MACROPHAGE PLASMA MEMBRANE AND SECRETORY PROPERTIES IN MURINE MALARIA

Effects of *Plasmodium yoelii* Blood-stage Infection on Macrophages in Liver, Spleen, and Blood

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Since the early work of Taliaferro and Mulligan (1), it has been apparent that macrophages (Mφ) are prominent in malaria infection, but little is known about the properties of Mφ in malaria and of their precise role in the host response. The outcome of plasmodial infection in the intact host depends on the parasite strain and variation, the animal species, and innate and acquired immune resistance mechanisms that are poorly understood (2, 3). Parasitized red blood cells (PRBC) are cleared by the liver and spleen during malaria infection (4, 5). Studies in vitro have shown increased ingestion of opsonized red cells by splenic Mφ (6) and killing of parasites by lymphokine-activated monocytes from uninfected individuals via an oxidative burst (7). In murine models, immunity is T cell dependent (8, 9), involving both antibody (Ab) and cell-mediated mechanisms (2, 10). Murine malarias have thus provided a unique means of studying the immune response to malaria both in vivo and in vitro (2, 4–6, 8–10).

We have used a strain of *Plasmodium yoelii* that gives rise to a blood-stage infection characterized by a single wave of parasitemia, self-cure, and acquired immunity, to examine the properties of Mφ from control and infected animals. Antigenic, endocytic, and secretory activities have been characterized on Mφ in liver and spleen and after isolation by collagenase digestion and adherence. We show that the host responds to circulating, parasitized erythrocytes by a dramatic accumulation of Mφ in the blood, liver, and spleen. Circulating monocytes and tissue Mφ from these organs display marked changes in surface and secretory phenotype, compatible with an important role in recovery from infection.

Materials and Methods

*Malarial Parasites.* Parasites of the *P. yoelii* 17X nonlethal strain were kindly supplied by the World Health Organization Reference Laboratory, Winches Farm Field Station, This work was supported by the Wellcome Trust and the Medical Research Council, United Kingdom.

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Abbreviations used in this paper: Ab, antibody; Ag, antigen; BCG, bacillus Calmette-Guérin; EC, endothelial cells; ElG, Ab-coated sheep erythrocytes; HMP, hexose monophosphate; IBA, indirect binding assay; Kc, Kupffer cells; Mφ, macrophage; MFR, mannoseyl, fucosyl receptor; NBT, nitroblue tetrazolium; PMN, polymorphonuclear leukocyte; PRBC, parasitized red blood cells; RAR, rabbit anti-rat; RPM, resident peritoneal Mφ; SAC, spleen adherent cells.
St. Albans, Herts, UK, and stored in liquid nitrogen. They were passaged in mice by injection of 100 µl of infected blood either intraperitoneally or intravenously via the tail vein. Parasitemia was scored by examination of Giemsa-stained smears from tail blood.

Animals. 8-10 wk BALB/c and C57BL/6 mice were infected with *P. yoelii* 17X. Swiss Pathology Oxford (PO) mice were used to prepare macrophage targets for binding assays.

Media and Reagents. PBS without calcium or magnesium was obtained from Oxoid Ltd., Basingstoke, England. DMEM (Gibco-Biocult, Paisley, Scotland) and RPMI 1640 tissue culture medium (Flow Laboratories, Irvine, Scotland) were supplemented with L-glutamine and gentamicin, 20 µg/ml. FBS was routinely heat inactivated for 30 min at 56°C unless stated. HBSS and Hepes buffer were from Gibco-Biocult. Deoxyribonuclease (Sigma I) was obtained from Sigma Chemical Co., Poole, England, and collagenase from Boehringer Corp., Lewes, East Sussex, England.

Monoclonal Antibodies. F4/80 is a rat IgG2b noncytotoxic mAb that binds to a plasma membrane glycoprotein of M, 160,000 and is specific for mouse Mφ (11, 12). F7/4 is a rat IgG2a mAb that binds to a polymorphic differentiation antigen (Ag) found on neutrophils from some strains such as the C57BL/6, but not on others, including BALB/c (13). The Ag is absent or barely detectable in resident peritoneal Mφ (RPM) or thioglycollate broth-elicited Mφ from 7/4+ strains, but is expressed weakly by monocytes of normal animals and by peritoneal Mφ after immune activation by bacillus Calmette-Guérin (BCG) or *Corynebacterium parvum* (12). M5/114, a rat anti-mouse mAb that detects an Ia epitope on I-A^b^ and I-E^k^ molecules (14), was from Dr. H. Waldmann, Department of Pathology, University of Cambridge. It was used to detect Ia antigen on C57BL/6 mice, which carry the H-2^b^ haplotype. Mac-1 (M1/70), a rat IgG2b mAb that binds to mouse Mφ, neutrophils, and natural killer (NK) cells and inhibits the binding of iC3b-coated sheep erythrocytes (E) to CR3 (15, 16), was from Dr. T. Springer, Harvard Medical School, Boston, MA. 2.4G2, a rat anti-mouse mAb that recognizes the trypsin-resistant mouse Fc receptor for IgG2b/IgG1 isotypes (17), was from Dr. J. Unkeless, The Rockefeller University, New York. MRC OX 12, a mouse anti-rat kappa chain mAb (12), was a generous gift from Dr. S. Hunt, Sir William Dunn School of Pathology, Oxford. F(ab')~2 fragments of rabbit anti-rat (RAR) mAb were used as described (12). All Ab were used at saturation unless otherwise stated.

Preparation of Macrophage Target Cells. For the F4/80 and Mac-1 Ag assay, glutaraldehyde-fixed adherent Mφ were prepared (18) from PO mice primed with thioglycollate broth. For the assay of Ag F7/4, femoral bone marrow cells from C57BL/6 mice were plated at 1.3 x 10^6 cells per well in DMEM with 10% FBS in poly-L-lysine-coated 96-well plates (Sterilin Ltd., Feltham, Middlesex, UK). Plates were centrifuged at 200 g for 5 min and fixed with glutaraldehyde, 0.125% (vol/vol) for 10 min.

Assays for Liver and Spleen Ag Content. For F4/80 and Mac-1 Ag, assays were carried out on Triton X-100 tissue extracts by absorption indirect binding assay (18). The J774.2 Mφ-like cell line was used to calibrate the absorption assay; 1292 fibroblast-like cells, which do not carry either Ag, were used as the negative control. Ag F7/4 was assayed by a similar method, except that organ extracts were made in 3% wt/vol Brij 96 and 1% deoxycholate, in which Ag activity is unaffected. Bone marrow suspensions from C57BL/6 mice containing ~50% neutrophils were used as target cells and for calibration of the absorption assay for F7/4 Ag. Cells or extracts from BALB/c bone marrow provided a negative control.

