Clin. exp. Immunol. (1987) **68**, 65–77

Lymphocyte migration in murine malaria during the primary patent parasitaemia of *Plasmodium chabaudi* infections

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(Accepted for publication 9 October 1986)

SUMMARY

Inoculation of adult C57/BC mice with 10^6 red cells infected with *Plasmodium chabaudi* induces an acute primary parasitaemia peaking around the 8th or 9th day and lasting 10–14 days. Concomitantly, the spleen enlarges to reach 6–7 times its normal weight by the 11th day. The major component of this increase is between day 9 and 11, due primarily to an increase in erythropoietic cells in the red pulp. Although initially the white pulp increases in size, by day 11 it shows partial lymphocyte depletion which coincides with the occurrence of massive absolute lymphocytosis in the peripheral blood. ^{3}H-Thymidine labelling *in vivo* suggests that this lymphocytosis is not due to lymphocytopoiesis. Collectively, these findings suggest a redistribution of lymphocytes. Lymphocyte migration was investigated around peak parasitaemia, using enriched populations of T and B cells labelled with ^{51}Cr. The traffic patterns of these cells were followed over 36 h. These studies show decreased uptake (or decreased retention) of T and B cells by spleens of infected mice. Concomitantly, there is increased retention of T and B cells in the liver and lungs of infected mice, suggesting a complex redistribution of these cells. Lymphocyte migration to lymph nodes was unimpaired in these animals. Similar changes in T and B cell migration do not occur in *Babesia microti* infections in C57/BL mice. We relate our findings to histological and histochemical changes in the liver and spleen of malarious mice and discuss the significance of these findings to immunosuppression in malaria and to the development of parasiticidal immunity.

Keywords lymphocyte migration murine malaria *Babesia* spleen liver

INTRODUCTION

The mechanisms of acquired immunity to malaria parasites are still incompletely understood (reviewed Wyler, 1982; Cohen & Lambert, 1984). During the acute and chronic erythrocytic phases of both human (McGregor & Barr, 1962; Greenwood *et al.*, 1972) and experimental malarias, immune responses to heterologous antigens are depressed, possibly as a result of T cell, B cell and macrophage dysfunction (reviewed by Weidanz, 1982). Lymphocytes undergo complex migrations through the spleen and other lymphoid tissues and thereby undergo the interactions with accessory cells necessary for the initiation of normal immune responses (Ford, 1975). The migration of lymphocytes in malarious animals is altered (Bitzan & Spira, 1978; Playfair & de Souza, 1982). This may be a consequence of acquired immunity to the parasite and/or the immunodepression

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accompanying the infection. Alterations in lymphocyte migration during malaria may also contribute to the pathological effects produced by the parasite.

Plasmodium chabaudi malaria is usually a non-fatal infection in mice; the mice suffering an acute primary parasitaemia lasting 10–14 days and subsequently showing one or two recrudescences. Using ^{51}Cr-labelled lymphocytes from mesenteric and axillary lymph nodes, Playfair & de Souza (1982) reported that during *P. chabaudi* infections, labelled lymphocytes showed increased migration to the liver (when assessed 24 h after cell injection). Similarly, there was increased migration of injected lymphocytes into the spleen of such animals apart from a period later on in the patent parasitaemia when lymphocyte traffic to the spleen declined. In our study we have extended the observations of Playfair & de Souza on *P. chabaudi* infections by using enriched populations of ^{51}Cr-labelled T and B lymphocytes and following the traffic of these cells over 36 h. We relate our findings to the histology of the spleen and liver of infected mice.

MATERIALS AND METHODS

Parasite

Plasmodium chabaudi. This (AS strain) was mosquito-passaged, cloned and cryopreserved in liquid nitrogen (Phillips & Wilson, 1978). Experimental mice were infected with blood obtained after one or two blood passages from cryopreserved parasites (Gray & Phillips, 1981). Mice were bled into heparin/Hanks (10 i.u. heparin per ml blood) and dilutions made with RPMI 1640 (Gibco) containing 25 mM Hepes (referred to as RPMI) and 5% fetal calf serum (FCS) (Flow Laboratories). Parasitized red cells (prc) were injected intravenously and unless otherwise stated the infecting dose was 1×10^6 prc in 0·25 ml RPMI. Parasitaemias were determined from mouse tail blood smears stained with Giemsa's stain.

Babesia microti. The Kings strain of *B. microti* was originally obtained from Professor F. E. G. Cox (Kings College, London). It was handled in the same way as *P. chabaudi.*

Mice

Inbred C57B1 mice (Centre for Tropical Medicine, University of Edinburgh) of the age and sex given in the test, were used.

Lymph node cells

Cervical, axillary, bronchial and inguinal lymph nodes were collected into Hanks' balanced salt solution. The lymphocytes were obtained by pressing the lymph nodes gently through a 60-mesh sieve and collected in heparized RPMI, without FLS. After two washes in the same medium, the lymph node cells were finally resuspended in RPMI containing 5% FCS. The cells were subsequently fractionated into enriched T and B populations using glass wool and nylon wool columns as described by McDonald & Phillips (1978). Vability was determined by the trypan blue exclusion test and was usually >90%. The proportion of T and B cells in the cell populations was enumerated as described by McDonald & Phillips (1978). The enriched T cell populations contained 70–82% T cells and 2–3% B cells. The enriched B cell populations contained 53–56% B cells and 12% T cells.

^{51}Cr labelling of lymph node cells

Cells were resuspended in RPMI containing 5% FCS to 10^8 cells per 1 ml and incubated with 50 μCi ^{51}Cr per ml, for 40 min at 37°C (Sodium Chromate, Radiochemical Centre, Amersham). After labelling, the cells were washed three times with RPMI and resuspended to give in different experiments $1–5 \times 10^6$ cells in 0·25 ml, which was the inoculum given to each mouse. Approximately 0·75, 4·5, 16, 24 and 36 h after receiving labelled cells, groups of 3–5 mice were killed and bood, lungs, liver, spleen and in some cases lymph nodes were removed and counted in a LKB Gamma

Counter. Results were expressed as the percentage of the total counts injected, determined by counting replicate aliquots of labelled cells for each experiment.

