hepatocytes and Kupffer cells. *J. immunol. Meth.* **27**, 159.

WARD K.N., WARRELL M.J., RHODES J., LOOANEESUWART S. & WHITE N.J. (1984) Altered expression of human monocyte Fc receptors in *Plasmodium falciparum* malaria. *Infect. Immun.* **44**, 623.

WILSON C.B., TSAI V. & REMINGTON J.S. (1980) Failure to trigger the oxidative metabolic burst by normal macrophages: possible mechanism for survival of intracellular pathogens. *J. exp. Med.* **151**, 328.

WOZENCRAFT A.O., DOCKRELL H.M., TAVERNE J., TARGETT GA.T.T. & PLAYFAIR J.H.L. (1984) Killing of human malaria parasites by macrophage secretory products. *Infect. Immun.* **43**, 664.

WYLER D.J. (1983) *The Spleen in Malaria. Ciba Foundation Symposium*, No. 94, p. 98. Pitman, London.