Immunoperoxidase Labeling of Cells and Tissue Sections. Tissue sections prepared after perfusion-fixation were immunoperoxidase labeled as described (12). Isolated Mφ were fixed with 0.125% (vol/vol) glutaraldehyde for 10 min and immunoperoxidase labeled with F4/80 and F7/4 by an identical procedure. Ab and strain controls were included where appropriate. For Mac-1 labeling of isolated cells, endogenous peroxidase was destroyed after, instead of before, incubation of cells with Mac-1 Ab.

Morphometric Estimation of the Volume Fraction Occupied by F4/80+ Areas in Liver Tissue. Liver tissue sections were taken from normal and infected animals and the volume of the fraction occupied by F4/80+ areas was determined (18).

Isolation of Blood Monocytes. Blood from mice killed by ether was obtained by cardiac
puncture into heparin (10 IU/ml). After low speed centrifugation (30 g for 20 min), the buffy coat was collected and cells were washed twice in PBS, resuspended in DMEM with either 10% FBS, autologous plasma, or serum as described, and plated in 16-mm wells on glass coverslips. After 1 h, nonadherent cells were removed with three changes of PBS.

Isolation of Liver Mφ. The procedure described by Lepay et al. (19) was modified. Mice were killed by ether and the portal vein cannulated with 0.3-mm-diam polythene tubing. The thoracic cavity was exposed and the right atrium slit open to allow efflux of perfusate. The liver was perfused with PBS containing 0.5 mM EDTA and 5 mM glucose at pH 7.4 warmed to 37°C for 8 min at a rate of 1.5 ml/min by an electric infusion pump (LKB Instruments Ltd., South Croydon, Surrey, England), followed by RPMI with 0.05% collagenase at 37°C for 20 min. The gall bladder was removed and the liver excised, teased gently, and the capsule removed. Cells were resuspended in 50 ml of RPMI with 10% non-heat-inactivated FBS and centrifuged at 30 g for 4 min to pellet parenchymal cells. At this stage, the nonparenchymal cell viability assessed with trypan blue was >98%, and parenchymal cell viability was >65%. The supernatant was collected and centrifuged at 30 g for 4 min four or five more times. The pelleted material that was routinely discarded after differential centrifugation contained up to 20% of the total Mφ yield per liver as determined by F4/80 immunoperoxidase labeling. The final cell suspension was washed twice in RPMI, resuspended in RPMI with 10% FBS, and plated at 1–2 × 10^6 cells per well in 16-mm wells (DK-4000; Nunclon Delta, Roskilde, Denmark) with or without plastic coverslips (Lab-Tek Div., Miles Laboratories, Inc., Naperville, IL). After 45 min incubation at 37°C in 5% CO2, nonadherent cells were removed by washing and gentle pipetting, up to four times. Experiments were carried out on live or glutaraldehyde-fixed cells (0.125% vol/vol glutaraldehyde for 10 min) after 2 h culture or cells were cultivated overnight before two further washes. In some experiments, preparations were enriched for endothelial cells (EC) by reducing the number of washes of adherent preparations.

Isolation of Spleen Adherent Cells (SAC). Mice were killed with ether, the hepatic portal tributaries ligated, and the portal vein cannulated with polythene tubing of 0.3-mm-diam. The spleen was perfused via the splenic portal vein with 5 ml HBSS containing 0.05% collagenase, 50 μg/ml deoxyribonuclease, and 5 mM Hepes. The aorta was severed above the diaphragm after visible distension of the spleen. Spleens were isolated, gently teased apart, and further digested at 37°C on a rotary wheel for 30 min. The cell suspension was sieved through a fine wire mesh and the effluent was layered on a column of FBS and centrifuged at 30 g for 20 min. The pellet was washed twice in large volumes of PBS with 5% FBS, resuspended in RPMI with 10% FBS, and plated at 0.5–1.5 × 10^6 nucleated cells per well in 16-mm wells, with or without glass coverslips. Viability was >98%. After incubation for 45 min, cells were washed once by gentle pipetting with warm HBSS containing 10 mM Hepes, and the adherent cells were cultivated further in fresh RPMI with 10% FBS. After 1 h, monolayers were washed three to four times with warm HBSS and 10 mM Hepes to remove nonadherent cells. Experiments were carried out at this stage on live or glutaraldehyde-fixed cells (0.125% vol/vol glutaraldehyde for 10 min) or after overnight incubation and two further washes.

Adherent Cell Counts and Protein. These were estimated by counting cell nuclei (20) and by the method of Lowry (21).

Indirect Binding Assays (IBA). (a) At saturation: Site numbers of Mφ Ag were estimated in a two-step IBA with first and second Ab at saturation (12). SAC and liver Mφ were harvested and plated in 16-mm wells with or without plastic coverslips at a final density of 1–2 × 10^6 adherent cells per well. Cells were fixed in glutaraldehyde, 0.125% vol/vol, for 10 min. Binding of first Ab was detected with 125I-F(ab')_2 OX12 or RAR (5–7 × 10^6 cpm/well, 25 μg/ml). Replicate coverslips were immunoperoxidase labeled with each first Ab and 300 cells were counted. From the differential count and the number of cell nuclei per well, the number of molecules of F(ab')_2 OX12 or RAR bound per labeled cell was calculated assuming an M, for F(ab')_2 of 1.0 × 10^6. (b) Trace IBA: Live liver Mφ at 2–5 × 10^5 adherent cells per well were incubated after 2 or 24 h culture with saturating Ab Mac-1 and trace amounts of 125I-OX12 F(ab')_2 (10^6 cpm/well).
Fc Receptor (FcR) Binding and Ingestion. E were coated with subagglutinating concentrations of polyclonal rabbit anti-E (E IgG) or mouse monoclonal anti-E IgG2a (Ab UM2) and IgG2b (Ab U88), gifts from Dr. B. Diamond, Albert Einstein College of Medicine, Bronx, New York. Mø preps were incubated at 50–100 E per adherent cell for 45 min at 37{\degree}C in 5% CO2, washed gently, fixed with glutaraldehyde (0.125% vol/vol), and immunoperoxidase labeled with F4/80 Ab.