Haematology

In two experiments injected mice were killed at intervals after infection, and blood cell counts made, using a Coulter counter, from cardiac blood collected in sequestrine tubes. Differential white blood cell counts were made from blood smears stained with Giemsa's stain. Packed cell volumes were determined in the standard manner using a haemacrit centrifuge.

Determination of the proportion of newly formed blood lymphocytes

Mice were injected with ^{3}H thymidine (TRA-61, Radiochemical Centre, Amersham UK, specific activity 185 Gbq/mmols), at a dose of 1 μCi/g body weight in three divided doses administered intraperitoneally at 8 h intervals. The aim was to label most newly formed cells during the period of ^{3}H thymidine administration (Ford, 1978). Mononuclear cells were prepared from heparinized cardiac blood samples by centrifugation over a Ficoll/Hypaque gradient (Pearson *et al.*, 1983). The degree of labelling of these mononuclear cell preparations was determined by Beta counting using an LKB liquid scintillation counter (Janossy *et al.*, 1973). Blood smears obtained from cardiac puncture of these animals were fixed in methanol and autoradiography carried out as described by Rogers (1967), using Ilford K5 emulsion. The autoradiographs were exposed for 4 weeks at 4°C, developed for 5 min in Kodak D19 at 20°C and stained with Giemsa's stain through the emulsion. The percentage of labelled lymphocytes was determined by doing grain counts (less background) over 500 lymphocytes.

Histology

Tissues used for α-napthyl acetate esterase (ANAE) and acid phosphatase (AP) staining were fixed in buffered formol-sucrose at 4°C for 24 h. Subsequently they were rinsed in Holt's gum-sucrose at 4°C for 24 h, snap frozen in liquid nitrogen and cryostat sections cut at 5 μm. Staining for ANAE activity was carried out as described by Dockrell *et al.* (1978). Briefly, the sections were incubated for 15 min in a solution of hexazotized pararosaniline in M/15 phosphate buffer, adjusted to pH 7·3. Staining for acid phosphatase activity was by the method of Yam, Li & Crosby (1971) using naphthol AS-BI phosphate (Sigma) as the substrate. Incubation was for 3 h at 37°C. Sections were counterstained with methyl green. Tissues for conventional histology and autoradiography were fixed in formol-saline and processed by standard paraffin-wax embedding.

Statistical tests

Student's *t*-test was used for assessing statistical significance except where stated otherwise.

RESULTS

The occurrence of pronounced lymphocytosis during the course of a primary patent P. chabaudi *infection in mice.* Table 1 summarizes the haematological changes and changes in spleen weight during the infection in groups 4 or 5, 3 months old male mice. Peak parasitaemia was reached around day 8 and the infection had become subpatent in most mice by day 19. The acute primary parasitaemia caused anaemia which was reflected in a reduction in red blood cell count and packed cell volume both of which reached a nadir 2–3 days after peak parasitaemia. The spleen weight increased with rising parasitaemia, reaching a maximum of 6–7 times the normal size in the mice killed on day 11, after which it diminishes in size but remained 2–3 times larger than normal even 40 days after infection. The greatest increase in spleen weight occurred around 10th and 11th days of infection at which stage the animals were rapidly recovering from their parasitaemia; the peak parasitaemia having occurred 2–3 days earlier. Of particular note was a massive leukocytosis 2–5 days after peak parasitaemia. The differential white cell count (Table 1) revealed that the leukocytosis was principally a lymphocytosis.

The source of the increased blood lymphocyte population. The above lymphocytosis could be due

Table 1. Haematological changes during infection of C57/BC mice with *P. chabaudi*

Day of infection*	White cell count ($\times 10^6$/ml)	Absolute lymphocyte count ($\times 10^6$/ml)	Red cell count ($\times 10^9$/ml)	Spleen weight (mg)
5	8·0 (1·1)	6·3 (0·92)	3·0 (0·39)	246 (27)
7	14·7 (4·5)	11·1 (3·2)	3·8 (0·25)	354 (10)
11	15·3 (3·4)	10·0 (1·2)	1·9 (0·03)	752 (22)
15	5·2 (0·25)	4·5 (0·32)	4·5 (0·32)	451 (37)
19	14·2 (6·0)	11·7 (5·2)	4·0 (0·05)	573 (113)
22	5·2 (1·0)	4·1 (0·47)	3·8 (0·38)	378 (56)
33	7·3 (0·99)	5·9 (0·8)	4·1 (0·19)	226 (13)
41	8·5 (1·4)	7·2 (0·9)	3·9 (0·1)	268 (23)
Control group	5·75 (0·84)	4·3 (0·19)	4·3 (0·2)	96 (8)

* Peak parasitaemia occurred on day 8 of infection. Mean and s.e. in brackets shown for groups of four or five mice at each time point.