Assays for Mø Secretory Products. Respiratory burst activity was elicited with 100 ng/ml PMA. Superoxide (O2-) release was assayed in duplicate by ferricytochrome c reduction, with controls containing 30 μg/ml superoxide dismutase (Sigma Chemical Co.) and cell-free blanks. Hexose monophosphate (HMP) shunt activity was assayed (22) in parallel. Fibrinolysis was assayed on 125I-fibrin plates (23) with 5 × 104 to 5 × 105 cells per well and with acid-treated dog serum as the source of plasminogen.

Assays for Mannosyl, Fucosyl Receptors (MFR). Binding and uptake of 125I-mannose-BSA were measured with and without mannan (from baker’s yeast, M7504; Sigma Chemical Co.) at 5 mg/ml (12). Liver Mø were fixed and immunoperoxidase labeled with F4/80 for autoradiography after uptake of 125I-mannose-BSA for 10 min at 37{\degree}C.

Nitroblue Tetrazolium (NBT) Reduction. NBT at 1 mg/ml in RPMI 1640 and PMA (100 ng/ml) were added to cells on glass coverslips, and preparations incubated for 20 min at 37{\degree}C in 5% CO2. Cells were then washed three times with PBS and fixed with 0.125% (vol/vol) glutaraldehyde for 10 min. Coverslips were viewed by phase contrast and bright-field microscopy to score NBT reduction and malaria pigment.

Results

Infection of BALB/c and C57BL/6 Mice with P. yoelii 17X

Infection of both strains of mice with the nonlethal strain of P. yoelii 17X resulted in a self-limiting infection that resolved after 16–19 d. The course of infection with 103, 104, or 105 PRBC/mouse, i.p. or i.v., was similar. Peak blood parasitemias were variable (15–45% of RBC) and occurred after 12–14 d with usually complete disappearance of blood parasites by 18–20 d (Fig. 1a). The BALB/c strain showed slightly increased susceptibility, with mortality rates up to 15% compared with C57BL/6 mice (mortality, <5%). Animals were completely resistant to rechallenge with 105 PRBC up to 100 d subsequently.

Infection with PRBC resulted in a peripheral blood monocytosis (see below) and a rapid increase in spleen and liver weight (Fig. 1b) in both strains. These organs were considerably enlarged with progressive accumulation of dark pigment visible macroscopically from day 3 to 4 of infection. Liver weight increased from 900 ± 100 to 1,600 ± 180 mg at the peak of infection, while spleen weight increased from 100 ± 30 to 1,600 ± 150 mg. Both organs remained enlarged and pigmented 100 d after infection. At the peak of infection and as noted by others (7), hematoxylin and eosin–stained sections of spleen tissue (not shown) revealed variable pigment deposition and hyperplasia of the red and white pulp with periarteriolar infiltration and prominent germinal centers. In liver, sinusoidal spaces were prominent with aggregates of dark pigment close to the sinusoidal lining and a few periportal cellular aggregates.

Further studies were undertaken to examine possible effects of malaria infection on Mø populations in various sites. Peritoneal Mø showed little change in morphology, surface Ag, or secretory properties (see below for markers used) after intravenous or intraperitoneal injection with PRBC, both on primary challenge and on subsequent rechallenge (not shown). Immunoperoxidase labeling with F4/80 Ab of glutaraldehyde perfusion–fixed tissue sections showed
prominent or increased F4/80+ labeling of Mφ in the liver, spleen, and bone marrow, but no significant changes in other organs surveyed (small intestine, kidney, brain, choroid plexus, heart, adrenals). Thus, the Mφ response to malaria infection appeared to predominate in the blood, liver, spleen, and bone marrow, and these tissues were analyzed further.

**Effect of Malaria Infection on Ag Markers Analyzed In Situ**

**F4/80 Ag.** Ab F4/80 was used to localize resident and recruited Mφ in different organs by immunocytochemistry and to quantitate changes in Ag content after infection.

(a) Liver. Immunohistochemical labeling of tissue sections from normal and malaria-infected BALB/c and C57BL/6 mice showed a marked increase in the number and size of F4/80+ labeled cells in the liver at the peak of infection (Fig. 2, a and b). The volume fraction occupied by F4/80+ cells determined by morphometry was 3.0 ± 0.33% in the normal liver compared with 12.3 ± 0.5% in the 13-d-infected liver. Thus, there was a fourfold increase in the amount of F4/80 Ag per unit volume of liver tissue or a sevenfold increase in the total F4/80 Ag per liver if liver weight increase is taken into account. F4/80+ labeling in the malaria liver, like the normal, was virtually confined to sinusoidal lining cells, which showed an increase in size and number of processes, and often contained prominent pigment. These cells were clearly adherent to the sinusoidal lining and in many instances filled the entire width of the sinusoid. Some showed attached erythrocytes (Fig. 2b) and evidence of ingestion. Only a few cells in
FIGURE 2. Immunohistochemical localization of F4/80 Ag (a, b) and F7/4 Ag (c, d, e) in normal liver (a, c) and liver from malaria-infected mice (b, d, e). × 400. Counterstained with Mayer's hematoxylin. (a) Sinusoidal lining F4/80+ Kupffer cells in normal liver from a C57BL/6 mouse. Some F4/80+ fine processes are seen extending from cell bodies. (b) F4/80+ cells in liver from a malaria-infected C57BL/6 mouse at day 14. There is an increase in the number of F4/80+ cells that contain malarial pigment, show greater size, and have spread to occupy a larger proportion of the sinusoidal lining. Sinusoidal spaces are apparently dilated compared to a. Some red cells (arrows) can be seen attached to the sinusoidal lining. Identical appearances for both a and b are also seen with BALB/c mouse livers by this procedure. (c) F7/4+ cells resembling PMN loosely attached to the sinusoidal wall in normal liver from the C57BL/6 (F7/4+) strain. These cells are extremely scanty in normal liver after perfusion fixation, and the field depicted represents the most dense region of F7/4+ labeled cells in the entire liver section. (d) F7/4+ cells resembling monocytes and Mϕ (single arrows) in liver from a malaria-infected C57BL/6 mouse at day 14. There is an increase in the number of F7/4+ cells that appear to be loosely attached to the sinusoidal lining. These do not contain malarial pigment, which can be seen as aggregates in F7/4+ areas of the section (double arrows). Most of the KC extending along the sinusoidal wall do not label with Ab 7/4. (e) F7/4+ cells (arrows) within and surrounding a periportal cell infiltrate in liver from a malaria-infected C57BL/6 mouse at day 14.
periportal infiltrates were F4/80+. Further, unlike the normal liver, in which a population of F4/80- endothelial cells (EC) is readily observed (24), a greater proportion of the sinusoidal lining region was labeled with Ab F4/80 at the peak of infection.