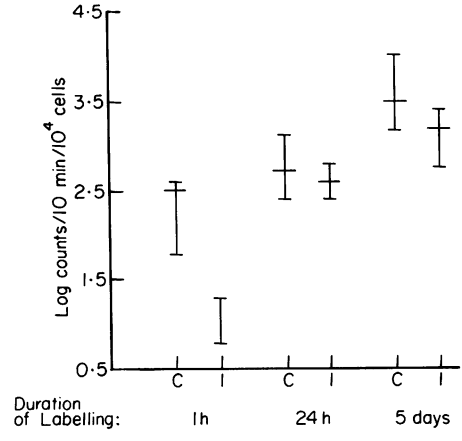

Fig. 1. ^{3}H-Thymidine uptake *in vivo* by peripheral blood mononuclear cells in murine malaria. Infected animals were labelled 1 h, 24 h or 5 days before peak parasitaemia in a synchronized *P. chabaudi* infection. Median and range shown for groups of five control or malaria infected mice. See results section for detailed description of protocol. The 1 h ^{3}H-thymidine uptake was significantly reduced in the infected group ($P > 0{\cdot}05$ by Wilcoxons rank sum test for paired samples). In the 24 h and 5 day labelling experiments the infected and control groups do not show a significant difference by this test. C, control; I, infected.

to (a) newly-formed lymphocytes entering the blood or (b) redistribution of the lymphocyte pool as occurs in *Bordetella pertussis* infection (Munoz, Arai & Cole, 1981). To investigate the first possibility, cohorts of malaria-infected mice were injected with ^{3}H-thymidine (see Methods section). One group received ^{3}H-thymidine for 5 days from day 7–11 of a primary malarial infection; the second group was labelled for 24 h from day 10–11 of malaria and the third group were injected with ^{3}H-thymidine 1 h before sacrifice on day 11. All groups were infected with malaria in parallel, and killed on day 11 (i.e. at height of their lymphocytosis). Peripheral blood mononuclear cells in malaria infected mice did not show an increase in ^{3}H-thymidine uptake compared to control animals labelled in parallel for equivalent periods before killing (Fig. 1). These results obtained by beta-counting were confirmed by counting the percentage of labelled lymphocytes in autoradiographs from cardiac blood smear (data not shown).

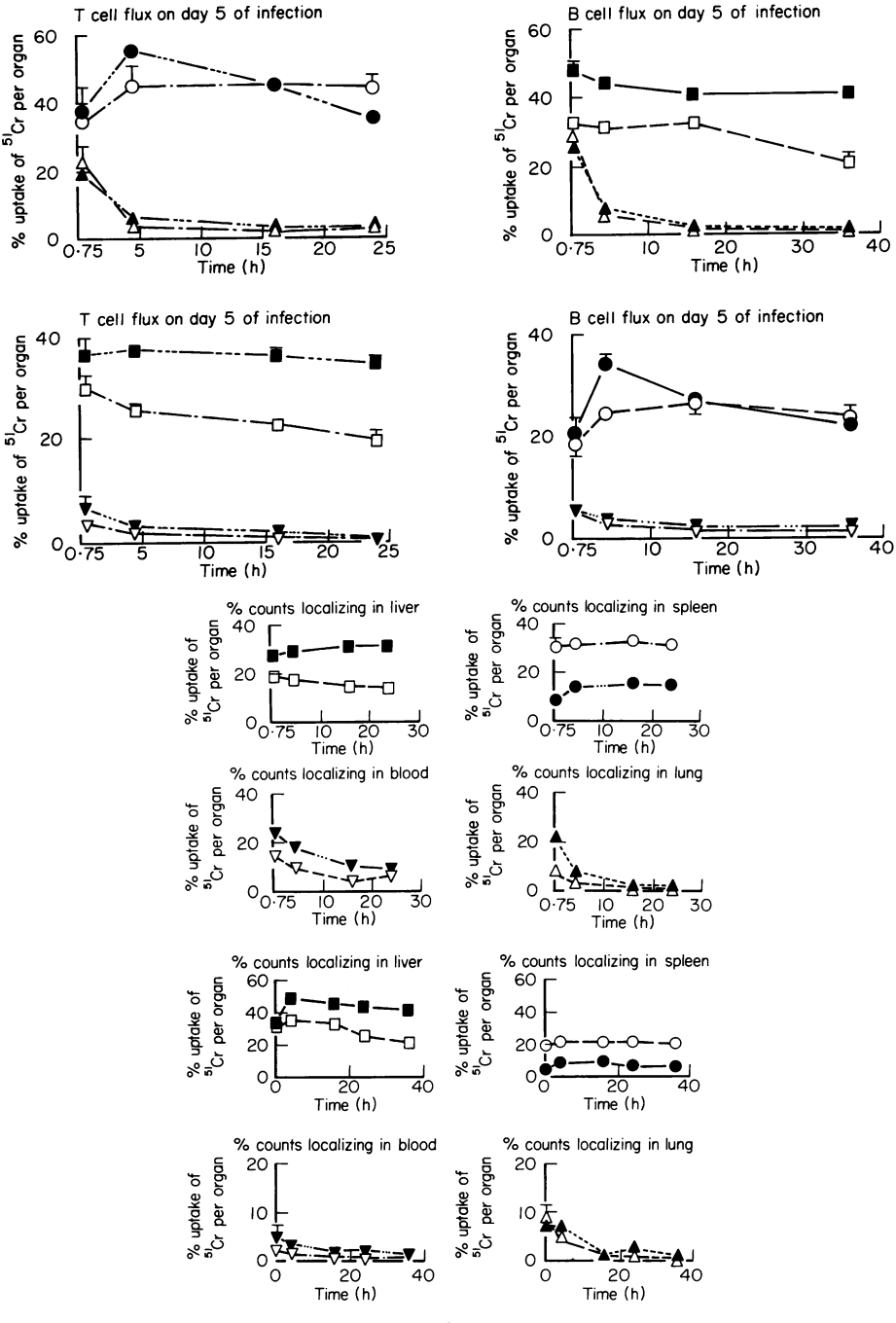

Fig. 2. Lymphocyte migration assessed by % uptake of ^{51}Cr during an infection with *P. chabaudi* between 0·75 h and 36 h after injection of ^{51}Cr-labelled enriched T or B cells. Mean and standard error (vertical bars) of ^{51}Cr uptake by various organs or 2 ml of blood are shown for groups of four to five infected or control mice. T cell migration was assessed at 0·75, 4·5, 16 and 24 h and B cell migration at 0·75, 4·5, 16 and 36 h, after cell injection respectively. Infected group: Liver (■); spleen (●); lung (▲); blood (▼). Control group: Liver (□); spleen (○); lung (△); blood (▽). (a) T lymphocyte migration on day 5 of *P. chabaudi* infection. (b) B lymphocyte migration on day 5 of *P. chabaudi* infection. (c) T lymphocyte migration at peak lymphocytosis during *P. chabaudi* infection (day (10/11). (d) B lymphocyte migration at peak lymphocytosis during a *P. chabaudi* infection (day 10/11).