(b) Spleen. In spleen there was increased F4/80 labeling, although the distribution of F4/80 within the splenic architecture (24) after malaria remained unchanged (not shown). F4/80+ areas were confined to the red pulp and periarteriolar regions, with weaker labeling of the marginal zone. Many of these cells were stellate, with a proportion containing malaria pigment. A few also showed red cell attachment.

(c) Bone marrow. Immunocytochemical analysis of the femurs of infected animals (not shown) revealed a similar pattern to that seen in normal bone marrow with prominent stellate F4/80+ cells and associated islands of hemopoiesis (24). Rounded F4/80+ monocytes were more prominent in sinus spaces than in uninfected controls.

Immunoprecipitation and SDS-PAGE of F4/80 Ag from the malaria liver revealed the same band of $M_w 160,000$ (not shown) as normal liver and other sources of F4/80 Ag (12), indicating that F4/80 Ag was unaffected by malaria infection. By absorption binding assay, total F4/80 Ag content in the liver and spleen of both BALB/c and C57BL/6 mice increased steadily in the course of infection, peaked coincidentally with parasitemia and resolution of the infection (12-16 d), and then fell in liver (Fig. 3a). At the time of peak infection, total F4/80 Ag in the malaria liver was increased eightfold relative to uninfected

![Graphs showing changes in F4/80, F7/4, and Mac-1 Ag content over time](image)
controls, in good agreement with morphometric estimation, whereas F4/80 Ag in the spleen rose 13-fold. Thus, both liver and spleen showed a marked increase in Mφ Ag content as a result of malaria infection in both murine strains studied. By immunocytochemical analysis of liver, this appeared to be due to an increased number of sinus lining cells, as well as increased Ag per cell.

F7/4 Ag. Since earlier studies in our laboratory (12) had shown that the myelomonocytic differentiation Ag 7/4 can be induced on peritoneal Mφ of 7/4+ strains by immune activation during other infections, we analyzed the expression of this Ag in liver and spleen in the course of murine malaria. Immunoperoxidase labeling of liver sections of uninfected C57BL/6 mice (a 7/4+ strain) showed scant labeling (Fig. 2c) confined to a few polymorphonuclear leukocytes (PMN) within the sinusoids. In the malaria-infected liver, an increase in the number of F7/4+ labeled cells was seen (Fig. 2, d and e). These were rounded, resembling monocytes rather than PMN, and carried little pigment. Most occurred singly within the sinusoidal space or loosely attached to the sinusoidal lining, and the majority of the sinus lining (F4/80+) population did not label with Ab F7/4. Some 7/4+ cells were found in periportal aggregates (Fig. 2e) that appeared to be cellular infiltrates rather than regions of hematopoiesis, since megakaryocyte nuclei were not present. In normal spleen (not shown), F7/4+ labeling was found on PMN in the red pulp, whereas after malaria infection, labeling was found on PMN and rounded monocytes rather than on the more stellate F4/80+ cells.

By absorption assay (Fig. 3b), F7/4 Ag was virtually undetectable in normal C57BL/6 liver tissue, but rose sharply during infection. F7/4 Ag was detected at low levels in normal spleen and increased by 11-fold within 4 d of infection. F7/4 Ag levels peaked in both liver and spleen at least 4 d before peak blood parasitemia and before maximal increase in F4/80 Ag content (cf. Fig. 3a). Results from the absorption assay of Ag 7/4 in organ extracts were in good agreement with immunoperoxidase labeling of tissue sections. No Ag was detectable by either assay in BALB/c mice, a result compatible with the polymorphic expression of this Ag (13). The increase in liver and spleen F7/4 Ag in C57BL/6 mice could be due to PMN and/or Mφ and was analyzed further on isolated cells, as described below.

Mac-1 Ag. To gain further insight into the nature of the inflammatory cell response to murine malaria, we examined expression of another myelomonocytic differentiation Ag, Mac-1. In spleen, this Ag is present on red pulp PMN and Mφ, but it has been reported (25) to be undetectable in normal murine liver by immunohistochemistry. In absorption assays with lysates from both C57BL/6 and BALB/c mice, Mac-1 Ag was detectable in small amounts in normal liver and in greater amounts in normal spleen (Fig. 3c). The total Mac-1 Ag content of these organs increased considerably, peaked, and fell in the same pattern as that seen for F4/80 Ag. Specificity controls showed Mac-1 Ag was undetectable in similarly treated detergent extracts from mouse brain, L cell fibroblasts, or rabbit liver. Immunoperoxidase labeling of Mac-1 Ag in glutaraldehyde perfusion-fixed tissue sections gave unclear results, probably due to poor preservation of tissue Mac-1 Ag. The increased Mac-1 Ag content in liver and spleen after malaria infection could be due to PMN and/or Mφ; analysis of this Ag on isolated Mφ is detailed below.
Properties of Isolated Mφ Populations

Blood. Infection with *P. yoelii* caused dramatic changes in the properties of circulating mononuclear phagocytes. Monocytosis in peripheral blood was marked, up to $2.0 \pm 0.5 \times 10^4$ monocytes/mm$^3$ blood, compared with $2.0 \pm 1.5 \times 10^3$ monocytes/mm$^3$ blood in uninfected controls. Blood monocytes after infection were heterogeneous in size and included a substantial population of large cells with endocytic debris, perinuclear pigment, and a striking capacity to spread on glass coverslips after isolation. These cells labeled more strongly with Ab F4/80 than monocytes from uninfected mice. Ag 7/4 expression by adherent blood monocytes from uninfected animals was weak, compared with more intense labeling of the smaller, rounded population of monocytes after infection. Large pigment-bearing monocytes also showed weak labeling with Ab 7/4. Since rapid spreading is characteristic of normal monocytes and of activated Mφ populations, and since components of the coagulation and complement cascades are known to enhance Mφ spreading (26), we investigated spreading by monocytes from malaria-infected animals in the presence of autologous plasma and serum, as well as in FBS, which reduces spreading by normal monocytes. Malaria infection markedly enhanced spreading of blood monocytes on glass in the presence of FBS. Plasma and serum from infected mice further increased spreading of monocytes from infected animals, as well as of RPM from normal mice. Single-cell analysis established that blood monocytes obtained after malaria infection also showed an enhanced ability to reduce NBT after challenge with PMA, compared with normal monocytes.