Migration of ^{51}Cr labelled lymphocytes. Five experiments were carried out in which ^{51}Cr labelled lymph node cells from non-infected syngeneic mice were transferred to malarious mice on day 9 or 10 and in two experiments on day 5 of a *P. chabaudi* infection. In repeat experiments the results were essentially the same and hence representative experiments are described.

Day 5 P. chabaudi *infection—a rising primary parasitaemia.* The infected recipient mice had a parasitaemia of around 8–12% on day 5 which rose to a mean of 35% the following day. The ^{51}Cr labelled enriched T and B cell populations were injected into the infected and control mice at 1700 h. The results are shown in Fig. 2 where it can be seen that T cells localized to a greater extent in the liver of infected mice ($P < 0{\cdot}01$) for up to 24 h after the cells were injected. There was a slight increase in T cell localization in the spleens of infected animals up to 4·5 h after cell injection ($P > 0{\cdot}1$), but this had declined to a level significantly below that of the control animals by 24 h ($P < 0{\cdot}01$). There were no significant differences in the T cell flux through the lungs or blood of these animals. For B cells there was significantly increased localization in the liver of infected animals ($P < 0{\cdot}01$) but the increased uptake in the spleen was statistically significant ($P < 0{\cdot}05$) only at 4·5 h, after which it paralleled changes in the control group. The blood and lungs showed similar ^{51}Cr uptakes in both groups of animals injected with labelled B cells. The total amount of label detected in the liver remained steady over 36 h after injection of T or B cells suggesting that cells entering the liver of infected mice appeared to remain there or were replaced as they left.

Day 9–11 of P. chabaudi *infection.* In this representative experiment, peak parasitaemia of around 45–50% was reached on day 8. The labelled cells were injected into the recipient mice at 1700 h on Day 9 when the parasitaemia had declined to around 20%. In the T cell recipients (Fig. 2c), in contrast to the day 5 mice, there was a significant drop ($P < 0{\cdot}01$) in the localization of the cells in the infected spleens (from 0·75 h though 24 h after cell injection) but throughout the experiment there was a significantly increased uptake into the liver ($P < 0{\cdot}001$). Higher counts in both lungs and blood were seen in the infected mice especially at the earlier time-points after cell injection. A similar pattern was found with the B cells although the increased localization in the infected liver in some experiments became significant ($P < 0{\cdot}01$) at varying time intervals greater than 4·5 h. The time course of these changes suggested that the failure of cells to accumulate in the spleen was caused by non-entry and not through later loss or by emigration.

In a preliminary experiment unseparated labelled lymph node cells from non-infected mice were transferred into infected mice on Day 10 of infection and the localization of these cells in the lymph nodes of the neck, the bronchial, axial, and inguinal lymph nodes was followed. There was no difference between infected mice and the controls (Table 2) ($P > 0{\cdot}05$), showing that B and T cell traffic into lymph nodes was not affected by *P. chabaudi* injection.

Transfer of lymph node cells into mice infected with Babesia microti. In mice infected with 5×10^6 parasitized red cells of *B. microti* peak parasitaemia of around 60% is reached by day 11 and then declines from about day 13 or 14. Peak parasitaemia is accompanied by profound immunosuppres-

Table 2. Migration of ^{51}Cr labelled peripheral lymph node cells into lymph nodes of *P. chabaudi* mice (day 10 of infection) (mean and standard error in parentheses are shown for each group ($n = 4$); control group values are in square brackets)

Time in h after cell injection	^{51}Cr uptake per 100 mg lymph node tissues	
	Mesenteric	Peripheral
0·75	0·87 (0·08) [0·6 (0·6)]	0·73 (0·08) [0·6 (0·2)]
4·5	2·2 (0·5) [2·0 (0·36)]	1·7 (0·19) [1·2 (0·12)]
19	5·25 (0·48) [6·25 (1·0)]	7·25 (0·25) [6·0 (0·4)]
36	8·3 (0·33) [7·0 (0·7)]	11 ± (1·1) [8·75 (1·31)]

Migration of lymphocytes into other organs was similar to the data presented in Fig. 2b for both groups.

sion (Gray & Phillips, 1983), anaemia and splenomegaly. Leukocytosis is not so pronounced. As a comparison with *P. chabaudi* two preliminary experiments were carried out with *B. microti* infected mice.

In the first experiment labelled T and B cell enriched populations from non-infected mice were injected into mice on day 10 of infection and in the second on day 14. The results from both experiments were essentially the same. There was a significantly increased uptake of T cells at 4.5 hours into both spleen and liver ($P < 0{\cdot}05$; data not shown). At 36 h there were also increases in B cell localization into liver and spleen. For the latter this was significant ($P < 0{\cdot}05$) on day 10 but was not significant on day 14 ($P < 0{\cdot}05$). On day 10 the labelling of lungs, blood and lymph nodes was similar in control and infected mice but on day 14 there appeared to be increased localization of T cells into peripheral lymph nodes ($P < 0{\cdot}05$). The important difference between the malarious mice at and just after peak parasitaemia and the mice at peak parasitaemia of *B. microti* infection is that in the latter there was an increased migration of both B and T cells into the spleen but in the former this was decreased.