Liver. Yields of Kupffer cells (KC) from normal liver were $2.0 \pm 1.0 \times 10^6$ cells/g liver for C57BL/6 mice and $5.0 \pm 1.0 \times 10^6$ cells/g liver from BALB/c mice, comparable to results obtained by others (19, 27, 28). After malaria infection, yields of Mφ were ~10-fold higher ($2.5-5.0 \times 10^7$ cells/g liver by day 14). Liver Mφ isolated after infection were more heterogeneous in size than KC from normal mice and contained numerous large, well-spread cells, many with clumps of perinuclear pigment and phagocytic debris. EC were the main contaminant of liver Mφ isolated by this method, but could be reduced to <15% of the adherent cell population by differential adherence and washing. EC were distinguished from liver Mφ by their lack of F4/80 Ag (see below) and by morphology, tending to be smaller, with a round or oval nucleus and absence of pseudopodia. The proportion of EC in preparations of malaria liver Mφ (<10%) was lower than that found in populations of normal KC (<15%) isolated under comparable conditions. Although EC were also found to bear receptors that mediate endocytic uptake (29, and see below) accumulation of malaria pigment was slight, relative to liver Mφ, even in heavily infected mice.

We next analyzed various Mφ surface and secretory properties in isolated normal and malaria-derived liver Mφ using freshly harvested, 2-h adherent cell cultures unless otherwise stated. Control experiments with collagenase-treated RPM showed no effects on the properties studied.

(a) Surface Ag. Table I, part A shows that 85–90% of 2-h adherent liver Mφ preparations from normal and malaria-infected mice were labeled by F4/80 Ab, and up to 95% of cells could be labeled after 24 h cultivation. Few normal KC were labeled by Ab 7/4 and, after malaria infection, there was increased labeling.
TABLE 1 A

Expression of Surface Markers by Isolated Liber Mφ

| Percent adherent cells | Normal       | Malaria-infected* |
|------------------------|--------------|-------------------|
| **Surface markers detected by:** |             |                   |
| Immunoperoxidase labeling |             |                   |
| F4/80                  | 85 ± 5 (+++) | 90 ± 5 (+++)      |
| F7/4                   | 17 ± 4 (+++) | 45 ± 17 (+++)     |
| Mac-1                  | 20 ± 3 (+)  | 67 ± 4 (+++)      |
| M5                     | 30 ± 3 (+++) | 10 ± 2 (+++)      |
| Indirect binding assay| F(ab')₂ second antibody bound |
| (molecules/positively labeled cell) | | |
| Normal                 | F4/80: 1.2 × 10⁵ | 2.5 × 10⁵ |
|                        | F7/4: 1.1 × 10⁵  | 2.4 × 10⁵ |
|                        | Mac-1: ND       | 3.0 × 10⁵ |
|                        | M5: 1.1 × 10⁶   | 3.7 × 10⁶ |

Yield total nucleated cells/spleen: 2.0 ± 0.5 × 10⁹
Yield adherent cells/spleen: 3.0 ± 0.6 × 10⁶

* Results pooled from days 9–14 of malaria infection.
² Immunoperoxidase labeling of liver Mφ after 2 h culture and of SAC on plastic coverslips after 4-h culture. Background labeling, <5%. Results are mean percent differential counts (500 cells) ± SD of three or more separate experiments. (+) weak labeling (+++) strong labeling.
⁴ F4/80, F7/4, and Mac-1 binding were detected with F(ab')₂ OX12 as second antibody, and M5 binding was detected using F(ab')₂ RAR. Liver and spleen Mφ were not compared under identical conditions.

25–65% of Mφ in different isolates, including cells with pigment. PMN, which are also 7/4⁺, were rare in these preparations. Similar results were obtained with Mac-1 labeling, which was weakly present on ~20% normal KC and increased on malaria-infected liver Mφ, with ~67% strongly labeled. M5 binding was
detected on a third of normal KC from C57BL/6 mice, but the proportion of labeled cells from malaria-infected mice of the same strain was reduced to 10%. If the increased liver Mφ yield is taken into account, the total number of Ia+ cells was increased by threefold. Saturation IBA showed that after infection, binding of all four Ab was increased two- to threefold per labeled cell (Table I A). Surface Mac-1 Ag could not be detected on normal KC by this method, but was detectable in trace IBA (not shown).

(b) FcR. Adherent cell populations were incubated with opsonized E and double-labeled with Ab F4/80 to examine FcR expression. The majority (90 ± 7%) of F4/80+ cells from normal and malaria-infected liver ingested ElG avidly, but two interesting observations were made. A substantial population of liver Mφ from normal (24 ± 4%) as well as infected mice (36 ± 3%) bound rather than ingested unopsonized E, similar to resident Mφ isolated from murine bone marrow (E receptor; Crocker, P., and S. Gordon, manuscript in preparation). Furthermore, unlike liver Mφ, EC bound but failed to ingest ElG. The isotype specificity of liver Mφ FcR was also examined. ~80% of normal KC expressed FcR for both IgG2a and IgG2b, scored by ingestion, and expression of both FcR was detectable in ~60% of liver Mφ from infected animals. The uptake of ElG2b could be effectively inhibited by the mAb 2.4G2, as expected.

(c) MFR activity. Studies in rodents have shown that mannosyl-terminated ligands such as 125I-mannose-BSA are rapidly cleared by sinus lining cells in liver, and both KC and EC have been implicated in this activity (30, 31). Although isolated Mφ from other sites are known to express mannan-inhibitable MFR (12, 31), there is no information on isolated liver Mφ. Since MFR activity is markedly downregulated in peritoneal and blood-derived Mφ by infections, such as BCG (32) or Trypanosoma brucei (T. brucei) (33), by lymphokines (34), or by IFN-γ (35), it was of interest to examine MFR activity by liver Mφ and EC isolated from normal and malaria-infected mice.

We first examined specific binding and uptake of 125I-mannose-BSA in routine liver Mφ preparations in which >80% of adherent cells were F4/80+. Adherent cells isolated from normal mouse liver displayed readily detectable MFR activity in vitro (Table II, part A), which was markedly decreased after malaria infection. Binding and uptake of 125I-mannose-BSA after infection (day 10) were 17 and 13% of control, respectively. Similar results were obtained when results were calculated per milligram cell protein (not shown). This striking decrease in MFR activity by malaria infection was not due to loss of cell viability and was maintained for at least 24 h in cell culture (not shown). Further studies distinguished MFR activity by liver Mφ (F4/80+) and EC (F4/80−). Adherent preparations were made with a higher proportion of EC and analyzed for MFR and F4/80 by single-cell analysis. Collagenase-treated RPM were included as controls. Table II B shows that both F4/80+ KC and F4/80− EC from uninfected animals accumulated 125I-mannose-BSA, and that mannan inhibited two-thirds of labeling by each cell type. In contrast with results obtained by single-cell analysis in situ (30, 31), KC were more heavily labeled in vitro than EC. Malaria infection (day 9) resulted in decreased specific uptake by liver Mφ, (~30% of uninfected control) as well as by EC (~10% of control) (Table II B). These results were in good agreement with the data in Table II A, and confirmed that KC and EC could each contribute
to mannosyl-specific endocytosis by normal liver, and that specific MFR activity by both cell types was markedly reduced during malaria infection.

(d) Respiratory burst activity. There is little evidence that Mφ generated during malaria infection display an enhanced respiratory burst and the capacity to destroy parasites or parasitized erythrocytes. Furthermore, KC from normal mice display low respiratory burst activity that is not enhanced in response to γ interferon (19). During infection by Listeria monocytogenes, the newly recruited population of monocytes contributes respiratory burst and microbicidal activity (36). We therefore measured the ability of liver Mφ from normal and infected animals to release O_{2} upon challenge with PMA (Table III A). \textsuperscript{14}CO_{2} release via the HMP shunt was measured in replicate wells in the same experiments, and single-cell analysis was performed using reduction of NBT (Table III B). KC from uninfected animals displayed a low level of respiratory burst activity; O_{2} release was lower than that by collagenase-treated RPM, and only 18% of KC

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**TABLE II A**

MFR Activity of Isolated 2-h-adherent Liver Mφ

| Cells used                        | \textsuperscript{125}I-mannose-BSA (ng/5 x 10^5 cells/30 min) labeling by: | Binding at 4°C* | Uptake at 37°C |
|----------------------------------|--------------------------------------------------------------------------------|------------------|----------------|
| Normal                           |                                                                               | 1.75             | 8.65           |
| Malaria-infected (day 10)        |                                                                               | 0.31             | 1.15           |

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**TABLE II B**

Autoradiographic Analysis of \textsuperscript{125}I-Mannose-BSA Uptake by Isolated Liver Mφ (F4/80+) and EC (F4/80-) in the Presence or Absence of Mannan

| Cells used                        | Mannan | F4/80\textsuperscript{2} | Grains/cell (±SD)\textsuperscript{3} |
|----------------------------------|--------|--------------------------|-------------------------------------|
| Normal                           | +      | +                        | 47 ± 17                             |
|                                  | +      | -                        | 22 ± 12                             |
|                                  | -      | +                        | 147 ± 46                            |
|                                  | -      | -                        | 68 ± 24                             |
| Malaria-infected (day 9)         | +      | +                        | 21 ± 17                             |
|                                  | +      | -                        | 3 ± 2                               |
|                                  | -      | +                        | 48 ± 29                             |
|                                  | -      | -                        | 8 ± 5                               |
| RPM                              | +      | +                        | 9 ± 7                               |
|                                  | -      | +                        | 43 ± 23                             |

* Specific mannan-inhibitable binding and uptake. Nonspecific binding was <10%. >80% of the adherent cells were F4/80+.

\textsuperscript{2} For these experiments, the preparations of liver-adherent cells used were enriched for EC (F4/80-). Percent F4/80+ cells for the three populations was: normal, 50%; malaria-infected, 80%; RPM, >98%.

\textsuperscript{3} Data represent mean number of grains ± SD associated with each cell type. >30 cells of each type were scored. Values include autoradiographic background, which was 5 ± 3 grains/cell. In control experiments, the MFR activity of RPM was not affected by collagenase treatment.
TABLE III A
Respiratory Burst Activity of Isolated Liver Mφ after PMA Stimulation

| Time in culture (h) | Normal | Malaria-infected (day 10) |
|---------------------|--------|--------------------------|
| 2                   | 1.0    | 3.6                      |
| HMP shunt activity (¹⁴CO₂ released) (10⁵ cpm/10⁵ cells/60 min)² |
| 2                   | 1.5    | 3.9                      |
| 24                  | 2.4    | 8.6                      |

TABLE III B
Single-cell Analysis: NBT Reduction after PMA

| Cell phenotype | Normal | Malaria-infected |
|----------------|--------|-----------------|
| F4/80⁺         | 80 ± 3 | 85 ± 2          |
| NBT⁺           | 18 ± 2 (+) | 60 ± 3 (+++)   |
| Pigmented      | 0      | 46 ± 2          |
| Pigmented and NBT⁺ | 0   | 29 ± 1          |
| Unpigmented and NBT⁺ | 18 ± 2 | 31 ± 2      |

* RPM treated with collagenase in suspension under similar conditions and after 2 h in culture gave superoxide release of 1.73 nmol/5 × 10⁵ cells/60 min at 37°C.

† RPM treated with collagenase in suspension under similar conditions and after 2 h in culture gave ¹⁴CO₂ release of 1.5 × 10⁴ cpm/10⁵ cells/60 min.

NBT reduction: (+), weak; (++), strong.

showed weak NBT reduction after PMA stimulation. After malaria infection, liver Mφ showed a substantial and stable (24 h in culture) increase in O₂⁻ release and HMP shunt activity (about threefold) and ~60% of these reduced NBT. We also asked whether pigment accumulation by Mφ after extensive lysis and uptake of PRBC might affect respiratory burst activity. However, the proportion of cells with strong NBT reduction was similar in liver Mφ with and without pigment (Table III, part B).

e Fibrinolytic activity. The malarial liver Mφ population displayed enhanced plasminogen-dependent fibrinolytic activity (over about fivefold) compared to normal KC, which showed very low levels of activity (not shown).

Spleen. Yields of SAC from control mice (Table I B) were comparable to those obtained by others (37, 38) and ~15-fold more adherent cells were recovered at peak infection. At least two-thirds of 2–4-h adherent SAC in both groups were F4/80⁺ Mφ; the normal population was heterogeneous in size, shape, and F4/80 labeling, which was undetectable in an additional subpopulation of SAC (up to 20%) which resembled immature Mφ by phase-contrast microscopy. F4/80⁺ Mφ in malaria-infected SAC showed increased size and spreading, and contained phagocytic debris and increased amounts of pigment at later stages of infection. F4/80⁻ cells in these preparations included lymphocytes, PMN,
dendritic cells, fibroblasts, and nucleated erythrocytes, many of which adhered to Mφ.