Histological and histochemical studies in P. chabaudi *infections.* Initial enlargement of the spleen included both increase in the size of the white pulp and more prominently of the red pulp. The major increase in splenic weight which occurs between day 10 and day 11 was due primarily to an increase in the red pulp, which displayed extensive (extra-medullary) haemopoietic activity. These erythroid precursors were negative for acid phosphatase but had a visible amount of non-specific esterase activity in their cytoplasm. Paradoxically, the white pulp at this stage is *reduced* in size and is depleted of lymphocytes (Fig. 3b). This lymphocyte depletion of the white pulp coincided with the occurrence of lymphocytosis of the peripheral blood (Table 1), suggesting that both features were a manifestation of a redistribution of the lymphocyte pool. After day 14 the white pulp rapidly regained its normal appearance and the red pulp size dimished with the waning of haemopoetic activity. It is only at this relatively late stage that germinal centres became prominent features in the white pulp.

During the course of this infection there were no pronounced or consistent changes in the bulk of the liver, though it acquired a slatey-grey pigmentation from day 5 onwards.

The first histological change observed in the liver was the appearance of malarial haemazoin in the Kupffer cells, which also appeared to have an increased content of acid phosphatase when compared with those of control animals. This occurred by 7 days after infection. By day 9 a new feature appeared. At this stage central veins and their tributaries become blocked with plugs of cells consisting chiefly of macrophages and a few lymphocytes (Fig. 3c). Some of these vessels showed prominent accumulations of parasitized red cells intermingled with the macrophages and lymphocytes (Fig. 3b). The areas adjacent to blocked central veins showed necrosis of liver cells 24 h later (i.e. day 10) and localized collapse of the hepatic connective tissue around these foci of necrosis was also evident. Around 9–10 days after infection portal areas of the liver were infiltrated with lymphocytes, many of them with single dot ANAE staining, characteristic of T cells (Dockrell *et al.*, 1978). Hepatic sinusoidal lymphocytosis was also evident. Autoradiographs of liver tissue from the animals labelled with ^{3}H-thymidine *in vivo* showed that many of the infiltrating lymphocytes found in the liver were labelled, indicating that they had recently passed through the S-phase (Fig. 3e). By day 14, the cellular infiltrates in relation to blood vessels had disappeared and the only remaining feature was the prominent Kupffer cells with their inclusions of malarial pigment.

DISCUSSION

We have shown that a marked increase in the blood lymphocyte count occurs shortly after peak parasitaemia, during a non-lethal *P. chabaudi* infection in C57BL mice. While Lelchuk *et al.* (1979) noted an increase in nucleated cells, especially of monocytes in mice infected with a non-lethal *P. yoelii* infection, the pronounced lymphocytosis we describe has not been noted as a regular feature of murine malaria (Strickland, De Silva & Sayles, 1979). ^{3}H-Thymidine labelling *in vivo* in the period preceding this lymphocytosis failed to show an increase in thymidine uptake by peripheral blood lymphocytes in these malaria infected animals. These data suggested that the lymphocytosis

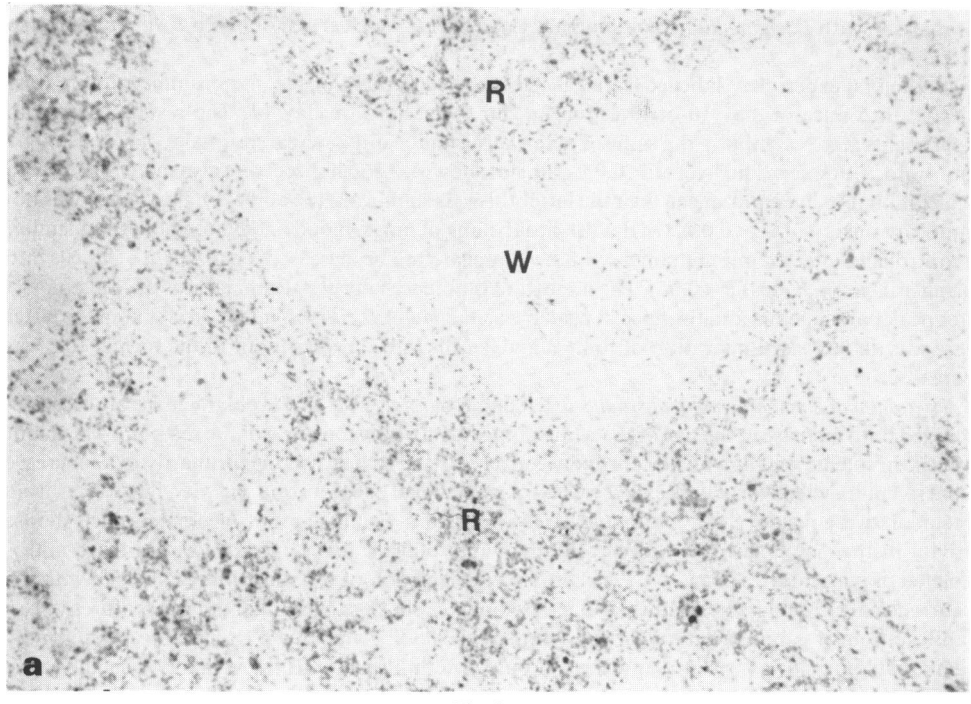

Fig. 3a.

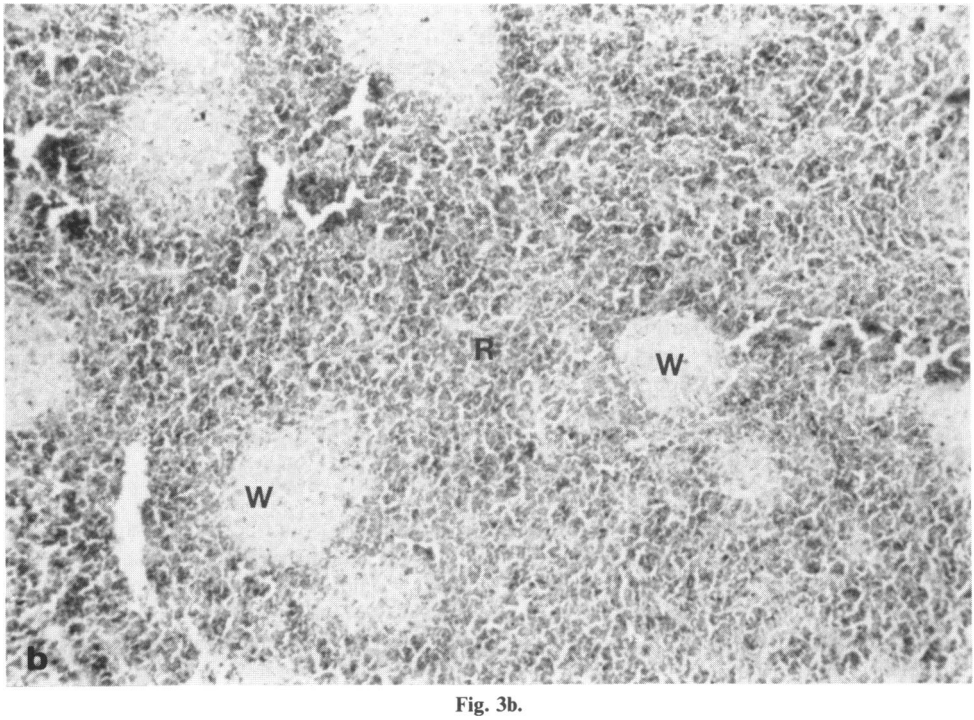

Fig. 3b.

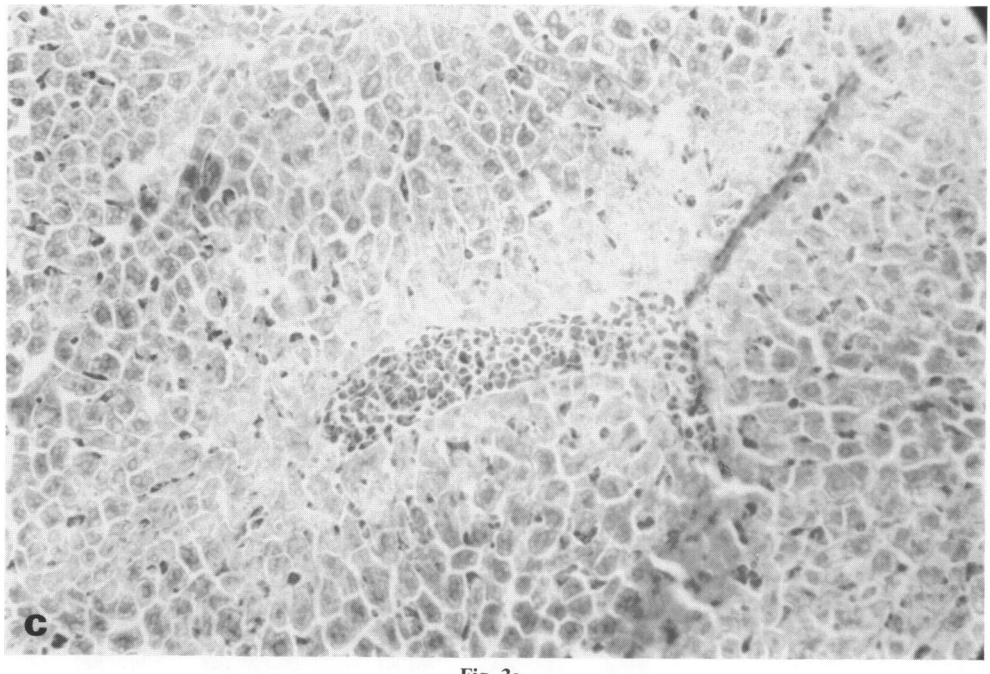

Fig. 3c.

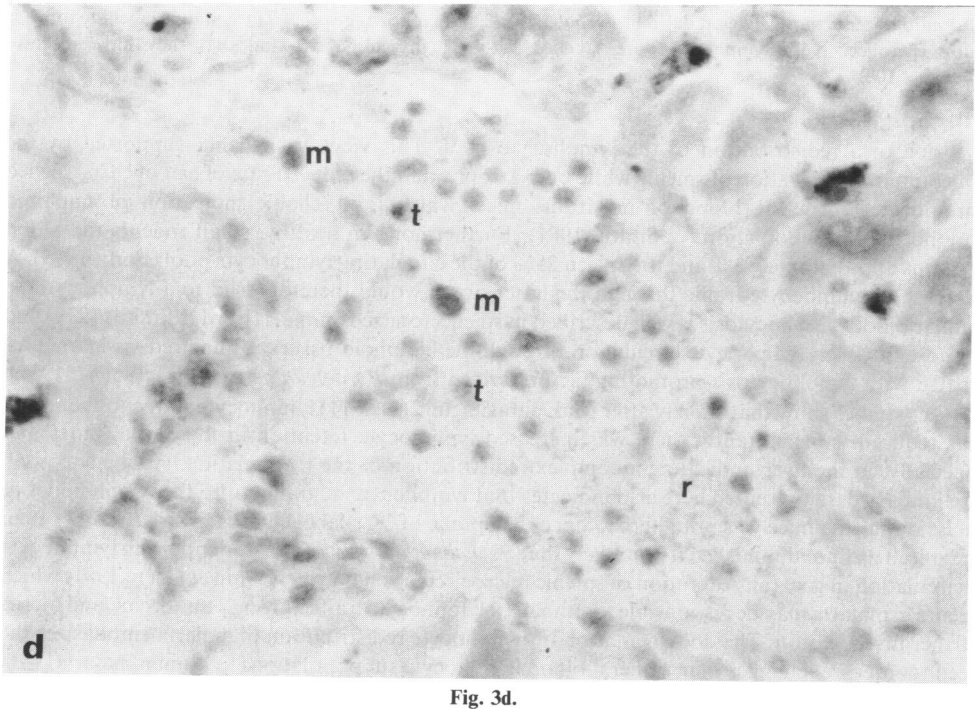

Fig. 3d.