(a) Surface Ag. Single-cell analysis and saturation IBA (Table I B) showed that the increased Mφ population induced by malaria infection expressed 50% higher levels of F4/80 Ag per cell than controls. These Mφ were Ag 7/4+ and Mac-1+, and a subpopulation (about half) expressed Ia Ag. The increased proportion of SAC that expressed Ag 7/4 after infection (88 vs. 27%) was not due to PMN contamination, which was <20% of adherent cell preparations. Many cells were more strongly labeled by 7/4 Ab infection, although average binding determined by IBA was 60% of controls. Labeling and expression of Mac-1 and M5 (Ia) by SAC were comparable irrespective of infection, and these data include binding by a minor population of PMN for Mac-1, and by lymphocytes and dendritic cells for M5.

(b) FcR. The majority of SAC (90% of F4/80+ cells) ingested both ElgG2a and ElgG2b, irrespective of infection. Binding of unopsonized E was somewhat increased after infection, from 14 ± 2% by normal SAC to 35 ± 4% by malaria-infected SAC.

(c) MFR. Isolated SAC from uninfected animals expressed specific MFR activity. 125I-mannose-BSA binding was 0.37 ng/5 × 105 cells, or 6.85 ng/mg protein (4°C, 30 min); uptake, 1.67 ng/5 × 105 cells, or 30.7 ng/mg protein (37°C, 30 min). Similar results were obtained after 24 h cultivation of SAC. Infection by P. yoelii had a marked effect on MFR expression by SAC. Both binding and uptake decreased sharply to <20% of levels of control cells by day 3 of infection, the earliest time point monitored, and remained low throughout the period of peak infection. Similar results were obtained whether calculated on the basis of cell number or protein content. MFR activity remained low when SAC were assayed after 24 h of cultivation. MFR activity was still ~50% reduced in SAC isolated 100 d after initial infection.

(d) Respiratory burst. SAC from uninfected animals displayed readily detectable respiratory burst activity after PMA stimulation: 5.05 nmol O2 released per 5 × 105 cells during 60 min at 37°C, 95.2 nmol O2 released per milligram protein during 60 min at 37°C; 4.2 × 104 cpm 14CO2 released per 5 × 105 cells during 60 min at 37°C, 6.0 × 105 cpm 14CO2 released per milligram protein during 60 min at 37°C. These levels were similar to those observed with collagenase-treated RPM and were ~50% of the levels displayed by untreated BCG-activated PM (not shown). Both measures of SAC respiratory burst specific activity were unchanged during malaria infection, irrespective of the duration of infection, for time points monitored up to the peak. Again, similar results were obtained whether results were calculated on the basis of cell number or protein content.

(e) Fibrinolytic activity. Normal SAC showed high levels of plasminogen-dependent fibrinolytic activity compared with RPM (about fivefold increase) and malaria SAC showed slightly lower levels (enhanced about threefold compared with RPM (not shown).

These studies with monocytes and Mφ isolated from blood, liver, and spleen established that cell numbers in these sites were markedly increased by infection, and confirmed that the myelomonocytic Ag 7/4 and Mac-1 detected in organ
extracts were expressed by Mφ as well as PMN. Properties characteristic of activation (i.e., spreading, enhanced respiratory burst, decreased MFR activity) were induced by \( P. yoelii \) infection but, in the spleen, some of these changes were obscured by the background activities of control Mφ populations.

**Discussion**

Our studies show that murine blood stage malaria has profound effects on the mobilization and functional characteristics of Mφ in particular tissues, giving further insight into the relative contributions of resident and recruited Mφ at sites of infection, and providing direct evidence that activated Mφ could contribute to host defense against malaria. The host Mφ response to \( P. yoelii \) infection was restricted to cells in contact with parasitized erythrocytes in blood and did not extend to Mφ in the peritoneal cavity. Mφ involvement in this murine malaria model therefore differs from the response to infection by intracellular pathogens such as BCG, in which modified Mφ and/or sensitized T lymphocytes are readily recruited into the peritoneal cavity, even after systemic infection (39). One consequence of this difference is that, in malaria, it is necessary to recover Mφ from spleen and liver by digestion for direct examination (cf. 4), since the yield from blood is limited. We have studied here only mature, adherent Mφ, and have made no attempt to assay nonadherent progenitors in bone marrow or spleen, although Mφ production is clearly stimulated by infection. Immunocytochemical screening of other tissue Mφ populations by Ab F4/80 revealed no obvious changes in the central nervous system or kidney, sites affected by malarial syndromes in man.

Accumulation of the Mφ-specific Ag F4/80 in liver and spleen was a major feature of \( P. yoelii \) infection, in parallel with the course of parasitemia and hepatosplenomegaly. Ag F4/80 content was measured by immunohistochemistry, absorption analysis of organ lysates, and by direct assay of isolated cells. Results were in good agreement, and at the peak of infection showed a large increase in total Ag (up to 20-fold), due to an increase in Mφ number (up to 10-fold) and in Ag content per cell (up to twofold). The marked monocytosis in blood favors the conclusion that much of the increase in liver Mφ was due to increased recruitment from the circulation. Local proliferation may be a concomitant feature, especially in spleen, where hematopoiesis can occur to some extent even in normal mice (37). Can the newly generated recruited Mφ in liver be distinguished from the initial tissue-resident population, as in \( L. monocytogenes \)-infected mice? (36). After malaria infection, these Mφ populations do not differ in F4/80 labeling, but the virtual absence of Ag 7/4 and Mac-1 on resident KC, and the expression of these Ag on blood monocytes and liver Mφ at an early phase of the infection provided markers for the recruited cells. This new population was characterized by enhanced respiratory burst activity, heterogeneous expression of Ag 7/4 and Mac-1, and an increase in size and spreading. The immunocytochemical analysis of Ag F4/80 and 7/4 distribution within the malaria-infected liver is consistent with this interpretation. Ab F4/80 labeled almost all cells along the walls of liver sinusoids, whereas Ab 7/4 labeled the more rounded cells (more recently recruited?) that adhered to sinusoids, rather than the extensively spread, more heavily pigment-laden cells. After cell isolation, only about half of the
F4/80⁺ liver Mφ were labeled by Ab 7/4. It is likely that monocytes retain F4/80 as they adhere and spread within liver sinusoids, whereas 7/4 and probably Mac-1 Ag are gradually lost during this process. In spite of the massive increase in spleen size and of Mφ number and Ag content, we were unable to distinguish the initial spleen resident Mφ population from newly recruited/generated cells by Ag marker analysis. The expression of low MFR and relatively high levels of respiratory burst and fibrinolytic activity by SAC from control animals is compatible with the presence of a less mature (37), recently derived (41), or partially activated Mφ population.