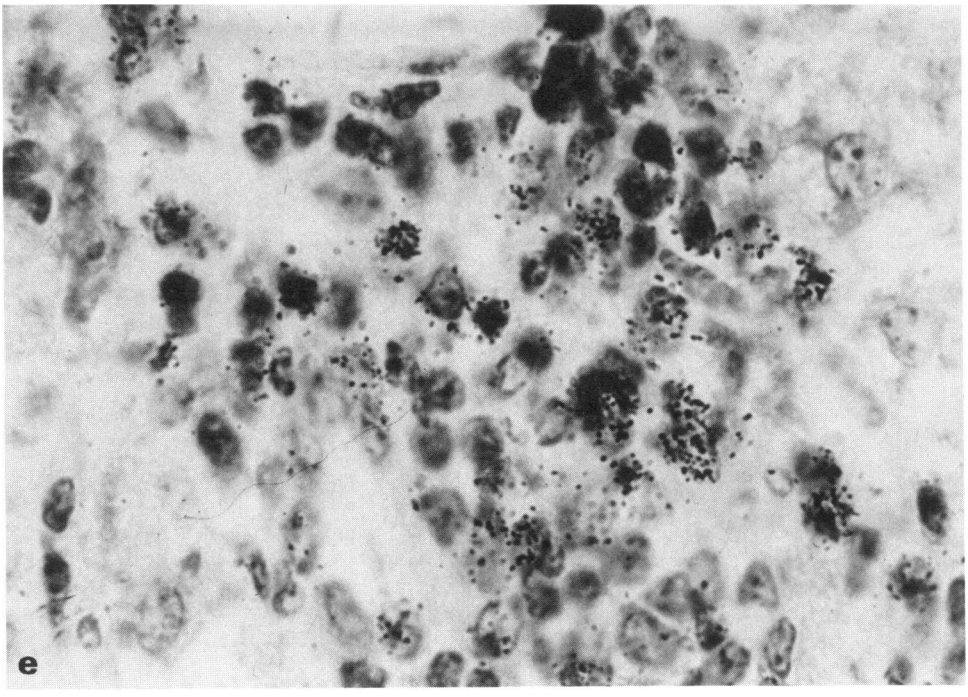

Fig. 3. (a) normal mouse spleen stained to show ANAE activity. (W, white pulp; R, red pulp) × 40. (b) mouse spleen from *P. chabaudi* infected mouse at day 10 of infection (day: note atrophy of white pulp, W; and increase in erythropoietic red pulp, R) × 40. (c) liver of *P. chabaudi* infected mouse at day 9 of infection with *P. chabaudi,* stained for acid phosphatase activity. × 40. Note sinusoid blocked with macrophages and loss of viability of surrounding liver cells. (d) another blood vessel in liver at day 9 of infection stained for ANAE showing macrophages (M), T cells with single dots of ANAE activity (T) and parasitized red cells (R) × 100. (e) autoradiograph of mouse liver at day 9 of *P. chabaudi* infection following *in vivo* ^{3}H thymidine labelling for 24 h before sacrifice. Note lymphocytes are labelled with ^{3}H thymidine showing that they have recently divided. × 100.

was due to a redistribution of the lymphocyte pool. This view was further supported by the concomitant observation of splenic white pulp atrophy and the reduced rate of exit of ^{51}Cr-labelled lymphocytes from the blood of malarious mice, which is a reliable indication of impaired lymphocyte migration (Ford & Smith, 1981). Furthermore, in healthy, small rodents the blood contains approximately 5% and the spleen 25% of the circulating lymphocyte pool (Ford, 1975). A decrease in lymphocyte traffic through the latter organ would therefore lead to a lymphocytosis. Similar blood lymphocytosis is well described in splenectomized animals (Ford & Smith, 1979) and in those in which lymphocyte circulation through the lymphoid tissues is impaired, as in animals treated with lymphocytosis-promoting factor derived from *Bordetella pertussis* (De Sousa, 1981).

Our studies show that shortly after peak parasitaemia (Day 11) lymphocyte traffic to the spleen was reduced; concomitantly there was increased lymphocyte retention in the blood, lungs and especially, in the liver, indicating a complex redistribution of the recirculating lymphocyte pool. Preliminary data presented here also indicates that lymphocyte migration into the lymph nodes of malaria infected mice was not altered. Since the uptake of ^{51}Cr-labelled cells by organs other than the spleen was not diminished it is unlikely that *P. chabaudi* produces a factor impairing lymphocyte recirculation in general. Alteration of splenic microcirculation (Wyler, Quinn & Chen, 1981) which occurs in malaria may be responsible for impaired lymphocyte traffic through this organ, and merits further investigation. The documentation of lymphocyte redistribution in malaria emphasizes the need to interpret changes in peripheral blood lymphocyte subpopulations in human malaria, with considerable caution. Thus T lymphopenia reported in human malaria (Kumararatne *et al.*, 1985)

may merely reflect redistribution but not loss of these lymphocytes. It is interesting to note that absolute blood lymphocytosis is seen in patients with the tropical splenomegaly syndrome, and changes in lymphocyte traffic patterns may be relevant to the immunosuppression occurring in these patients (Greenwood, 1978).

Data from previous studies of lymphocyte traffic in malaria are not strictly comparable with the present investigation as these workers either used pooled lymphocytes from peripheral and mesenteric lymph nodes (Playfair & De Souza, 1982), thymocytes (Brissette, Coleman & Rencricca, 1978), or measured lymphocyte uptake at only one time interval following transfer (Bitzan & Spira, 1978). Mesenteric lymph nodes contain a high proportion of lymphoblasts which take up relatively more ^{51}Cr than small lymphocytes and migrate to gut associated tissue, adding to difficulties in interpretation of data (Ford, 1975). Determination of lymphocyte distribution at a single time point after cell transfer is unreliable in assessing a dynamic process such as lymphocyte migration (Ford & Smith, 1981). The results of the detailed study of lymphocyte traffic in murine malarias by Playfair & De Souza (1982) are essentially similar to that presented here, taking into account that these workers used mixed T and B cell populations which have different tempos of migration (Ford, 1975).

Localization of lymphocytes in the liver is not due to damage by ^{51}Cr. It may be argued that the increased localization of lymphocytes in the liver was due to radiation damage caused by the ^{51}Cr (Ford, 1978). We think this is unlikely for the following reasons: (a) the accumulation of radiolabel (^{51}Cr) in the liver persisted up to 36 h after injection. ^{51}Cr from non viable lymphocytes is rapidly excreted and not reused (Ford, 1978); hence the label is unlikely to have been retained in the liver if it was not associated with viable cells. (b) Histological examination of liver tissue from infected animals consistently showed lymphocytic infiltrates in this organ (as described in the results section), providing independent confirmation of increased lymphocyte accumulation in the liver.

Differences in ^{3}H-thymidine uptake by liver and blood lymphocytes in malarious mice is interesting. The lack of ^{3}H-thymidine uptake by blood lymphocytes while there is evidence of extensive cell division in the liver, appears to be contradictory. Data on ^{3}H-thymidine uptake by peripheral blood cells was obtained by repeated injection of radiolabel using a standard protocol (Ford, 1978) that labels most newly formed cells, to three groups of malaria-infected mice comprising five animals in each group and a parallel group of control mice. The livers for autoradiography were obtained from the same mice as above. This must mean that lymphocytes undergoing cell division did not migrate into the blood stream in substantial numbers between day 7 and 11 inclusive, of a primary *P. chabaudi* infection or that there was selective retention of immunologically stimulated lymphocytes in the liver. A corollary is that antigen and lymphocyte growth factors stimulating lymphocyte mitosis were locally concentrated in the liver of these malarious mice.

What is the significance of the reduced lymphocyte migration through the spleen and the significant increase in T and B cell traffic to the liver in these malaria infected animals? The lack of an anatomically intact spleen prejudices the control of parasitaemia in human and experimental malaria (Grun, Long & Weidanz, 1985). It is also well established that the complex migratory pathways of lymphocytes through the spleen are necessary for cell interactions needed for normal immune responses to blood borne antigens (Ford, 1975), which are depressed around peak parasitaemia (Weidang, 1982). However, the animals studied by us all recovered from their primary parasitaemia. Playfair and colleagues were the first to document increased cell migration to the liver during murine malaria infections, and they went on to show that leuocytes accumulating in this organ during malarial infection were crucial to the development of protective immunity. This view is supported by the adoptive transfer studies of Pearson *et al.* (1983). Splenectomy increases traffic of lymphocytes through lymph nodes and non-lymphoid tissues (Ford & Smith, 1979). Similarly, the impaired entry of lymphocytes into the spleen observed in our experiments may actually increase the delivery of committed immunologically competent cells to the liver where parasite destruction is taking place. A considerable body of evidence (Dockrell & Playfair, 1984; Brinkman, Kaufman & Simon, 1985; Allison & Eugui, 1983) supports the view that toxic oxygen metabolites produced by activated macrophages play a key role in the destruction *in vivo* of malaria parasites. The production of toxic oxygen metabolites by macrophages is enhanced by lymphokines secreted

by antigen specific T lymphocytes, i.e. through a delayed-type hypersensitivity reaction (Brinkman *et al.*, 1985). We have shown that during the phase of control of an acute primary parasitaemia, the liver contains intravascular cell accumulations containing varying proportions of T cells, macrophages and parasitized red cells. It is therefore tempting to speculate that these focal cell accumulations are a major site of parasite destruction via a delayed-type hypersensitivity response. Surrounding liver cell destruction may at least in part be due to bystander cell damage by toxic oxygen metabolites produced by macrophage activation. Focal liver necrosis around cell accumulations in the central vein, has been previously reported in murine malaria (Van Zon, Eling & Jerusalem, 1978; Clark & Clouston, 1980) and in *B. hylomysei* infections (Hussein, 1977). Possible explanation for the liver necrosis are ischaemia or the production of toxic oxygen metabolites by activated macrophages as discussed earlier. Adoptive transfer experiments using congenic rat strains bearing T and B cells differing in cell surface allo-antigenic markers (Hunt & Fowler, 1981; S. V. Hunt, pers. comm.; Kumararatne *et al.*, 1985) would allow the role of T cells, B cells and macrophages from the spleen and liver to anti-malarial immunity, to be analysed in a systematic fashion.

In preliminary experiments with *B. microti,* the localization of labelled normal lymphocytes appeared to be different from that in mice with *P. chabaudi.* At peak parasitaemia and just after, there was no exclusion of labelled lymphocytes from the spleen but there was an increased localization in both liver and spleen of T cells (at 4·5 h) and B cells (at 36 h). It is well established that mice infected with *B. microti* at this time are severely immunodepressed (Gray & Phillips, 1982). Hence in the malarious mice, immunodepression is accompanied by reduced migration of lymphocytes to the spleen but not in mice infected with *B. microti.* However, since our studies did not examine the microcirculatory pathways taken by lymphocytes traversing through splenic tissue of *B. microti* infected mice we cannot exclude the possibility that lymphocyte traffic patterns through this organ are altered in this disease. Autoradiographic studies using ^{3}H-adenosine labelled lymphocytes would be needed to answer this point (Ford, 1975).

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