The monocytes found in blood after malaria infection resembled activated Mφ in size, spreading, expression of 7/4 Ag, and enhanced respiratory burst activity. These cells contained pigment and phagocytic debris derived from erythrocytes, and resembled Mφ isolated by digestion from liver and spleen. It is not clear whether cells acquired these features in the bone marrow, blood, or even in peripheral sites before reentering the circulation. The Mφ circulating in infected animals showed a dramatically increased intrinsic ability to spread on artificial substrata, and preliminary studies indicated that plasma proteins, possibly complement, could enhance this activity. The role of specific antibody in modulating these and other Mφ functions also requires further study.

Although liver Mφ isolated from normal animals expressed virtually no CR3 Ag (Mac-1), they displayed relatively high levels of FcR and MFR, other receptors involved in endocytosis. An interesting F4/80⁻ population of less adherent cells was also present in liver preparations, and because of distinctive morphology and marker expression (MFR⁺, and binding without ingestion of IgG), resembled EC previously isolated from rodent liver (29). Unlike KC and liver Mφ after infection, which expressed FcR for both IgG isotypes, these EC expressed only the FcR IgG2b (S.-H. Lee, unpublished results). Additional studies are needed to establish the relationship of liver EC to other sinus-lining cells and to EC in other tissues.

The strongly F4/80⁺ Mφ in isolated SAC may correspond to the prominent F4/80⁺ population in splenic red pulp, but the origin of these isolated Mφ and their relationship to resident cells in spleen and elsewhere also need to be defined further (24). In view of their heterogeneous morphology and Ag expression (F4/80 > Mac-1 > Ia > 7/4), and low levels of MFR activity, single-cell studies are needed to analyze expression of different markers by SAC subpopulations and to relate cells that express MFR in vitro to those that selectively accumulate polysaccharides in the intact spleen (38).

The differences between Mφ populations in normal liver and spleen were masked by the large increases in Mφ in both organs after P. yoelii infection. Malaria-induced Mφ were similar in liver and spleen and their phenotype resembled that of peritoneal cells induced by other infectious agents, such as BCG (32) and T. brucei (33). Apart from high levels of respiratory burst activity, the malaria-derived Mφ displayed a marked, early, and sustained decrease in MFR activity. Interestingly, liver EC MFR activity was also reduced by infection. These results are compatible with the action of both cell types of a cytokine such as IFN-γ, which is known to downregulate Mφ MFR selectively (35). However, we have no evidence that IFN-γ levels were locally or systemically increased in our
system. The absence of substantial Ia induction on Mφ and the marked 7/4 Ag increase (a marker that is not induced by IFN-γ in vitro; S. Gordon, unpublished results) indicate that Mφ activation by malaria is not necessarily or entirely due to T lymphocyte products. A role for additional mediators possibly derived from complement and antibody, and the effects of massive erythrophagocytosis must also be considered. Since normal monocytes lack MFR (35), bear 7/4 Ag, and express high respiratory burst activity (40), the immaturity of the Mφ populations recruited to liver and spleen by *P. yoelii* infection could contribute significantly to the activated phenotype observed. Finally, our studies indicate that, in organs such as the spleen, which in the normal animal can contain Mφ with features of activation, the host response to infection may depend on rapid expansion of this Mφ population, as noted by others (42), as well as on further modulation of the cytotoxic activity of individual cells.

Mφ isolated from animals at peak parasitemia have the capacity to destroy parasitized erythrocytes by phagocytic and extracellular effector mechanisms that involve known plasma membrane receptors (FcR, CR3) and toxic oxygen radicals. There is evidence in both human (43) and murine (44) malaria that parasitized erythrocytes may be opsonized with cleaved C3 products and IgG, and, whereas the phagocytosis of complement-coated erythrocytes does not initiate a respiratory burst (45), binding of E1gG does promote erythrocyte destruction by ingestion and/or extracellular lysis. Our studies confirm that CR3 are not deficient in murine malaria, although hypocomplementemia has been reported to contribute to defective complement-mediated clearance at late stages of another rodent malaria (46). It is not known whether other opsonins or opsonin-independent mechanisms contribute to direct recognition and lysis of parasites and infected erythrocytes. In addition, recruitment of large numbers of biosynthetically active Mφ to liver and spleen could result in extensive local and systemic release of potent mediators of inflammation such as IL-1 (47) and tumor necrosis factor (48). The roles of these and other Mφ activities in the immune response to malaria remain to be established.

**Summary**

We have studied the effect of infection with the blood-stage of *Plasmodium yoelii* 17X, a nonlethal parasite, on plasma membrane antigens, receptors, and secretory properties of macrophages (Mφ) in murine liver, spleen, and blood. mAb F4/80 (Mφ specific), F7/4 (a marker for immature and immunologically activated Mφ, as well as neutrophils), and Mac-1, which binds to the type 3 complement receptor, were used to measure the distribution and total content of antigens in situ and to assay surface expression of antigens on Mφ isolated by collagenase perfusion-digestion and adherence. We also examined respiratory burst activity after stimulation with PMA, FcR activity, Ia antigen expression, and binding of 125I-mannose-BSA and unopsonized sheep erythrocytes by isolated Mφ.

In the normal animal, spleen Mφ expressed Mac-1 and F7/4 antigens and relatively high levels of respiratory burst activity, in contrast to Kupffer cells in liver, where all three features were virtually absent. The introduction of parasitized erythrocytes into the circulation resulted in a large influx of F4/80+ Mφ.
into the blood, liver, and spleen, where local Mφ proliferation could also contribute. Liver Mφ during malaria infection showed increased Mac-1 and 7/4 antigen and an increased respiratory burst potential compared with uninfected controls. Increases in total, but not specific activity of FcR, Ia antigen, and binding of unopsonized sheep erythrocytes were found in spleen and liver Mφ populations after infection. In both populations, there was an early but persistent marked reduction in specific binding and uptake of 125I-mannose-BSA.

These results confirm and extend observations that normal Kupffer cells are relatively homogeneous in morphology, surface markers, and anatomical location, in contrast to Mφ in normal spleen, and that both of these populations differ from resident Mφ elsewhere, including the peritoneal cavity. In the course of infection by *P. yoelii*, Mφ with high levels of opsonic receptors (CR3, FcR) and respiratory burst potential are mobilized in large numbers at specific sites such as liver and spleen, in accordance with an important role for Mφ in the clearance of parasitized erythrocytes from blood.

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